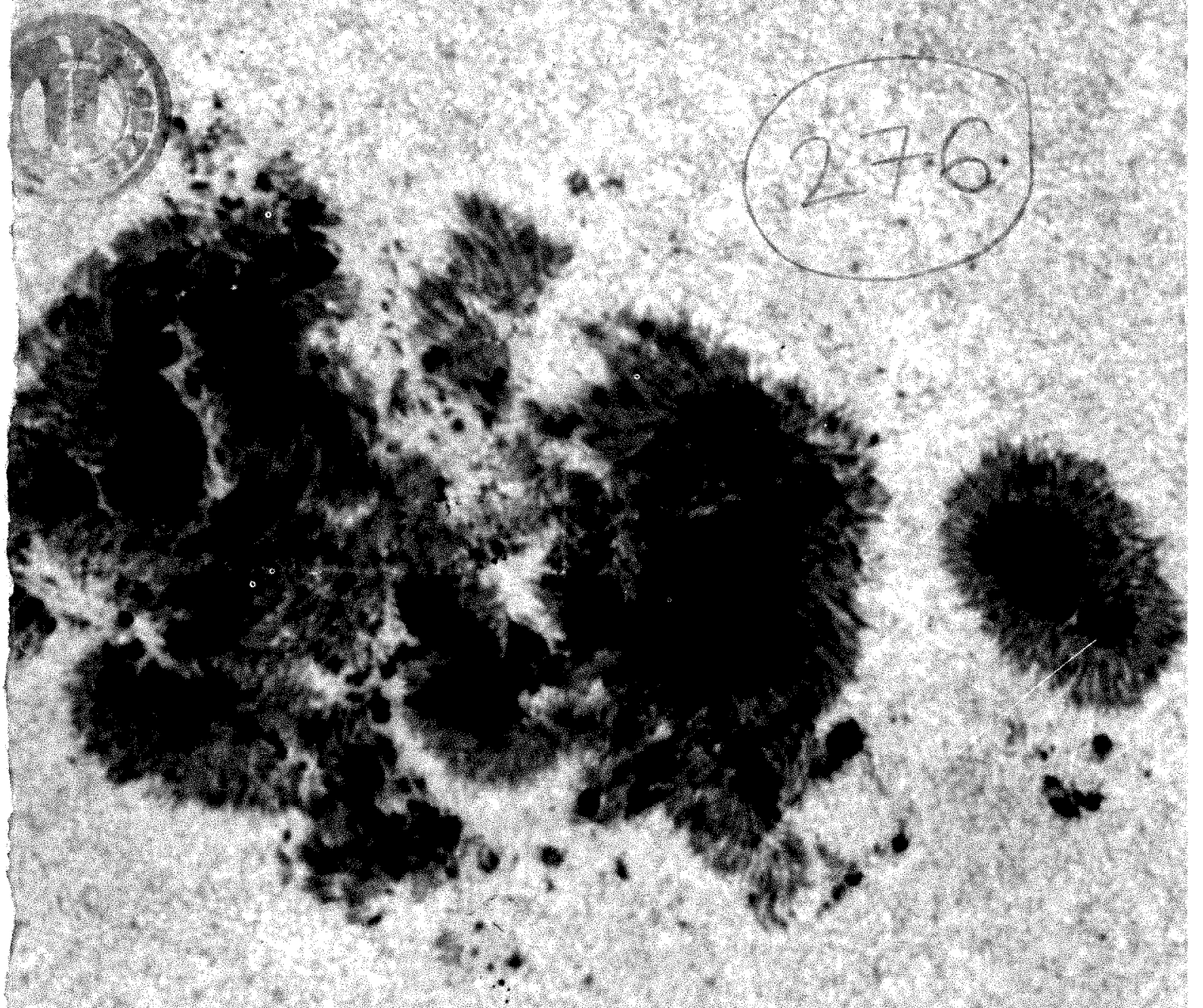
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Cover picture

A large sunspot group photographed at Hale Observatories on 17 May 1951. Statistical evidence for a link between sunspots, solar luminosity and terrestrial climate is presented on p.24.

Vol. 280 No. 5717 5 July 1979

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Volume 280 5 July 1979

Nuclear powers must act quickly 1
British scientists wait for crucial decision that could ease pressure on promotions 2
Dearer oil puts brake on Third World growth 2
Experts agree on Mediterranean pollution treaty 3
Proxmire must answer libel charge 4
NASA bets 50-50 on space shuttle next summer 4
New warning on risk from antibiotics in animal feeds 4
In brief 5
Alpha-fetoprotein screening: too hot to handle? 6
Handle us with care, say chemical industries 8
Correspondence 10

NEWS AND VIEWS

The legacy of Xenophon/Aetiology of natural scrapie/Photogenic impurities/Budgeting for volcanoes/Assessing effects of marine pollution/Alpha-particle decay of giant resonances/Virus evolution during persistent infection 11

REVIEW ARTICLE

A unifying model for the G1 period in prokaryotes and eukaryotes — *S. Cooper* 17

ARTICLES

Redshift dependence of colour and space density of radio galaxies — *P. Katgert, H.R. de Ruiter and H. van der Laan* 20
Solar luminosity and the sunspot cycle — *R.H. Dicke* 24
$^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ ratios from the Azores and their significance in LIL-element enriched mantle — *C.J. Hawkesworth, M.J. Norry, J.C. Roddick and R. Vollmer* 28
An exceptional intraplate earthquake sequence in Meløy, Northern Norway — *H. Bungum, B.K. Hokland, E.S. Husebye and F. Ringdal* 32
Recognition and initiation site for four late promoters of phage T7 is a 22-base pair DNA sequence — *N. Panayotatos and R.D. Wells* 35

LETTERS

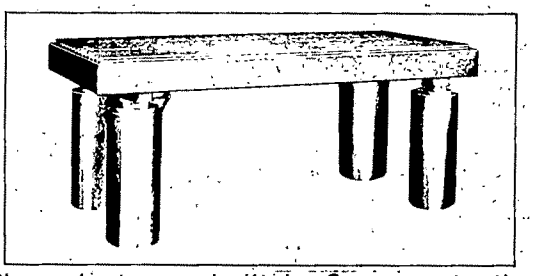
On the detection of large-scale inhomogeneities in the Universe — *A.C. Fabian and R.S. Warwick* 39
The possibility of accretion onto radio pulsars — *G.A.E. Wright* 40
The size of Jupiter's electrically conducting fluid core — *R. Hide and S.R.C. Malin* 42
A relationship between solar activity and planetary albedos — *G.W. Lockwood and D.T. Thompson* 43
Drag reduction in fibre suspensions: transitional behaviour due to fibre degradation — *W.D. McComb and K.T.J. Chan* 45
Ferromagnetism in ancient copper-based coinage — *B.K. Tanner, D.W. MacDowall, I.B. MacCormack and R.L. Smith* 46
Confirmation of the Suess wiggles: 3200–3700 BC — *A.F.M. de Jong, W.G. Mook and B. Becker* 48
Sulphur uptake from the atmosphere by forest and farmland — *I.E. Galbally, J.A. Garland and M.J.G. Wilson* 49
Magnetic measurements used to assess sediment influx at Llyn Goddionduon — *J. Bloemendal, F. Oldfield and R. Thompson* 50

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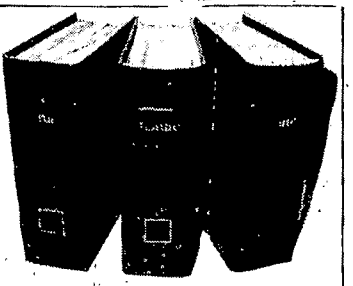


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^{210}Pb dating of annually laminated lake sediments from Finland	*P.G. Appleby, F. Oldfield, R. Thompson, P. Huttunen and K. Tolonen*	53
Cinnamic acid inhibition of detritus feeding	*I. Valiela, L. Koumjian, T. Swain, J.M. Teal and J.E. Hobbie*	55
Isolation and cultivation of diatom symbionts from larger Foraminifera (Protozoa)	*J.J. Lee, M.E. McEnery, M. Shilo and Z. Reiss*	57
Changes in the circuitry of the kitten visual cortex are gated by postsynaptic activity	*J.P. Rauschecker and W. Singer*	58
Orientation of horizontal cell axon terminals in the streak of the turtle retina	*R.A. Normann, H. Kolb, M. Hanani, E. Pasino and R. Holub*	60
Involvement of cyclic GMP phosphodiesterase activator in an hereditary retinal degeneration	*Y.P. Liu, G. Krishna, G. Aguirre and G.J. Chader*	62
Evidence that cyclic GMP regulates membrane potential in rod photoreceptors	*W.H. Miller and G.D. Nicol*	64
Opposite effects of intracellular Ca^{2+} and glucose on K^+ permeability of pancreatic islet cells	*J.C. Henquin*	66
Variability of cell cycle times measured *in vivo* in embryonic chick retina by continuous labelling with BUdR	*V.B. Morris, R. Cowan and D. Culpin*	68
Decreased response to calcitonin in osteopetrotic microphthalmic mouse bone	*G.N. Kent, D.G. Walker and D.V. Cohn*	71
Melittin shares certain cellular effects with phorbol ester tumour promoters	*R.A. Mufson, J.D. Laskin P.B. Fisher and I.B. Weinstein*	72
Quantitation of the adaptive response to alkylating agents	*P. Robins and J. Cairns*	74
Adaptive response to alkylating agents involves alteration *in situ* of O^6-methylguanine residues in DNA	*P. Karren, T. Lindahl and B. Griffin*	76
Hormonally specific phosphorylation of cardiac troponin I and activation of glycogen phosphorylase	*L.L. Brunton, J.S. Hayes and S.E. Mayer*	78
High-frequency generalised transduction by bacteriophage T4	*G.G. Wilson, K.K.Y. Young, G.J. Edlin and W. Konigsberg*	80
Inability of circular mRNA to attach to eukaryotic ribosomes	*M. Kozak*	82
Evidence that *Euglena* chloroplasts do not export tRNAs	*S.D. Schwartzbach, W.E. Barnett and L.I. Hecker*	86
Molecular basis for proton-dependent anion binding by soybean leghaemoglobin *a*	*J. Trewhella, P.E. Wright and C.A. Appleby*	87

BOOK REVIEWS

Cancer, Science and Society (John Cairns)	*Richard Peto*	89
Gravitation, Electromagnetism and Quantized Charge: The Einstein Insight (D.M. Pisello)	*Maurice Bazin*	91
Science and the Sociology of Knowledge (M. Mulkay)	*Stuart S. Blume*	91
Mysterious Universe: A Handbook of Astronomical Anomalies (W.R. Corliss)	*David W. Hughes*	92
The Physics of Vibration (A.B. Pippard)	*D.J. Thouless*	92
Newly on the market		vii

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nature

5 July 1979

Nuclear powers must act quickly

FOR the next few months the question of ratification of the SALT 2 treaty by the US Senate is bound to occupy the attention of most Western arms controllers and politicians with an interest in arms control. Then early next year, regardless of the outcome of the SALT debate, their thoughts will have to be diverted to the five-yearly review conference of the Non-Proliferation Treaty (NPT) planned to take place in May 1980. And it is certain that the NPT conference will be the occasion for yet more bitter questioning by the non-nuclear-weapon states of the seriousness of commitment of their nuclear counterparts to Article VI of the NPT, which said that all parties to the treaty undertook to "pursue negotiations in good faith on effective measures relating to cessation of the nuclear arms race at an early date and to nuclear disarmament . . . ".

A successful outcome to the SALT debate would certainly tone down the criticism to a certain extent. But it is clear that what the non-nuclear-weapon states really want is a weapons-test ban. Diplomatic wording of the agreed statement at the end of the 1975 NPT review conference could not conceal exasperation at continued testing. "A considerable number of delegations" wanted a ban for a specified time, with a view to negotiating eventually a "universal and permanent cessation of all nuclear weapons tests". Such a treaty has yet to come about.

True there is now US-Soviet agreement to restrict tests to a maximum of 150 kilotons yield. (The UK uses the US test-site for its one or two explosions per year so is also bound by this agreement; the French and Chinese are not.) True there are US-UK-Soviet talks proceeding at present on a possible short-term treaty. True there has been some softening of the previous Soviet intransigent position on monitoring stations on its own soil. But there is as yet little evidence that there will be a solid treaty by the time of the NPT conference. Two points are as yet unresolved.

The first is rather silly. Some months back the Soviet Union proposed that for the three-year period, now regarded as the most that a ban would be negotiated for, each party to the tripartite talks would have ten seismic monitoring stations on its soil to verify compliance — the data to be mutually available. This was a big step forward for the Soviet Union, but did they really mean ten stations on British territory? Apparently they did. British territory does not amount to much these days outside the UK. There would be no scientific point in more than two stations in the UK to monitor compliance, and for the rest the instruments would have to be placed in Hong Kong (useful for keeping an eye on the Chinese, were the noise levels not so high) and sundry remote islands, probably equally noisy and therefore useless. As the monitoring posts are supposed to be the best that technology allows, the bill for this futile operation could not be less than tens of millions of pounds. The British, who on the whole wanted to take a back seat in these negotiations and not get in the way of a superpower agreement, suddenly find themselves pushed up front in rather a ridiculous way. Scientific honesty requires that they resist this pointless waste of resources; political necessity requires that they not let the Soviet Union make capital out of it.

The second sticking point is more substantial. After a three-year total ban, what comes next? On the surface the question is what to call the review conference at the end of the period, but the real issue is whether a three-year ban could ever be converted into a longer one. Yes, say the Russians, if the Chinese and French behave themselves and if some way can be found of coping with 'peaceful' explosions (a thorny problem). No, say the Americans, we can't be tied; there is enough domestic opposition as it is to any ban at all — the weapons laboratories are pushing the issue of stock-pile testing harder year by year and claiming (not without dissenting voices) that weapons untested for long periods will be unreliable. Aldermaston, with its trans-Atlantic ties, would say the same if allowed to be asked, but the British are burying any internal differences in a united front of keeping the options open for longer term ban. Mrs Thatcher is known, however, to be showing some interest in the UK test ban position; it is on the cards that Britain might eventually show less enthusiasm for long term restraint. Whatever agreement is finally reached, it is indisputable that in the US, the President will have to sell it very hard by means of vigorous 'safeguards' such as more, not less, financial support for the weapons laboratories.

Time, it hardly needs saying, is running out for a convincing treaty to be prepared before the NPT conference, especially as the multilateral Geneva Disarmament Conference will want to take a look at it. Non-nuclear-weapon states are, rightly, going to work up a head of steam over the failures to prevent vertical proliferation whilst they have been restrained from horizontal proliferation. Their position of restraint is not made easier by the continual gossip about the nuclear capabilities of South Africa, Pakistan and Israel. Unless the three negotiating nations put a lot more goodwill into their discussions in the next few months they will deserve all the obloquy they will certainly get. □

British scientists wait for crucial decision that could ease pressure on promotions

In two weeks the Science Research Council is to make a decision on a controversial plan aimed at easing the critical staff stagnation problem which now affects universities in the UK. On 18 July, its central council will decide whether or not to give the go-ahead to a special release fellowship scheme that is intended to help more senior research workers vacate tenured posts which can then be filled by young scientists.

The age structure at UK universities has been deteriorating since the higher education expansion of the early 1960s, when new departments were created and were manned at senior levels with young scientists. In recent years, economic recession has ended the creation of additional posts and the distribution of posts has been frozen.

Indeed, in some science departments there have been no new appointments for the past ten years. Where there have been vacancies these have been filled from within departments, thus halting the crucial flow of ideas and talent between universities.

Now, in an attempt to stop this stifling of fresh talent, the SRC is seriously considering the establishment of special fellowships which would fund research workers advanced in their careers and allow them to leave university posts to concentrate solely on research without administrative responsibilities. Their vacated positions would then be filled by young scientists and over subsequent years allow the phasing-in of new research workers.

However it is stressed that the scheme is not meant as a form of premature retirement for ageing researchers, and only those who are active and still have important scientific contributions to make will be considered for funding.

The proposal to be discussed by the SRC this month is for 15 fellowships to be set up in the first year of the programme with further annual increments of 10 new awards. This could then provide a total of 45 special release fellowships within four years.

But there are several difficulties associated with this plan. For one thing, it has not yet been agreed whether the SRC should pay for the senior researcher's salary or if it should pay for the junior post. The difference could represent a variation of about 50% in final costs and consequently estimates vary between £300,000 and £450,000 a year for the ultimate price of the full fellowship scheme.

However the council is at present particularly concerned about its future budget allocations and may be very reluctant to commit itself to such a level of funding. At its meeting, it may decide to cut the numbers of fellowships or may even postpone any decision until September when it hopes to learn its financial guidelines for the coming years.

Indeed, some council members are very cautious about the whole idea of the scheme, believing it be the role of the University Grants Committee to provide proper staff structures within departments. Combined with these other factors, this view puts the council's coming decision very much in the balance.

Robin McKie

Dearer oil puts brake on Third World growth

THE effect of the current energy crisis on Western nations has attracted considerable international attention but precious little has been said about the non-OPEC Third World countries. Energy experts who gathered at a forum on 'Third World Energy Strategies and the Role of Industrialised Nations' organised recently (20-22 June) by the Royal Institution in London, described an extremely critical energy situation developing shortly in these countries, which could almost threaten their very survival.

Their predicament was well illustrated by a case study of Kenya presented by T. S. Tuschak, a UN energy consultant based in Nairobi. Mr Tuschak pointed out that Kenya consumed in 1978 a little less than 12 million barrels of oil (equivalent to about one week's consumption in Canada). This small quantity of oil accounted for about 85% of the energy consumed by the country's modern sector. About 80-82% of this oil was consumed in essential sectors like industry, agriculture and goods transport. The rest was accounted for by private automobiles, which too in many cases were not inessential because of an underdeveloped public transport.

The possibility of oil savings in the essential sectors is at best 7%. Consumption by private transport can at best be reduced by about 10%. Kenya, therefore, has few options but to maintain oil imports at near current levels, and will possibly even have to increase them if the economy continues to grow.

Tuschak estimates that for $1/barrel increase in oil price, the country's export earnings would have to increase by about 1% over the 1978 level. This expansion in exports may sound small but could well be impossible considering the global slow down in economic growth.

Coffee and tea represent 50-60% of Kenya's total exports and they are about the only commodities in the country capable of any significant expansion in world markets. Their export would have to increase by as much as 30% in a year to cover the incremental foreign exchange needs. "The only way to meet this bill," said Tuschak, quite despondently, "is to further impoverish the country, by paying for oil by funds destined for development". Few developing countries will be able to afford an oil price of $20-22/barrel.

The other possibility, of course, is for Western countries to bale out the developing countries. But this option brought forth only a spirited discussion, virtually no agreement.

Stephen Bosworth, the US Ambassador to Tunisia, and former chairman of North-South negotiations on energy at Paris, plainly warned Third World energy planners that Western aid and loans from international financing institutions are unlikely to rise fast enough to meet the needs generated by oil price rises. Nor is an oil sharing system which gives preferences to developing countries is likely to come into operation he said. The world will have a slower rate of growth in the future. "We have seen that Western parliaments react unfavourably to any real transfer of resources in those conditions."

The discussions on alternate energy sources that could replace oil revealed two important Third World concerns; first, that the Third World is extremely unhappy with the constant preaching by Western scientists and technologists that unconventional energy sources like wind and solar power, and fuelwood plantations are best for it. All Third World energy experts gathered in London agreed that these energy sources would be good for developing countries but they would first want them to be fully tried and tested.

Secondly, they pointed out, none of these sources can provide an alternative to oil in the short-term which is really the critical period for the non-oil producing Third World.

Dr A. K. N. Reddy of the Indian Institute of Science at Bangalore threw an outright challenge to the developed countries: "Teach us to live without oil but by doing it yourselves". That the developed countries must first put their own house in order was a demand that was echoed by many Third World participants.

Dr Joao A. Meyer, of the State University of Campinas in Sao Paulo, criticised Western efforts for following their traditional approach to technology transfer even in the new field of non-traditional energies. They are once again trying to sell gadgets, instead of trying to

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Fledgling transport systems could be hit by petrol shortages

help developing countries solve their own problems, he said; the only difference now is that the accent is more and more on solar power plants instead of nuclear plants.

"Often we do not even know whether immature energy technologies are being pushed upon us", Dr Meyer alleged. "And where we have developed technologies indigeneously, for instance, the production of alcohol from sugarcane, we find ourselves besieged with claims of patent infringements and demands for royalties."

Among alternate energy sources, discussions centred on wood, which provides over three-quarters of the Third World's population with its energy needs. For these people, the wood crisis is worse than the oil crisis, and certain to become even more acute. Kenyan experts revealed that wood scarcity in their country is already so acute that serious thought is being given to providing electricity for cooking in rural areas to replace the use of firewood. "Until 1973, we thought we had the wood problem licked", said India's energy planner Sankar. "We were subsidising the use of kerosene in villages to replace the use of firewood. But then in 1973 the carpet was pulled out right under out feet."

The answer is to grow more wood; but the forum discovered that first, scientists themselves do not have adequate knowledge about fast growing trees and forestry techniques, and second, even if existing knowledge could be applied right away, a British expert pointed out, it would be 1996 before rural areas could get wood. Problems of social organisation in Third World villages will prevent rapid diffusion of innovations. India's progress with biogas plants to meet rural cooking energy needs has also been very slow.

There was, however, total unanimity on the need for developing countries to plan their future energy use carefully and to discover new sources. Once developing countries identify their energy problems, and choose the technologies they want to solve them, then the developed countries can help them in developing those technologies instead of passing on to them packages that imitate successful ones in the West, said Professor Ignacy Sachs of the University of Paris.

However, the forum learnt that though very soon most Third World countries would be setting up ministries of energy, few of them had either the relevant information systems or the trained personnel with analytical capabilities to make proper economic, social and technical choices in the energy sector. It is here that developed countries and international organisations can help most, felt the Third World participants.

Professor M. S. Wionczek of Mexico suggested the establishment of an institute for advanced studies in energy policy, possibly similar to the Institute of Theoretical Physics at Trieste, a unique institution which provides scientists from developing countries an opportunity to meet and discuss with leading scientists, including Nobel laureates from developed countries. Venezuela's Minister of State for International Economic Affairs, Mr M. Perez-Guerrero, suggested a new institute be set up within the UN to help Third World countries in energy planning.

But there were no solutions to the problem that Third World experts had themselves identified: what do developing countries do to solve their energy problems in the immediate future? As India's Sankar put it: "I find no dearth of experts prepared to chart out an energy strategy for the year 2000. My problem, however, is 1980 and 1981. Who will help me solve that?"

Anil Agarwal

Experts agree on Mediterranean pollution treaty

Scientists from 14 of the 18 Mediterranean states unexpectedly agreed last week on the text of a treaty to control land-based sources of pollution — but the rate of implementation of the treaty will depend on how fast scientific work can be completed.

"The deadlock is over" said the director of the United Nations Environment Programme's regional seas unit, Stjepan Keckes, this week, "but it is not the breakthough that some newspapers have reported".

One of the main obstacles had been a disagreement between the northern, industrial nations, and the southern underdeveloped states, over whether to impose uniform emission standards on polluters, or to define common goals for coastal water quality. The underdeveloped states argued that the former would penalise the growth of new industry in an area which presently had little industry and little pollution; while the north contended that the latter would allow the south to develop cheap and heavily polluting industries which would compete with more expensively controlled northern firms. In the end the underdeveloped states won the argument, and the treaty specifies coastal water quality standards. "This is the most logical arrangement" Keckes told *Nature*. "It is the same pragmatic scheme you operate in Britain".

The treaty will go to a specialist diplomatic meeting in Athens in April for signature. Ratification comes later.

"These are the first steps", said Keckes, a marine scientist who is also in charge of the scientific assessment of the state of the Mediterranean. "But we don't have enough evidence yet. We can define water quality for bathing, using a microbiological standard — we've been measuring on 150-200 beaches — and later we hope to define heavy metal limits for shellfish growing" he said. "But the rate of implementation of the treaty will depend on how fast we can define stanards scientifically."

Progress is therefore limited by the money UNEP can spend on its pollution monitoring programme, 'Medpol'. Earlier this year, a full meeting of the contracting states voted to delete Medpol's air pollution research programme (see 15 February p 506) — whereas air, Keckes feels, may be the main source of the central Mediterranean's metallic pollution.

"The research programme needs an additional boost and additional money" said Keckes. He hopes to present a strong case for restarting the air pollution programme to the next full meeting of contracting states in February 1981.

Robert Walgate

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Proxmire must answer libel charge

THE US Supreme Court last week decided that Senator William Proxmire could not claim Congressional privilege in making derogatory remarks about a scientist's research in press releases and news broadcasts announcing his Golden Fleece award. The award is presented regularly to esoteric-sounding research projects supported by federal funds.

The court also decided that, in claiming libel damages for remarks made by the Senator in presenting such an award, a scientist should not be considered a "public figure" by virtue of having received public funds, and therefore was not required to demonstrate that the remarks had been made with deliberate malice.

The Supreme Court's decision was based on an appeal made by Dr Ronald R Hutchinson of Kalamazoo, Michigan, in connection with a libel suit that he had filed against the Senator following the award of a Golden Fleece to the National Aeronautics and Space Administration for supporting Dr Hutchinson's research on the latent aggression of laboratory monkeys.

Labelling the research as being to study "why monkeys clench their teeth", Mr Proxmire claimed in a speech to the US Senate in 1975 that "the good doctor has made a fortune from his monkeys and in the process made a monkey out of the American taxpayer", a charge he repeated in a newsletter to his constituents and a subsequent radio television interview.

Dr Hutchinson subsequently filed a libel suit, claiming that Mr Proxmire's actions had caused him to be held up to public scorn and resulted in a loss of respect in his profession, as well as "loss of income and ability to earn income in the future".

The suit was rejected by a lower court partly on the grounds that Dr Hutchinson had become a "public figure" by soliciting government grants and publishing articles in professional journals, which required him to demonstrate "actual malice", and partly because Senator Proxmire claimed legislative privilege in making the remarks.

In last week's decision, however, the Supreme Court stated that, although local newspapers had reported Dr Hutchinson's receipt of research grants, and although he was quoted in the press in reacting to the Golden Fleece award, neither of these was sufficient to make him a "public figure".

"His access [to the media] such as it was, came after the alleged libel and was limited to responding to announcement of the award. Those charged with alleged defamation cannot, by their own conduct, create their own defence by making the claimant a public figure," the court announced.

On the question of legislative privilege, the court ruled that this could not be used by the Senator as a defence, since there was no evidence of past Congressional concern to grant absolute privilege for remarks made outside the legislative chambers, since neither the news-letters nor the press release were "essential to the deliberation of the Senate", and neither was protected as part of the responsibility of members of Congress to inform the public of their activities.

In the light of these decisions, the Supreme Court directed that the case be returned to the Court of Appeals "for further proceedings consistent with this opinion". In a dissenting opinion, Mr Justice Brennan declared that in his opinion "public criticism by legislators of unnecessary governmental spending whatever its form, is a legislative act shielded by the Speech or Debate clause". □

NASA bets 50-50 on space shuttle next summer

OFFICIALS of the US National Aeronautics and Space Administration told a Congressional committee last week that the first orbital flight of the reusable space shuttle is not now likely to take place until next summer at the earliest, and that this may mean at least four satellites scheduled for early shuttle launches will have to seek alternative ways of getting into orbit.

Dr Robert Frosch, the agency's administrator, told the House Science and Technology Committee that technical delays in the development of the shuttle's engines and in fitting its heat-resistant surface tiles meant that there was now only a one-in-five chance of launching the shuttle by the end of the first quarter of 1980, a launch originally planned to have taken place this spring, and a 50-50 chance of a first flight in the second quarter.

"This slip means our first operational flight, which had been planned for February 1981, will be delayed until September 1981," Dr Frosch said. "This means some of our early users will have to shift to use the backup expendable launch vehicles to get their satellites into orbit." (It also means that it will now be necessary to revise the launch schedule for the European Space Agency's Spacelab, which was due to be launched by Space Shuttle in August 1981).

Among the satellites whose launches will be affected by the new shuttle delays is a geosynchronous operational environment satellite which was planned to be launched by the National Oceanographic and Atmospheric Administration; shifting from a shuttle launch to an expendable launcher could increase the cost from about $10 million to $22 million, and the same would apply to two commercial communications satellites that were to have been among the first payloads. □

New warning on risk from antibiotics in animal feeds

THE US Congress' Office of Technology Assessment reported last week that antibiotic resistance is continuing to increase in the US population, and warned that, although quantative relationship remains uncertain, the continued use of antibiotics in animal feeds seems to be contributing to this process.

The OTA's warning came in a report on "Drugs in Livestock Feed" prepared at the request of the Congress following the Food and Drug Administration's proposal in 1977 to ban the use of penicillin and sharply restrict the use of two tetracycline drugs in animal feed. According to the industry, sales of antibiotics and similar antibacterial agents added to animal feed exceeded $180 million last year; and the OTA estimates that the FDA's proposed restrictions could increase retail meat and poultry prices by between 3% and 28% in one year.

However the OTA also says that antibiotics in animal feed contribute to a "growing pool of drug-resistant bacteria", and that physicians are now reporting reduced effectiveness of the drugs in treating disease; and it points out that many of the drugs on which restrictions are proposed could be replaced with alternative drugs that are already approved by the FDA.

It is up to Congress, the agency concludes, to weigh the economic costs of banning the drugs in animal feeds against the health risks of allowing their continued use. "These decisions involve value judgements that cannot be based simply on monetary considerations. And the lack of scientific certainty on the magnitude of both the probable health risks and the attributed increases in meat production makes the formulation of a balance-sheet approach difficult," the OTA report says.

The short-term consequences of a ban on the drugs could be significant, since production might decrease, leading to higher prices. But the OTA says that the long-term consequences are less certain, probably resulting in small decreases or no changes in production and small increase in both consumer prices and overall producer incomes.

Dr Joyce Lashoff, OTA's assistant director for health, emphasised at a press conference last week that the OTA made no specific recommendations about how Congress should act, merely listing a range of options. Giving a personal view, however, she said that she believed the availability of substitutes meant that the economic impact of the regulations proposed by the FDA would not be great enough to offset the potential risk to human health if the use of antibiotics remained unrestricted. □

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news in brief

New agency proposed to handle nuclear waste: A loss of confidence in the US Department of Energy's ability to come up with a co-ordinated nuclear waste disposal programme means that the problem should be handed over to a new committee of federal and state representatives, the General Accounting Office, the investigatory arm of the US Congress, announced last week. In a report prepared at the request of Senator John Glenn, the GAO pointed out that a number of states had already passed various forms of legislation concerning how nuclear waste issues should be handled, but that the federal government had so far failed to come up with an acceptable permanent storage plan for high level wastes. This was becoming a growing obstacle to the development of nuclear power. "We believe it is very unlikely that making the Department of Energy the responsible lead agency to plan and co-ordinate the programme will establish public trust and confidence", the GAO report states.

German mathematician fights 'Berufsverbote': The case of Horst Eckart Gross, 36, who was excluded from a job as assistant professor at the University of Oldenburg, has entered a new phase. West German authorities are now arguing that the recent public campaign in support of Gross' employment is further evidence of his "unconstitutional behaviour". Gross was nominated for a position teaching the history and professional practice of mathematics by the normal university committees in February 1977. The Verfassungsschutz, the West German body responsible for vetting political opinions of government employees, delayed the appointment for almost a year and then said in its final report that there was information which gave reason to doubt Gross' loyalty to the "Freiheitliche Demokratische Grundordnung" (free and democratic constitution). The information in question consisted of Gross' activities in a German-Cuban friendship society which has local groups in more than 30 West German cities. Members of the organisation include trade unionists and members of parliament. Gross is also accused of membership of the German Communist Party and of past membership (1967/68) in the SDS.

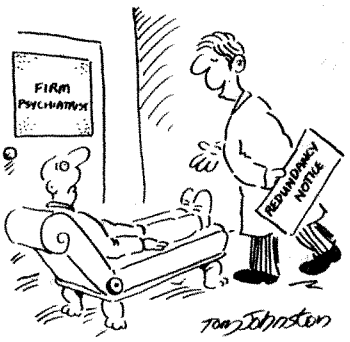
The council of the University of Oldenburg passed a strong resolution in support of Gross on 11 January 1978 protesting at his examination by the Verfassungsschutz and calling for his immediate employment. "This Berufsverbote case shows perhaps more clearly than any other the unconstitutional methods used by the political police", it said.

Cuba to build nuclear reactor: Cuba is proceeding with a plan to build the first nuclear power plant in the Caribbean. Mr Luis Beltran, minister in charge of the electrical power industry, announced last week that construction on the Soviet-designed reactor would start this year at Cienfuegos on Cuba's southern coast. The reactor is of the pressurised water type similar to the one involved in the Harrisburg accident. Mr Jorge Perez-Lopez, a US government economist, claims that the Soviet design lacks two safety features which are common to PWRs built in Western Europe and the US. Writing in the magazine Cuban Studies, Perez-Lopez says that Soviet PWRs lack a backup cooling system and an outer casing to contain possible leaks. Perez-Lopez suggests that Cuba and the Soviet Union have a mutual interest in developing Cuban nuclear power at this time. The Soviet Union conserves its oil supplies for trade on the world market and Cuba lessens its financial dependence on Soviet oil.

'Plenty of jobs in the UK': According to Neil Macfarlane, minister of science in the new Conservative government, jobs are going begging in the UK. Speaking at an Association of British Science Writers lunch, Macfarlane stated that "for people with the right skills there are plenty of jobs". When asked whether this included scientists on short-term contracts, Macfarlane declined to comment, agreeing with his host, Roger Lewin, deputy editor of *New Scientist*, that "the issue was too complicated for a brief discussion".

Meanwhile, the Department of Education and Science has just released a recruiting pamphlet, Science at Work, intended to attract school students to science careers. The pamphlet is one of a DES series whose "main emphasis is on educational courses and qualifications that lead to jobs". It will be circulated to all schools, colleges and careers offices in the UK. The booklet specifically cites teaching as a career option. "To ensure the continuity of science progress, there is always a need for scientists as teachers in schools, colleges, polytechnics and universities." Also listed are possible careers in microelectronics and librarianship. *Science at Work*. Department of Education and Science, Room 2/11 Elizabeth House, York Rd, London SE 7PH. Free.

Europeans are afraid of new science and technology: A public opinion poll shows that 80% of the people in the EEC fear for the future of the world they live in because of the "despoiling of natural life and the countryside through pollution of all kinds". A second major fear (67%) is of widespread unemployment caused by automation. A majority (53%) also expressed concern about new technologies in housing, traffic and food and the introduction of drugs that "may severely affect the human personality". The study had been requested by the EEC Commission from European Omnibus Survey, a group of eight specialised institutes in the member countries. Four countries — Germany, Denmark, the Netherlands and the UK — rank pollution as their number one concern and four others — France, Belgium, Ireland and Luxembourg — rank unemployment first. The Italian public fears an overall deterioration of living conditions. This includes the conviction that science is not being used for the benefit of the population as a whole.



"And just how long have you had this fear of unemployment caused by automation?"

The poll shows that even though only 10% considered themselves sufficiently educated to discuss science competently, the majority insisted on being involved in major development choices. The nuclear power questions show that the public has been convinced that curtailing nuclear power could mean loss of electricity. But at the same time, 36% are still opposed to nuclear power compared to 44% who favour it. The poll disagrees sharply with a 1977 survey, which showed "clearly" that there was no mistrust of science. *The European Public's Attitudes to Scientific and Technical Development*. Commission of the European Communities. Brussels.

ETSU finds most renewable energy sources safe: The UK's Energy Technology Support Unit has given an environmental clean bill of health to most renewable energy sources. In a preliminary report to the Department of Energy, the unit finds that the impact of solar and wind energy conversion is "likely to be relatively small". The impact of tidal energy and wave energy is considered to be more complex. Wave energy converters could interfere with herring and salmon migration paths and with ship navigation.

The report lists 13 areas of concern about tidal energy conversion, including effects on ports, freshwater fisheries, pollution dispersion and agriculture; £1.5m has been devoted to the further study of these effects. *Environmental impact of the renewable energy sources*. ETSU, Harwell, OX11 0RA.

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Alpha-fetoprotein screening: too hot to handle?

US manufacturers want to market pre-natal screening kits for anencephaly and spina bifida. But can US medicine cope with the risks? **David Dickson** reports

WHEN Dr Donald Kennedy returned to Stanford University last Friday after a two year spell as Commissioner of the Food and Drug Administration, he left unresolved a classic but little-publicised example of the problems society faces with potentially hazardous technologies: namely whether — and if so under what circumstances — the FDA should approve the marketing of techniques capable of warning an expectant mother that she is bearing a child with a serious birth defect.

The techniques in question use a radioimmunoassay to measure the level of alpha-fetoprotein (AFP) in the mother's blood serum. A high level of AFP indicates that the foetus has an above-average — although still relatively small — chance of suffering from a neural tube defect (NTD), manifest at birth as either anencephaly or spina bifida. A precise diagnosis can subsequently be achieved using ultrasonography and amniocentesis.

If properly used, this series of tests could theoretically pinpoint the one or two children in a thousand born in the US with a neural tube defect at a sufficiently early stage of pregnancy to permit an abortion, if the mother so chooses. A wrong diagnosis, however, or a misintepretation of preliminary test results, could lead to the abortion of a healthy foetus.

It is a situation in which even obstetricians have expressed doubts about their present ability adequately to handle the whole testing procedure. The FDA, to which a number of manufacturers have submitted applications for licences to market AFP screening kits, now faces the problem of building sufficient checks into the licensing process to minimise the risks involved. In doing so, some fundamental questions have been raised about the ability of an essentially free-market medical system to provide the coordination, support and oversight needed to make this possible.

The discovery that neural tube defects are associated with high levels of AFP in the amniotic fluid was first made in the early 1970s by two scientists at the University of Edinburgh in Scotland. It is thought to occur because the defect allows the protein, which is only found in foetal blood, to leak into the surrounding amniotic fluid.

Early tests showed that measuring AFP level by amniocentesis provided a highly accurate guide to the chances of a foetus suffering an NTD. And subsequent tests in the United Kingdom also indicated that merely measuring AFP levels in the mother's blood could provide an initial clue to such a likelihood.

The problem, however, is that a correct diagnosis based initially on serum testing requires a number of separate further steps, each of which has to be carried out with a high degree of accuracy. Firstly the AFP level in the maternal serum must be measured between the 16th and the 18th week of pregnancy; if the AFP reading is abnormally high (typically considered to be in the top 5% of mothers tested), a second measurement is made, although the woman still has only a one-in-fifty chance of carrying a defective foetus.

A second high reading, indicating a 10% chance of a defect, leads to further testing to discover the reason. Ultrasonic examination of the womb can usually indicate if the foetus is anencephalous, or if the high AFP is caused by twins or premature death. If none of these is found, anmiocentesis — which is not in general applied earlier, since the risk of accidentally inducing an abortion is higher than the general one-in-a-thousand risk of bearing a defective child — is used as a final check.

A $25 million business

In theory this complete screening procedure, if made generally available, could lead to a significant decrease both in the 2000 to 3000 children who are born and survive every year in the US with spina bifida, and in the anguish to the parents of those (in particular the anencephalics) who do not survive at birth. A national programme has therefore been urged by many, and is already being studied in Britain, to minimise what is currently the most common serious birth defect. Manufacturers envision a multi-million dollar market for their screening kits and reagents; and private laboratories a comparable market for their testing services.

Three main concerns, however, have been raised by the prospect of the techniques being freely marketed and becoming generally available. The first is the general opposition of the politically powerful anti-abortion lobby, critical of what one Right to Life committee member last week described as any "search and destroy" mission involving pre-natal screening.

The second concern is on the economics of mass screening, and revolves around cost-benefit arguments. Research workers at the Center for Disease Control at Atlanta, Georgia, have calculated that a screening programme offered to all pregnant mothers in the US, with a 40% acceptance, would cost about $25 million a year; but the averted annual cost would be about $50 million. Others challenge these figures, arguing that the risks are insufficiently known to enable realistic quantification.

It is the third consideration, however, which is presently causing the greatest headache to FDA officials, namely how to ensure adequate quality control of the whole process, and not just of the screening kits, to minimise the risks involved. For as one FDA official points out: "The use of the test is so integral to obstetric practice that it is hard to look at this purely as a technique without also taking into account the way that it is going to be used."

Such concerns, focused in particular on the dangers of causing large numbers of unnecessary abortions, have four main aspects:

- how to ensure adequate counselling for the parent, both with respect to gaining initial "informed consent" and to correctly interpreting the test results at various stages;
- how to ensure physicians are sufficiently informed about the techniques to be able to interpret laboratory results properly, and provide follow-up guidance;
- how to ensure that the complete range of testing facilities is available to every mother taking the test, so that a decision to abort, for example, is not made solely on a positive first test due to the lack of sonography or amniocentesis facilities (or an inability to pay for both); and
- how to ensure sufficient accuracy in the information provided to physicians by testing laboratories.

Although such issues have been debated in academic circles for a number of years, alarm bells only started ringing at the FDA last Autumn after a hearing of a review panel of the agency's Bureau of Medical Devices on applications to market screening kits which have been developed by a number of manufacturers, including Hoffman la Roche, Abbott Laboratories, and the Wampole Division of Carter-Wallace Inc.

Shortly after the hearing, Mrs Carol Bucholz, a director of the Spina Bifida Association, wrote a strongly-worded letter to Dr Kennedy complaining that, as "the obvious consumer population" the association had been neither informed of nor represented at the meeting.

Members of the association mounted a vigorous letter-writing campaign to the FDA. And in their opposition to unrestricted licensing, they were soon joined by other groups and institutions. The director of the Center for Disease Control, for example, Dr William H Foege, wrote that without proper control, AFP screening could lead to unnecessary abortions and false-negative diagnoses which would "continue to be made until successful malpractice suits alter medical practice to stop these mistakes".

The Health Services Administration an-

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nounced that it did not feel adequate facilities existed to provide the necessary sonography and anmiocentesis services if the screening technique became suddenly available on a national basis. "We therefore recommend deferral of the approval for the production and marketing of the test kit at this time", the head of the HSA wrote to Dr Kennedy.

Perhaps most significantly, opposition to unrestricted licensing was also expressed by two professional medical bodies, the American College of Obstetricians and Gynaecologists, and the American Society for Human Genetics. ACOG admitted openly that, in addition to sharing the concerns of others, it did not feel its members were sufficiently prepared to carry the responsibility which the general availability of AFP screening techniques might impose.

"Our position is that we need to have a realistic protocol and set of guide-lines prepared both for the physician and for the patient. It is going to take institutional approaches and a lot of education to learn how to use this technique properly, and we are concerned that it should not be turned loose until we have worked out our line of approach," Dr Ervin E Nichols, director of practice activities at ACOG, told *Nature* last week.

Many of these views were aired at a further public hearing of the medical devices panel in February of this year. However the director of the Bureau of Medical Devices, Dr David Link, subsequently recommended to the Commissioner that licences be granted without broad restrictions on marketing, and subject only to requirements for labelling, reporting, and the provision of information to physicians and patients.

But things were already stirring at the top of FDA. In March the agency announced that it was rejecting an application from Abbott Laboratories to have its screening kit reclassified from a category of medical devices that require pre-market testing on an individual basis, to one which merely required compliance with pre-determined standards.

In announcing its decision, and overriding the recommendation of an advisory panel that the reclassification be granted, the FDA stated that it was "not convinced that a standard would provide reasonable assurance of the safety and efficacy of the device" if it were reclassified as requested. It added that "the petitioner did not state how FDA could assume that ultrasonography or equivalent procedures, i.e. amniography, would be used for determining gestational age", essential information for correct interpretation of the test data.

The various groups have now met with top officials at the FDA to state their case. And it appears that, despite the continued opposition of some manufacturers, the FDA is now prepared to add restrictions to the licences that it issues. A possible protocol is now being developed by ACOG and the American Academy of Pediatrics. And the big question is how far restrictions will go; for, given that all parties in the debate accept the need for a high degree of quality control, the issue remains of how in practice this should be ensured, in other words how sufficient public accountability can be achieved.

On several points both the manufacturers seeking licences and those seeking restrictions on such licences are broadly in agreement. For example ACOG is now working on appropriate forms of physician and technician education, with considerable support of the manufacturers (some of whom have already produced trial educational material to this end).

Who is in control?

The main source of controversy, however, is over who should control the quality control process. Manufacturers and laboratories see quality control as essentially a technical issue; they would like the federal government to agree on standards of accuracy, and then merely be required to meet these standards. Critics, however, such as the Washington-based Health Research Group, argue that this is insufficient; they want more direct public involvement and accountability, perhaps through either federal or stage agencies, in monitoring the way that laboratories conduct the tests, requiring for example, regular inspections rather than occasional proficiency testing.

Direct regulation of laboratory practices, however, is taking the FDA into unchartered areas. At present, government regulation is a "loose, patch-work affair" according to Mr Robert Leflar of HRG, with responsibility for different types of laboratories divided between different agencies. The FDA is confident that it can devise an adquate control system, possibly through the CDC; but has yet to announce details of how this will be done.

One approach which, some feel, would act as a half-way house to general marketing of the screening techniques would be through carefully-controlled pilot programmes. One successful pilot programme has already been in operation for several years on Long Island, run by Dr James Macri of the State University of New York at Stonybrook; another is being planned under the auspices of the State of Maryland's Commission on Hereditary Disease, set up in 1973 to guide methods for the detection and management in hereditary disorder in Maryland.

Listing the problems that surround the accurate use of AFP screening techniques, Dr Neil A Holtzman of the Johns Hopkins Medical School in Baltimore told the February FDA hearing that the commission "does not believe that these problems can be solved judiciously or expeditiously if tests are offered by private laboratories". The pilot project soon to be launched with the cooperation of obstetricians and parent groups in the Baltimore area will be organised through the Laboratories Administration of the state's Department of Health and Mental Hygiene.

One advantage of a state-run project is that it can provide the close coordination between different individuals and institutions that is essential for the success of the screening test, but might otherwise be lacking in a more laissez-faire arrangement. "The solution (to unnecessary risks) will require the multiple agencies of the Public Health Service to cooperate, and PHS to cooperate with the private sector". Dr Foege of CDC wrote to the FDA, urging that a plan for such a policy "must be worked out before a kit or kits are marketed."

In Britain, such requirements have the advantage of being able to work through the National Health Service, and screening is now offered by a number of regional health authorities. In the US, where any moves towards "socialised medicine" are still strongly resisted, such tight coordination is a relatively new departure. "The need for quality control means that the various agencies will have to be part of a cooperative system of testing much broader than we are used to. The problems are difficult, but not, we think, impossible," one FDA official said last week.

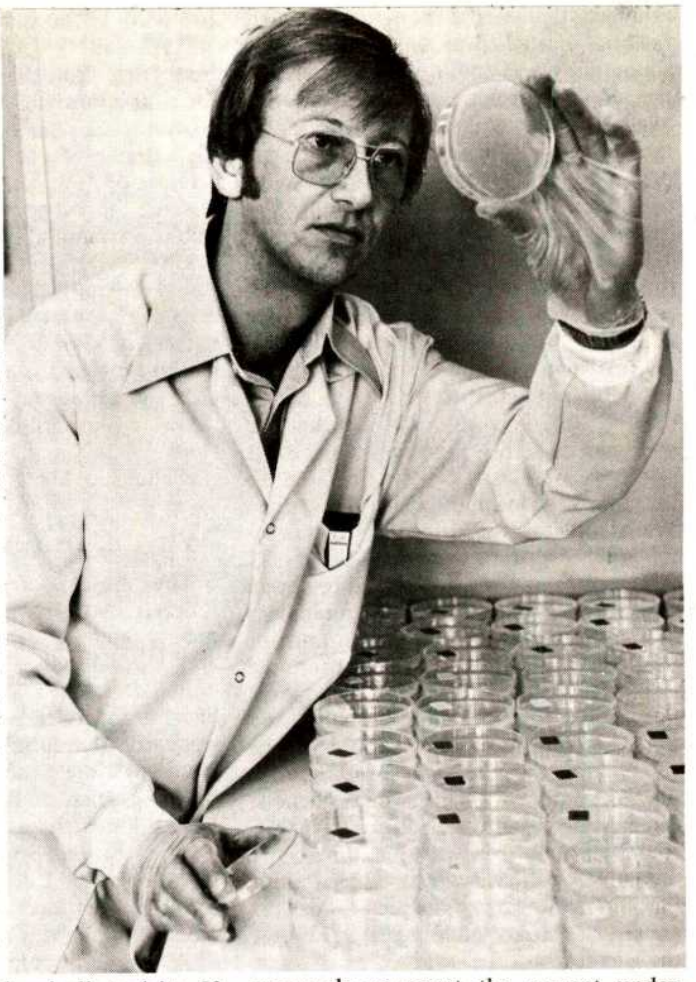
FDA is expected to announce within the next few weeks the path that it proposes to take through this complex of issues. Its decision will be closely scrutinised; Mrs Bucholz of the Spina Bifida Association, for example, has warned that if restrictions on licences "are not carefully designed and planned for with due consideration we will not only withdraw our support of alpha-fetoprotein screening but will actively work to inhibit AFP testing".

On the other side, laboratories and manufacturers could well seek recourse to legal constraints if they feel FDA is imposing unwarranted restrictions on their trade. But in the current absence of restrictions, some small laboratories are already offering AFP screening on a "bootleg" basis, with minimal control over either the quality of their test results or the way they are interpreted and used.

Thus what was until recently a largely ethical debate on the social implications of genetic screening has become an overtly political issue. In the case of nuclear power — perhaps not strictly comparable, but one where the same type of issues about controlling risk have arisen — it took the Three Mile Island accident to awaken broad public debate on the dangers of leaving the "control of quality control" too firmly in the hands of private enterprise, with insufficient public accountability. "We hope that the FDA will not make the same mistake" one observer said last week. □

Handle us with care

Alastair Hay reports on the reaction of chemical companies in the UK and US to safety legilsation and control of toxic substances



THE chemical industry is reacting in no uncertain manner to toxic substance control measures, introduced by both the US and UK governments as a result of recent public pressure and originating in concern over industrial pollution.

In the US in particular, the industry is objecting to proposals for testing the toxicity of new substances. Singled out for attack is the 1976 US Toxic Substances Control Act, which authorises the Environmental Protection Agency (EPA) to obtain production and test data from industry on selected chemical substances and mixtures, and to regulate the substances when needed.

The chemists are acutely aware of the political pressure to curb pollution, and their reaction to these proposals is not an outright condemnation of the philosophy of control, but a more subtle questioning of the validity of some of the measures designed to achieve it. Two issues are central to the chemical industry's concern: the variation found in the government control measures of different countries; and the scientific validity of testing procedures for toxic substances.

Britain's Health and Safety Executive (HSE) is sensitive to the protests from industry, but as the public guardian of the nation's safety, it is determined to achieve safer working conditions. The HSE policies the 1974 UK Health and Safety at Work Act, which requires industry — as the employer — to ensure that workplaces are safe. However, the means whereby this safety is assessed is still a matter of debate.

Last year the HSE issued a discussion document on a Notification Scheme for Toxic Substances, which would provide an agreed procedure for toxicity studies. There was no shortage of response, and this reaction has been taken into account by the Executive in the preparation of the next stage of the scheme: a consultative document. Views on this will again be sought before the notification scheme eventually becomes law.

The approach of the HSE to the regulation of toxic substances is in marked contrast to that of the EPA. Although the EPA guidelines for toxicity testing have yet to be finalised, one early draft suggested a specific battery of tests for assessing the toxicity of compounds. The HSE proposals on the other hand, are more flexible and selective. Mr Ted Langley, who has overall responsibility for the HSE notification scheme, outlined the philosophy behind the required toxicity testing as "the need to have a minimum set of data to enable movement to be made to the next stage (of development of a chemical) rather than a rigid set of tests, which may be very expensive." According to Langley the essence of the HSE approach is flexibility to "identify the hazard."

The HSE and EPA also differ in their approach to toxicity testing. The Executive's policy is based on an instalment plan, where sub-acute toxicity data generated over a 28 day study period will provide sufficient information to enable evaluations to be made about a chemical's toxicity. If the data generated in this period suggest the need for further research of a long term nature the HSE will then insist that this be done. The EPA, on the other hand, envisages a one-off, all-embracing package, which requires that a battery of tests, both short and long term, be undertaken before a chemical will be considered for registration.

The Chemical Industries Association (CIA) is the collective voice of the British chemical industry with a membership ranging from the huge multinational companies like ICI to very small concerns. On the question of safety the Association is on record as "fully supporting" a notification scheme for new chemical substances, but not unnaturally, it has some reservations about the new HSE proposals and even graver ones about the EPA Toxic Substances Control Act.

Mr Roy Granger is Director of Company Operations for the CIA and, as such, responsible for communicating the Association's views on the new regulations to the HSE and EPA. Granger does not mince his words when commenting on their proposals. On the Toxic Substances Control Act he says the "EPA shopping list of tests is unjustified" and that the CIA does not "want a rigid programme of tests." Granger approves of the HSE proposals which he says are based on a dialogue between the Executive and industry. In Granger's view the HSE proposals represent the current understanding of experts in the UK of the value of tests to assess toxicity.

In reply to the HSE and EPA proposals the CIA says it is concerned that UK proposals may differ from those being considered by the European Economic Community. It is also worried that the cost of doing the testing — and particularly the EPA tests — may prove so prohibitive for small or medium size chemical companies that it will be a barrier to innovation and to the introduction of new products on the market.

Furthermore, the CIA says that there is an acute shortage of toxicologists available to evaluate the tests; that intermediates in the chemical process should be exempt from legislation; that more thought must be given to the confidentiality of company data; and that the UK proposals which require notification for chemicals marketed in quantities above 1 tonne per annum are too stringent, and the figure should be raised to 10 tonnes. Raising this figure, says the CIA, would exempt many products and certainly most development chemicals from legislation.

For its part the HSE has let it be known that it is anxious to ensure uniformity in European legislation so that British manufacturers will not be unfairly penalised, and that HSE proposals may ultimately have to be modified to achieve these ends. The number of toxicologists available to evaluate data is not regarded as a long term

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problem by the HSE, which points out that if necessary, it can look to Department of Health and Social Security toxicologists for advice. The HSE is building up its expertise in the field by an intensive "in house" training programme. The Executive will comment on intermediate products, confidentiality and tonnage in its consultative document.

The CIA claims in its submissions to the HSE and EPA that the views it expresses are those of its members. One such is Yorkshire Chemicals, a medium sized company based in Leeds, and dealing mainly in dyestuffs. It employs some 900 people and has an annual turnover of £22 million. Mr John Dawson, research director for Yorkshire Chemicals, agrees with most, although not all, of the CIA's views, saying for a start that the cost of toxicity testing is high. He feels that some of the new tests required by the HSE and EPA are "not justified" for dyes because, in his opinion, the tests required should be related to a product's final use.

To the best of his knowledge dyes themselves have never killed anybody, although he concedes that in the past his industry has used products such as ß-napthylamine and benzidine — which are carcinogenic in man — as intermediates. But he insists that toxic products such as these are no longer used.

Dawson's view is not shared by David Douglas, newly appointed head of the HSE unit responsible for collating information on toxic substances. Douglas insists that Dawson is "out of the mainstream of thinking on this one," as there is a risk to people from the breakdown products of finished dyes.

Many medium size chemical companies use outside research institutes, such as the Huntingdon Research Centre, to carry out toxicity testing. The dye stuff industry also shares much of its research data by operating collectively though the Ecological and Toxicological Association of Dyestuff Manufacturers (ETAD).

According to Dawson, the limited range of tests recommended by ETAD to assess the safety of their products are sufficient to assess the toxicity of dyes. In Dawson's view, "better hygiene could obviate the need" for some tests such as chronic oral dosing studies, and he doubts whether the CIA recommendation on raising the minimum tonnage of chemicals requiring testing would have a great effect on Yorkshire Chemicals.

Although many chemical companies complain about the cost of the HSE's proposed notification scheme, Ted Langley points out that the outcry is perhaps unnecessary. If, he says, industry is carrying out its obligations under the 1974 HSW Act to protect its workforce, then it should already be doing much of the testing which will be a feature of the new notification scheme. The difference, however, is that there will now be an agreed set of tests to do, and the results will have to be submitted to the Executive for evaluation. □

How ICI tests for toxicity

Few chemical companies can be as well prepared to undertake toxicity testing as ICI. The company finances its own laboratory, the Central Toxicology Laboratory (CTL), at Alderley Edge in Cheshire. CTL has a budget of £6 million for 1979. With a staff of nearly 300 the laboratory works in close collaboration with ICI's Occupational Medical Service (OMS) and both CTL and OMS have had substanial influence on the philosophy behind the HSE approach to chemical toxicity testing in the UK.

The science of toxicology is still in its infancy, according to scientists at CTL. They believe that research into new methods for assessing toxicity is essential to generate new tests which may be more rapid but, most important, are valid and reliable. Dr Iain Purchase is an Assistant Director at CTL and he believes that both the search for new test systems and work on understanding toxic actions are essential programmes for any such laboratory.

Such research, he believes, provides the right blend of academic work and routine testing which allows toxicologists to develop and execute an alternative to the recipe book approach to toxicology. Furthermore it provides the laboratory with a good understanding of the advantages and limitations of tests to assess toxicity.

It is this experience which makes CTL so influential. One area in which the laboratory has had considerable impact — and particularly on the HSE — is in the field of short term *in vitro* tests for predicting carcinogenicity. A cell transformation assay developed by Dr Jerry Styles at CTL is at least as reliable as the Ames test for detecting potential carcinogens, and in fact complements it. With some known carcinogens such as Butter Yellow the Ames test fails to identify the chemicals as potential carcinogens whereas the Styles assay will do so; and the reverse is also true. On this basis, the CTL group advocates that where short term tests are used to assess carcinogencity, as a minimum requirement the two tests should be used as a screening net. The HSE is in agreement with this suggestion.

Styles and his colleage at CTL, Dr John Ashby, have recently argued that *in vitro* tests have distinct limitations and that the evidence they produce is not sufficient to allow quantitative assessments about carcinogenic potency. They are, however, ideal for use in a qualitative fashion for identification of potential carcinogens. The point was well taken by the HSE, which believes that carcinogenic potency is best assessed by long term animal studies.

With CTL bearing the responsibility for the testing of ICI industrial chemicals, some of which may be toxic and potentially carcinogenic, rigorous laboratory hygiene is essential. To achieve this CTL uses a concept pioneered by ICI to assess safety on its industrial plants — the hazard and operability (haz-op) study. Purchase reports that the studies were time-consuming, but worthwhile. They involved a detailed assessment of every stage in the testing of a chemical from its synthesis to the analysis of animal tissue following long-term feeding tests. Flow charts are constructed detailing the stages and every conceivable mishap is considered and precautions instituted, either to prevent it occurring or to contain it (for example in the case of spillage of a carcinogen). Purchase believes that this kind of analysis ought to be carried out in all laboratories dealing with toxic substances, be they chemicals, viruses or bacteria. He says that the analysis ensures far more professionalism in the laboratory.

Things are more complicated when it comes to dealing with an ICI process which involves the use of chemicals which might be carcinogenic. Although this is not a new problem for ICI, it is one which is becoming more complex. To deal with it the company as instituted an additional reviewing step in the form of a carcinogen panel. With a membership embracing the four disciplines of medicine, biology, chemistry and law, the panel meets fortnightly and since its inception in 1975 has reviewed some 150 chemicals. The panel's brief is to review all relevant data, from the literature and within ICI, and to advise on the chemicals which may pose a risk and the action that is required to deal with it.

Purchase says that the philosophy behind the actions of ICI and much of the chemical industry is that "hazards can be contained". Dr Eric Longstaff, one of the panel members, says that in 9 out of 10 cases it is an ICI division which approaches the panel for information and advice. If the carcinogenicity of a chemical is established and it is involved in an ICI process then the panel's review will probably ensure containment of the chemical.

Work at CTL embraces a far wider field than simply testing for carcinogenicity. CTL's toxicologists recognise that the need for good toxicity testing on chemicals is a fact of life, and while they admit that the ICIs of this world have the skills available to perform the tests required by Governments, their real concern is whether they have the capacity to cope with the work-load the testing will involve. The oustanding question is whether the smaller chemical companies, with fewer resources, will be able to cope too. □

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correspondence

How accurate was the carat?

SIR, — The carat, a well known jeweller's measure of weight, is generally defined as the weight of a seed of the carob tree *(Ceratonia siliqua;* Leguminosae) or about 200 mg *(Webster's New World Dictionary).* It is traditional to view seeds as having very low intra specific variation in weight, and therefore not surprising to find them used as standards of commercial weight. But how reliable was this species of seed as a standard?

The carob is a small tree native to North Africa and has the largest dry and hard seed of any Mediterranean plant. In September 1978, I haphazardly collected 12 freshly fallen pods from the ground below a healthy carob tree growing in a courtyard of the Hebrew University, Jerusalem. In April 1979, the seeds were removed from the indehiscent pods and weighed. There were ten seeds that weighed only 0.07 to 0.13 g, and I discarded these seeds from further analysis because they were variously malformed and would be noticed by any discerning merchant or shopper. The remaining 156 seeds could not be sorted into groups of heavier or lighter seeds, and this set had a mean weight of 0.198 g (s.d. = 0.024), remarkably close to the supposed weight of the carat.

However, there is no reason to believe a merchant would be so stupid as to use average (randomly selected) carob seeds as balance weights. There were 48 seeds (31%) in the sample that looked normal but weighed less than 0.2 g. All a merchant would have to do is use a gravitational sorting system on a large sample of seeds to obtain a pool of carob seeds up to 25% underweight to use when making sales. The other end of the weight distribution contained 64 (41%) that weighed 0.21 to 0.24 g. These heavy seeds would have been most useful when making purchases. In short, only 28% of the seeds in a carob seed crop would have given an honest measure. This study was supported by a subsidy from the Israeli government.

Yours faithfully,

DANIEL H. JANZEN

University of Pennsylvania, Philadelphia, Pennsylvania, USA.

Science is not all whizz

SIR, — Few would disagree with your leader article (10 May, page 89) that science in the UK is depressed. More precisely, scientists who have chosen to make a career in research are depressed. The danger is that the full effects of the lack of career structure for research scientists has not yet made sufficient impact on scientific output in this country to force any action on the problem. By the time it does, it may be too late. The experienced scientists who should play a major part in research teams of the future will have been dropped by the wayside because research councils and charities are no longer prepared to take on the "burden" (as they describe it) of funding the older person.

Your challenge to the new government to find effective, creative employment for Britain's postdoctoral fellows is to be welcomed. But is it centres for "whizz-kids" that we really need? Advancement in scientific research rarely requires the flash of genius, but rather a methodical and pains-taking approach. Most research scientists are ordinary men and women of average intellect trying to do a job like any other professional. How good they are at their job depends critically on the kind of training they have received. It is about time we got away from the notion that only geniuses can produce good science, and the corollary, that only scientists of "exceptional ability" should be funded. We are not arguing for the support of mediocrity, merely the acknowledgement that scientific research, like other professional careers, should have room for a wide variety of people with differing abilities and contributions to make, not just jobs for "whizz-kids".

Yours faithfully,

SUSAN BARLOW

ARMS, Guy's Hospital, London

Physics and Ghana

SIR, — Your article (10 May, pages 96-97) rightly emphasised the relative irrelevance of electron transport in thin manganese films to scientific problems in Ghana.

However, as the supervisor of the physicist concerned during his studies, partly in Ghana and partly at Sussex on a faculty exchange, I would like to add that we also carried out an extensive literature search and correspondence with the Timber Research Association relating to possible physics-based problems in Ghanaian forestry. These formed the basis of many long discussions during his time at Sussex.

Yours faithfully,

A. D. C. GRASSIE

University of Sussex, UK.

DPAG, GMAG and the trade unions

SIR, — Reg Bird (26 April, page 776) uses the argument that, because a fatal accident occurred under the supervision of DPAG, which has no trades' union nominees, whereas no comparable accident has occurred under GMAG, which has TUC nominees, therefore the accident free record of GMAG is a consequence of this presence. It seems a pity that fallacious reasoning which would never appear in your scientific papers is allowed to exist in your correspondence columns.

Yours faithfully,

ALAN D. B. MALCOLM

St Mary's Hospital Medical School, London, UK.

Nuclear power does not lead to nuclear weapons

SIR, — Professor Rotblat with his phrase "the life blood of modern society" (31 May, page 370), recognises the need for adequate energy supplies, yet he seems reluctant to accept that nuclear power should make a contribution.

Even though his proposed World Energy Organisation would *(inter alia)* "advise countries of the type of energy most suitable for them — including nuclear if deemed necessary", Professor Rotblat's obsession with the proliferation of nuclear weapons, the extreme views he put forward in his lecture as reported in *Nature,* 1 March, page 4 ("In the long run nuclear energy is not compatible with the survival of civilisation") and his hostility towards the IAEA make it clear that nuclear power would play little part in his WEO.

Although my arguments (26 April, page 776) have failed to convince Professor Rotblat, the facts seem plain. There is no case where a country has developed nuclear explosives by diverting material from a civil power station. In the ten year period 1945-54 three countries (USA, USSR and UK) developed nuclear explosives; between 1955 and 1964 another two countries (France and China) and between 1965 and 1974 one further country (India). Over this same time span the world nuclear generating capacity grew, and by 1974 there were 54,000 MW in operation in 19 countries. There are now 100,000 MW in operation in 22 countries, and if stations under construction are also included, the figures rise to 400,000 MW in 33 countries. This indicates that there is no direct link between peaceful nuclear power and nuclear weapons. This point comes out even more clearly when it can be shown that for each of the five nuclear weapon states the explosion of their first bomb preceded, not followed, the entry into service of their first prototype commercial power reactors (Table 1).

The plutonium for the Indian nuclear explosion of 1974 was obtained from a research reactor. In addition it is widely believed that Israel and South Africa are probably capable of making nuclear weapons, but neither country has a commercial nuclear power station in operation.

It could be argued that the greater a country's dependence on nuclear energy for essential electricity the less likely it would be to jeopardise its relations with international suppliers by breaking international agreements. Quite contrary to the innuendo of Professor Rotblat's final sentence, most nations have not become nuclear weapon states: 104 of them have acceded to the Non-Proliferation Treaty.

It is essential that every effort is made to find ways to prevent the misuse of nuclear energy; a refusal to accept its benefits is not one of them.

Yours faithfully,

G. H. GREENHALGH

A Power for Good, London, UK.

Table 1 Dates of first nuclear bomb and first nuclear reactor for the five nuclear weapon states

Country	Date of first bomb exploded in atmosphere	Date of first prototype power reactor in service	Commercial name
USA	1945	1957	Shippingport
USSR	1949	1958	Troitsk
UK	1952	1956	Calder Hall
France	1960	1964	Chinon
China	1964	No firm evidence of a power reactor in service	

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news and views

The legacy of Xenophon

from Robert W. Cahn

THE US National Academy of Sciences gave the name COSMAT to a massive study it sponsored of the profession of materials science and engineering (MSE), published in five thick volumes in 1974 and 1975. It is a remarkable compilation; because of its wide terms of reference, and especially because of its historical and international emphasis, it is of value far beyond the originally intended readership in its target profession. The report is difficult to locate and few people in Britain have read it, and to make at least a small extract accessible to a wider readership, a learned periodical, appropriately named *Materials Science and Engineering*, has published a special issue entirely devoted to a revised and updated version of two chapters from one of the volumes (*Materials Science and Engineering: Its Evolution, Practice and Prospects*, Elsevier-Sequoia, March 1979).

The heart of the extract is a forty-page essay, by Melvin Kranzberg (editor of the journal *Technology and Culture*) and Cyril Smith (the world's leading scholar in the history of metallurgy), on 'Materials in History and Society', complete with a bibliography of 85 items which alone would justify acquisition of the volume. The emphasis is predominantly on the history of the use and understanding of metals. The essay is a masterly examination of the ways developments in the manufacture, purification, variegation and fitness for purpose of materials influenced, not only the tenor of daily life, but matters of great social impact such as the spread of agriculture; the balance of affluence between patricians and plebians (as 'aristocratic' bronze gave way to 'democratic' iron); the spread of the printed word and the printed image; the materials testing institutions which were a precondition of safe machinery and all that implies. The later parts of the essay are more concerned with the gradual development — remarkably delayed, and significant only in the twentieth century — of a scientific and analytical approach to materials. Réaumur, in the early eighteenth century, was the first modern materials scientist, but his ability to relate traditional practice to advanced science was not followed, for the love of mathematical physics applied to highly simplified models was at that time irresistible and the obscurantism of the alchemists had repelled the disciples of Newton.

Kranzberg and Smith's survey is followed by a section with its eyes wholly on modern times: Richard Claassen and Alan Chynoweth, two industrial scientists, write on 'MSE as a Multi-discipline'. Specifically, they trace the routes by which physicists, chemists and engineers of various persuasions combined to create the profession of MSE, and they do so primarily through a collection of case histories of technical developments (heatshield design for space vehicles; the development of the transistor; plastic coatings on razor blades; artificial fibres; optical lightguides, and others). These stories, combined with an analysis of the implications for the organisation of interdisciplinary research projects, provide the flavour of this section.

Kranzberg and Smith, the historians, quote the Greek historian Xenophon ("What are called the mechanical arts carry a social stigma and are rightly dishonoured in our cities") and Plutarch ("For it does not of necessity follow that, if the work delight you with its grace, the one who wrought it is worthy of esteem"), to indicate what the ancients thought of craft and skills applied to materials. Of all the ancient exponents of the 'mechanical arts', the slave miners of antiquity were perhaps the most abjectly oppressed, miserable and shortlived.

Today, once again, 'mechanical artists' and miners in particular are apt to be regarded by wielders of temporal power as the carriers of social stigma. This surprising conclusion emerges with great clarity from another recent publication, the proceedings of the 1978 American Mining Symposium, the subtitle of which was 'Politics, Society and the Mineral Industry'. The proceedings are published in *Materials and Society* (**3**, 1; 1979). Several of the lectures are germane to the matter in hand: these are written by a metallurgist, a congressman, a copper mining expert, a coal mining expert and J. Allen Overton, the president of the American Mining Congress, who finds himself under the necessity of acting as a fulltime lobbyist. Their complaint — the word is too weak, it is a succession of cries of despair — is the unchecked growth of regulation and of the body of state employees who enforce regulations and steadily add to their number. The chairman remarked that the 'political outcry' as he termed it, at the symposium was unpremeditated and uncolluded and the more credible for it. The tone is exemplified by two extracts from Allen Overton's lecture: "Time and again, we see no attempts to consider trade-offs or weigh the cost of regulations against their supposed benefits. This is the work of zealots, and the only guideline they know is their own self-proclaimed virtue. The repeated failure — I should call it the obstinate refusal — to take acount of the economic consequences mean that some regulations can have ruinous impact.". And again: "Finally, there is bewilderment and foreboding about the sheer mass of regulations, their frightening complexity, the paralysing uncertainty about what the bureaucrats will propose tomorrow, and how the rules will be changed in the middle of the game ". The changing of rules in the middle of the game is exemplified by William Simon, a former US Secretary of the Treasury, in another recent publication (*A Time for Truth*, McGraw-Hill, 1978): "Manufacturers of children's sleepwear were forced by the government to process children's sleepwear with a flame-retardent chemical. When the companies shifted over to the costly new process, the FDA banned the flame-retardant chemical as a suspected carcinogen, and the manufacturers were ordered to recall their merchandise and to compensate buyers".

In such an atmosphere, it becomes understandable why American oil policy is in such disarray: those who fully understand the situation in prospect plead for decontrol and a partial release from the burden of regulation (McKetta's lecture on American energy policy at the symposium is a case in point), but their

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word is widely distrusted by politicians precisely because they are experts. Xenophon stalks among us again. The proponents of regulation, and those who support and encourage them, are all too often convinced that experts are venal and devoid of public spirit (just as the free citizens of ancient Greece regarded their bonded craftsmen). When the mining specialists plead public interest, the fact that the plea comes from them at once invalidates it. But the inevitable consequence is that the same judgment is being applied to the regulators themselves: they have by now as great an interest vested in the increase of regulations as the protesters have in their abatement. Not only that: those miners, MSEs, and may others to whom by implication Xenophon's cold dictum is attached, conclude that their honesty is disbelieved by some of the most powerful in the land, and when a professional's integrity is systematically impugned, he is driven at last towards political extremes; at the least he will come to distrust the regulators' own honesty of purpose. The prospect is not enticing, and the American crisis of regulations should give us in Britain pause. □

Aetiology of natural scrapie

from H. B. Parry

THE crux of the problem raised by Kimberlin recently in News and Views[1] is the principal means by which natural scrapie of sheep is disseminated. My report[2] which stimulated him, was confined to providing new evidence for a heriditary factor and to demonstrating a new practical means of genetic control under current agricultural practice. Kimberlin's account[1] summarising the published laboratory studies of his colleagues[3] widens the issue of aetiology without considering relevant evidence from the epidemiology of the natural disease or from the possible pathogenesis of the characteristic sequential localised neuronal decay.

There are two main aetiological factors. First, a neurotoxic agent is present in the tissues of animals with the pathological lesions of scrapie and is transmissible artificially[4]. This is commonly called a 'slow virus', or more accurately a transmissible spongiform encephalopathic agent (TSEPA) from the pathological nature of the special neurotoxic damage developing in inoculated animals. In the absence of any reliable means of detecting the TSEPA in the living animal, we have no data on the occurrence of the agent in any sheep population. Second, there is a hereditary factor, probably a single autosomal recessive gene, with an 'all-or-none' effect on the development of the primary neuronal decay, for which no simple peripheral 'marker' reaction is known. However, indirect inferences of the gene's likely presence can be made on the basis of the results of test-mating procedures and meticulous breeding records[1].

Without such data, assessments of recessive allele frequencies are notoriously hazardous when based solely on the statements of individual flockowners and shepherds[5-7]. In my view the term 'scrapie-free' should mean free of the scrapie-allele, at least to a probability of less than 1 in 100; such sheep are available.

Spread of scrapie

The inferences of natural spread among experimental flocks, quoted by Kimberlin, are contrary to my field experience within breeding groups. Using recording methods designed to reveal such spread, I detected none, which is in accord with the evidence of many reliable observers since 1750[5-7] and with the results of other less well controlled current field observations. No spread has followed the introduction since 1950 of British sheep, which developed the disease, into certain countries that are free of the disease and have not operated any quarantine or slaughter policy. One therefore seeks possible explanations for the anomalous conclusions quoted by Kimberlin. There are four main considerations.

First, the data he quotes relate to four British sheep breeds, all of which have had substantial scrapie attack-rates at some period since 1900 and three very seriously in some sectors of their breeds since 1950 until the present time. In these circumstances the designation 'scrapie-free' applied to a sheep, a flock or a breed without meticulously supervised and relevant records, covering at least one decade before and continuously during the experimental period, lacks any precise scientific meaning. This is because the TSEPA cannot be detected and the recessive scrapie allele is likely to be widespread but clinically 'silent' in these breeds and may at any time without prior evidence reach frequencies leading to clinical manifestation[2]. Thus the group designated 'scrapie-free' is likely to consist of mixed populations of the three genotypes. The authors quoted by Kimberlin provide no indication that the proper safeguards against these possibilities have been enforced. The meaningful interpretation of results from such populations on the present evidence is thus not possible.

The second consideration is that the results quoted are commonly presented as totals of all matings between so-called 'scrapie-free' and 'scrapie-affected' groups without any attempt to assess presumptive recessive genotype frequencies in each group. Third, the aggregation of the results of different sire-progeny groups as accumulated totals masks information relevant to their proper evaluation, for example, the possible proportions of the three recessive genotypes and allele frequencies in different sectors of the breeding population, the degree of in-breeding, occurrences of associated sub-normal health and reproductive efficiency, which may affect the totals manifesting scrapie and their ratios. Fourth, the American experience, quoted by Kimberlin, kindly made available by Dr. J. L. Hourrigan, when analysed, as far as the data allow, on a provisional genotype basis, are approximately compatible with my own. I therefore have serious reservations of the validity of the conclusions derived from the experimental flocks quoted as evidence for the normal dissemination of natural scrapie by the spread of a communicable agent.

Artificial scrapie and genetic control

The artificially induced form of scrapie disease is probably not identical to the natural form and the precise transferring of the results of the one to the other is questionable. In most of the studies quoted by Kimberlin the source of inocula of scrapie TSEPA material designated SSPB/1 was derived from 40 affected sheep brains. After 20 years this is now very much more toxic than any TSEPA we have been able to demonstrate in the natural disease on first transfer.

These reservations do not preclude extrinsic factors operating in natural scrapie, merely that their possible occurrence requires more precise definition. Any aetiological hypothesis must also provide an acceptable explanation of the specific characteristics of the neuronal degeneration in the natural disease[8]. □

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Photogenic impurities

from Henry Slayter

TWO papers, published together in the *Journal of Biological Chemistry* (**254**, 1747; 1979) come essentially to the same conclusion concerning the structure of pyruvate carboxylase examined by electron microscopy. Goss *et al.* of the University of Adelaide and Cohen *et al.* of Case Western Reserve and the National Institute for Medical Research, London, have both applied negative staining to this molecule, obtaining different results from those previously reported by Valentine *et al.* (*Biochemistry* **5**, 3111; 1966) using the same technique. (Two of the coauthors of the latter group were also coauthors of the original Valentine group.)

Negative staining was first described and applied by C.E. Hall and later applied skillfully and to great advantage by many investigators (including, especially, Valentine and R.W. Horne) in the study of biopolymeric structures. In this method, the purified preparation is mixed with concentrated electron-dense 'stain', usually a 1 — 5% solution of uranyl acetate, uranyl formate, sodium phosphotungstate or sodium phosphomolybdate. The preparation is air-dried to form a thin layer supported on 10-nm carbon films. Stain, which is excluded by biopolymer components, penetrates voids, thus outlining the structure. Electron contrast results from the greater electron scattering power of the stain (average density about 7) compared with that of the specimen.

Inherently, this technique is a simple and effective method for examining macromolecules larger than about 5 nm in diameter — but pitfalls abound. Resolution is limited by granularity of the stain, alteration of specimen structure by dehydration, extent to which contrast can be induced in small subunits, and, to an indeterminate extent, specimen damage in the electron beam. Other difficulties relate to the effects of these rather unusual chemical salts on the specimen (due to *p*H and ionic strength requirements, the reducing nature of the salts, the presence of divalent cations and so on). Penetration of the specimen by stain can produce misleading measurements of dimensions or loss of fine structure. As in any method for the examination of individual macromolecules, absolute specimen purity is vital.

Valentine *et al.* originally described the pyruvate carboxylase molecule as a distinctive square-planar tetramer of globular subunits. Several lines of evidence supported this premise. The highly photogenic square-planar configuration appeared in enzyme preparations isolated from several animal species. Dimensions obtained by electron microscopy were consistent with the measured molecular weight of 500,000, consisting of four similar or identical subunits of molecular weight 125,000. The square-tetramers exhibited cold-lability, a well characterised property of chicken liver pyruvate carboxylase.

Valentine, however, also showed that the same enzyme from yeast had a rhombic appearance (Valentine, *Proc. fourth Regular Conf. (Europe) on Electron Microscopy, Rome* **2**, 3; Tipografia Poliglotta Vaticana, Rome, 1968). Now, the new reports show that a similar structure characterises pyruvate carboxylases of animal origin, and that the square-planar molecule is a contaminant commonly found in such preparations. That this contaminant was so much more evident than the enzyme itself is explained, in part, by the demonstration of Goss *et al.* that this structure is actually an octamer in which the thickness (and therefore the relative contrast) of each subunit is doubled.

Both new studies confirm that pyruvate carboxylase consists of four subunits. Cohen *et al.* suggest a rhombic structure with major and minor dimensions of 19 nm and 16 nm respectively, and a centre-to-centre distance between adjacent subunits of 7.3 nm. (They report that exact dimensions vary by as much as 10 % depending on the substance used as negative stain.) Goss *et al.* describe the molecule as a 'splayed tetrahedron' with a long axis of approximately 17.7 nm and a short axis of 15.2 nm with centre-to-centre distance again 7.3 nm. The main difference between the two interpretations is whether the subunits lie in a plane (rhombic structure) or are without regular mutual separation and are not coplanar (splayed tetrahedron).

Cohen *et al.* indicate that their data cannot be fully interpreted with regard to the question of subunit arrangement in three dimensions, but Goss *et al.* suggest that one pair of subunits is displaced slightly above the plane of the second pair. Examination of the field of negatively stained molecules presented by the latter authors shows that only a small number of images can be so interpreted. Thus, while their speculative model is most interesting, I must admit difficulty in accepting with certainty either the proposed three-dimensional arrangement or the detailed tear-drop model of subunits. Further understanding of the structure of this enzyme must await preparation of crystals and their examination by iterative electron microscopy or X-ray crystallography.

Anti-biotin antibody and pyruvate carboxylase are known to form soluble complexes. Cohen *et al.* also present micrographs of this complex, which indicate that the antibody binds to the surface of the tetramer at the same general location as does avidin. Thus, additional evidence is presented that the rhombic tetramers are in fact a biotin-containing protein.

In perspective: some will remember that similar interpretive difficulties have arisen in past studies of negatively stained enzyme preparations — brought on, likewise, by impurities of a significantly more photogenic character than the plainer principal subject. (DNA-dependent RNA polymerase, first reported to be amorphous globular protein, was credited by a second group with a distinctive polyhedral structure. A third group showed up the polyhedron as a mere contaminant and found the polymerase to be an amorphous globular protein.)

What can be learned from all this — perhaps — is that while carefully applied electron microscopy can be of tremendous and unique importance in providing structural information, interpretation must always be carried out with extraordinary care. Awareness of the artefacts to which biopolymers are prone is a must, and investigations should include parallel studies in solution which pose the same questions asked of the electron microscope. Above all, very careful attention must be given to the purity of the preparation. Even a few per cent of contaminating molecules, especially those of the more photogenic sort, can — given the harsh treatment inherent in this method — be interpreted as being a few per cent of survivors of the structure sought. □

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Budgeting for volcanoes

from Peter J. Smith

WHAT is the energy budget of a volcano? Or to be more explicit, what proportions of a volcano's energy are carried away by the various modes of energy transport? What, for that matter, are the appropriate transmission mechanisms? These are the sort of conceptually simple questions one would expect some nineteenth century volcanologist to have answered, albeit incorrectly; but whether he did or not, the same questions are still being asked in 1979, possibly because they are not as easy to come to terms with as might be supposed. Certainly they are not answered entirely satisfactorily by McGetchin and Chouet (*Geophys. Res. Letts.* **6**, 317; 1979) even now, as these authors freely admit.

But to start at the beginning, for many years McGetchin and Chouet have been studying the behaviour of Stromboli, the famous volcanic island that rises about 920m above the Mediterranean some 75km

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north of Sicily. Since records began this volcano has been in a "more or less continuous state of episodic activity, characterized by well-collimated jets of incandescent gas loaded with molten fragments of basaltic vitric lapilli" — a characteristic "fire-fountaining" type of activity that has come to be known as Strombolian though not limited to Stromboli. In jargon, this is known as the volcano's "normal mode", the "steady state episodic mode"; and it is with this type of behaviour that McGetchin and Chouet are chiefly concerned.

No reliable heat flow values have been obtained in the vicinity of Stromboli; but from their own downhole temperature measurements, McGetchin and Chouet estimate that above sea level the volcano is emitting something like 6 MW in the form of heat conducted up through the ground. This is about 84 % of the total energy emitted. By comparison, all other forms of energy transmission are small and most are negligible. Next in magnitude comes the heat carried by ejected gas (700 kW, or 9.8 %), then the heat carried by ejected particles (2.8 %) and the heat radiated from the vent (also 2.8%). Seismic energy released amounts to 0.42 % of the total. Finally there is the kinetic energy of ejected gas (0.0091 %), acoustic power (0.0014 %) and the kinetic energy of ejected particles (0.00034 %).

Needless to say, these estimates depend on assumptions and are therefore open to correction. But even if the results are perfectly accurate, the conclusions that McGetchin and Chouet reach are highly dubious, as they themselves note. The most significant phrase in the last paragraph is probably "above sea level". The fact is that Stromboli is largely submarine; yet the underwater region has been ignored, and with it the large amount of heat likely to be transferred by the convective power of circulating groundwater.

The question of the "normal mode" of volcanic activity is also crucial. It is well known that from time to time Stromboli departs from its usual behaviour and undergoes violently explosive eruption. During the explosive eruption of September 1930, for example, the mass of material ejected was equivalent to that produced during 300 years of normal activity. These violent phases, though unusual in the duration sense, strongly influence the energy/mass equation. So should they be included in the sums, or ignored on the grounds that they are atypical?

On the face of it, McGetchin and Chouet seem not to have achieved very much so far; their figures are, at best, misleading and in any case refer only to one type of behaviour from one particular volcano at one particular period. On the other hand, they have gone some way towards the solution of a fundamental scientific problem, demonstrating in the process that the simplest questions to formulate are not necessarily the easiest to answer. The issue may also be of practical importance to the extent that volcanic heat is being investigated as a possible source of geothermal energy. □

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Assessing effects of marine pollution

from B. L. Bayne

STUDIES of the chemical and biological aspects of pollution should go hand in hand. The chemical assessment of estuarine and marine pollution involves the analysis of water, sediments and biota to determine the levels of contaminants in the ecosystem. Because of technical difficulties of analysing water and sediments (Phillips *Env. Pollut.* **13**, 281; 1977), chemical monitoring is usually concentrated on the biota to provide time-averaged values for the biologically available contaminants (Goldberg *et al. Env. Conserv.* **5**, 1; 1978). The biologist is therefore given the dual responsibility of elucidating the effects, if any, of the observed levels of contaminants in living material, and co-ordinating his efforts with those of the chemist in order to provide an overall assessment of environmental quality.

It is not surprising, therefore, that there has been a recent increase in interest in procedures for measuring the biological impact of pollution (International Council for the Exploration of the Seas (ICES) Cooperative Research Report 75, 1978; *Phil. Trans. R. Soc., Lond.* **286**, in the press; ICES Workshop on Pollution Effects Monitoring, in the press). Much of the considerable research effort is based on the effects of pollution on the individual organism; from this work two general points have emerged which facilitate the planning of further research.

First, the non-specific effects of pollution can generally be characterised in terms of a stress syndrome following the paradigm of the general adaptation syndrome of Selye (*Stress*; Acta Inc., Montreal, 1950). This syndrome includes physiological (mostly involving energy gains and losses, and hormone balance), pathological and biochemical responses, the details of which may vary between species, but which, taken together, make possible a quantitative assessment of animal health that can be used to rank different populations according to the biological impact of environmental changes (Bayne *et al. Phil. Trans. R. Soc., Lond.* **286**, in the press). It is axiomatic that the observed biological responses must constitute reduction in ecological fitness for a pollution effect to be registered.

The second general point to emerge from recent studies is the widespread occurrence of mechanisms of detoxification in marine (and other) organisms. These include the cytochrome P450-linked mixed function oxidation reactions (Bard and James *Biochem. biophys. Perspec. mar. Biol.* **4**, 126; 1978), the production of metal-binding proteins, or metallothioneins (Brown *et al. Env. Conserv.* **4**, 213; 1977) and various cytological mechanisms, such as lysosomal encapsulation, that sequester some foreign compounds (Allison in *Lysosomes in Biology and Pathology*, ed. Dingle and Fell **2**, 178; Elsevier, Amsterdam 1969). These are specific responses to particular classes of contaminant and they can provide evidence of the causative agents involved in situations where measurements of the general stress effects indicate a decline in animal health. The concept of linked non-specific and specific effects of environmental agents is consistent with attributes of the general adaptation syndrome.

It has therefore become possible, by making certain measurements of the general stress response of animals, and by linking these on the one hand to measures of potential detoxification processes and on the other to the results of chemical analyses of the body burden of contaminant, to assess the impact of pollution on the organism. However, the transition from laboratory demonstrations of this kind to practical involvement in environmental management is complicated by the problem of biological variability. The response of the individual organism is seldom a simple function of the intensity of the pollution 'stimulus', because the reactivity of the animal may vary, depending on a combination of intrinsic and extrinsic factors. This inherent variability must at present be accommodated in sample design, by optimising the sampling strategy over time and space.

The merit of such studies on individuals, however, is the early warning that can be signalled concerning environmental impact; individuals respond to sub-lethal stressors long before the effects are apparent in the population or ecosystem. Nevertheless, there is an urgent need also for studies on pollution effects at the level of the ecosystem, if

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only because the problems of variability at the level of organism or population may thereby be reduced (Mann and Clarke *J. Fish. Res. Bd Can.* **35**, 791; 1978). Attributes of ecosystem function that may warn of impending damage are few at present. Indices of diversity and species richness, although controversial, have been effective in some circumstances (McIntyre in *Ecology of Marine Benthos*, ed. Coull, 301; University of South Carolina Press, Columbia, 1977; Sanders *J. Fish. Res. Bd Can.* **35**, 717; 1978.). Greve and Parsons (*Helgol. Wiss. Meeresunters.* **30**, 666; 1977) have discussed the implications of a change in the ratio of diatoms to flagellates as an index of pollution effect, and Gray (*Phil. Trans. R. Soc., Lond.* **286**, in the press) has postulated that changes in the form of the cumulative species distribution curve indicate the effects of environmental disturbance. Measures of the productivity of various trophic levels, and of various size-fractions of the fauna, in both the pelagic and the benthic components of the ecosystem, may also be useful.

Further research is urgently needed and can be framed in certain generalised notions of biological response to stress. When this is coupled with better chemical understanding of the form and the biological availability of pollutants, it should be possible to provide integrated measures of environmental quality as a basis for legislation. □

Alpha-particle mode dominates giant resonance decay

from P. E. Hodgson

A GROUP at the National Bureau of Standards in Washington DC has recently made an important advance in an old technique, the use of virtual-photon spectra to determine the multipolarity of nuclear excitations (Wolynec, Dodge & Hayward *Phys.Rev.Lett.* **42**, 27; 1979). This has been used to study the nickel isotopes, and has led to the surprising conclusion that alpha-particle emission is the dominant decay mode of the E2 isoscalar giant resonance.

It is well known that when electrons are decelerated, for example when they are deflected by passing through a nuclear field, they produce photons, a process known as bremsstrahlung. These photons may be absorbed by the nucleus itself, initiating a nuclear reaction. In this case the photons are not emitted, and are referred to as virtual photons. The theory of these processes is well understood, and it is possible to calculate the virtual photon spectra associated with the excitation of the E1 and E2 giant resonances, and hence to determine the energies and strengths of these resonances from the measured yield curves. The accuracy of these analyses has now been increased greatly by the use of the distorted wave Born approximation to calculate the spectra of the virtual photons.

In the new experiments, targets of the nickel isotopes ^{58}Ni, ^{60}Ni and ^{62}Ni were bombarded with electrons from 16 to 50 MeV, and the yields of protons and alpha-particles were measured at 48°, 90° and 132°. The spectra of these decay particles peak at 5 MeV for protons and at 8 MeV for alpha-particles, and have the asymmetrical shape characteristic of emission at energies around that of the Coulomb barrier.

The total cross-section for proton emission as a function of electron energy is shown in Fig. 1, and it is compared with calculations using the E1 virtual photon spectrum and the (γ,p) cross-section known from another experiment. The excellent agreement between the curve A and the experimental data supports the correctness of the analysis.

In the second part of the experiment, a tantalum foil was interposed in the electron beam in front of the nickel target. The photons emitted from bremsstrahlung in the tantalum then cause photo-disintegration of the nickel, so that the yield from these reactions is added to the electrodisintegration yield. The cross-section for the emission of protons with this foil in place is also shown in Fig. 1 (curve B), and agrees with the calculated sum of the electrodisintegration and photodisintegration cross-sections.

The cross-section for the emission of alpha-particles was measured in the same way, and the results are shown in Fig. 2. As

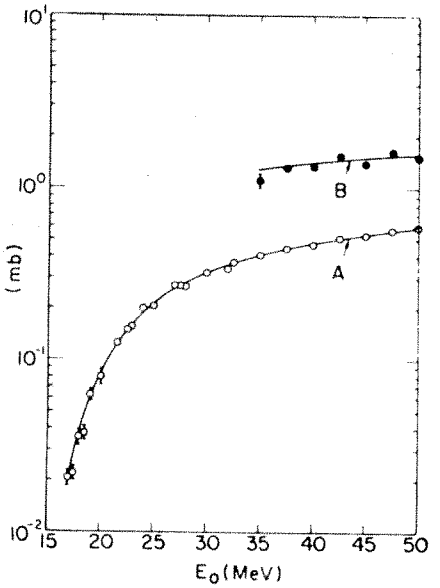


Fig. 1 Total cross-section for the production of protons as a function of the energy of the electrons incident on a ^{58}Ni target. The curve marked A refers to the case without, and B to the case with, a tantalum foil.

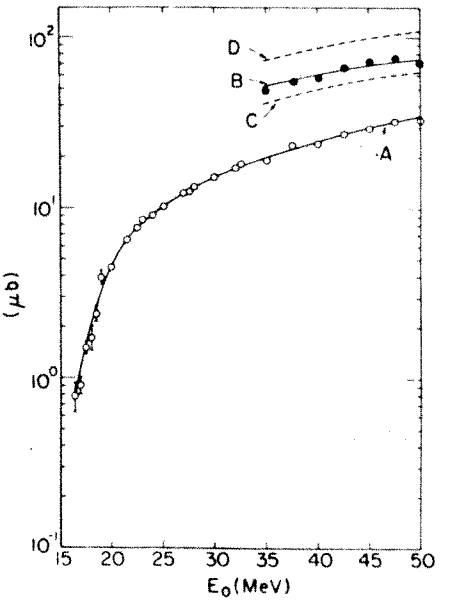


Fig. 2 Total cross-section for the production of alpha-particles as a function of the energy of the electrons incident on a ^{58}Ni target. The two sets of results are as in Fig. 1, and the assumptions made in obtaining the curves B, C and D are described in the text.

before, curve A refers to the measurement without the tantalum foil, and curve B to that with the foil. In the latter case there are three calculated curves: B was obtained using the E1 and E2 virtual photon spectra, C using the best E2 fit and D with the best E1 fit. Of these curves, only B agrees with the data, showing that both the E1 and E2 components must be included to fit the cross-section for alpha-particle emission.

These analyses show that the E1 components in the (γ,α) reaction have an energy of about 20 MeV and width of 6 MeV and account for only about 1% of the E1 absorption cross-section for each of the nickel isotopes. The E2 components have similar energies and widths, 16 MeV and 4 MeV, but account for more than half the energy-weighted isoscalar sum rule for ^{58}Ni and ^{60}Ni, and nearly a third for ^{62}Ni. Comparing with the values of 55% and 63% obtained from inelastic alpha-particle scattering for ^{58}Ni and ^{60}Ni, this leads to the conclusion that alpha-particle emission is the dominant decay mode for the E2 isoscalar resonance.

This is a very striking result, and it suggests that the E2 giant resonance has in some sense an alpha-particle structure. One possibility is that it consists of an alpha-particle coupled to a nuclear core. To test such a possibility, it will be necessary to make detailed calculations to see if it is in agreement with the experimental data. This latest application of the virtual photon method will certainly stimulate further studies to improve its accuracy and to apply it to other nuclei. □

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Virus evolution during persistent infection

from C. R. Pringle

IN certain circumstances cells in culture can survive infection by viruses which normally kill them, and the cells continue to multiply still harbouring the infecting viral genome. Nuclear DNA viruses and reverse transcribing RNA viruses persist by integration into the host genome, or by regulated association with the host cell nucleus. The phenotype of the cell may be changed as a consequence and the cell may become oncogenic. However, many cytocidal RNA viruses like the rhabdoviruses and parainfluenza viruses which multiply entirely in the cytoplasm are able to establish a persistent infection in susceptible cells. The molecular mechanisms concerned in the moderation of normally lethal infections in which there is no nuclear involvement are attracting increasing interest. Three factors have been associated with persistent infection. These are temperature-sensitive (*ts*) mutation which impairs virus replication at normal incubation temperature, defective interfering (DI) particles which have portions of the viral genome deleted and suppress the multiplication of homologous complete virus, and production of interferon. The most recent analyses indicate that these factors are interdependent, and that interferon production may hold the balance. Sekellick and Marcus (*Virology* **85**, 175; 1978; **95**, 36; 1979) have shown that many *ts* mutants of the rhabdovirus vesicular stomatitis virus (VSV) are good inducers of interferon in competent cells at restrictive and semi-permissive temperatures, and that the ability of DI particles to protect cells from the lethal action of infectious virus (as distinct from their ability to inhibit viral replication), is mediated by interferon. Interferon levels in persistently infected cultures are usually low, but addition of anti-interferon serum frequently results in an increase in virus production and cell destruction (Youngner *et al. J. Virol.* **28**, 6 1978; Ramseur and Friedman *Virology* **85**, 253; 1978). However, persistent infection can be established in cell lines such as Vero and BHK-21 which are genetically deficient in some component of the interferon system. Another important factor may be the progressive selection of a population of semi-refractory cells, because a change in karyotype has been observed in a culture of BS-C-1 cells persistently infected with respiratory syncytial virus (Pringle *et al. J. Virol.* **28**, 199: 1978).

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Persistent infection has been given new significance by the results of surveillance over five years of a BHK-21 cell culture persistently infected with the Indiana serotype of VSV, with oligonucleotide fingerprinting used to detect changes in the genetic constitution of the virus (Holland *et al. Cell* **16**, 495; 1979). The data reveal that the virus has undergone extensive and progressive non-lethal mutational change during this period. The implication is that the mechanisms which moderate virulence to produce persistent infection may simultaneously promote rapid virus evolution.

VSV seems to be genetically stable during repeated cycles of lytic infection. Clewley *et al.* (*J. Virol.* **23**, 152; 1977) found that strains of VSV derived from different field isolates were readily distinguishable by oligonucleotide fingerprinting, although most of the oligonucleotides were conserved. On the other hand one strain maintained independently in six laboratories for 16 yr was unaltered. Likewise Holland and his colleagues could detect no changes in their strain of VSV after eight sequential cycles of cloning in BHK-21 cells, forty cycles of lytic infection in the same cells, and five lethal infections of adult mice. The virus also remained unaltered after growth for 2 months in *Aedes albopictus* cells, although passage in insect cells was known to generate high freequencies of *ts* mutants. These results are not unexpected, however, because 85–90% of all sequence changes would not be detectable by oligonucleotide mapping, because only the larger oligonucleotides (10–15% of the total) obtained by T1 ribonuclease digestion would produce unique spots after polyacrylamide gel electrophoresis in two dimensions.

A very different pattern was observed in the persistently infected culture. This carrier culture had been established by a maturation-defective *ts* mutant of VSV (*ts* G31) in the presence of a DI particle derived from this same mutant. Thus all the genome alterations observed in this culture were a consequence of mutation in the progeny of a single virus particle. The DI particle associated with *ts* G31, which is the smallest of the VSV DIs with approximately 90 % of the genome deleted, was considered to be an essential factor in establishing persistence, although during propagation of the culture it was subsequently replaced by a succession of other DI particles. These DI particles had different oligonucleotide patterns from the original DI of *ts* G31. Infectious virus recovered from the culture after continuous propagation for 14, 21 and 42 months showed 5, 8 and 11 map changes, respectively. At any particular time one DI particle predominated and different clones of infectious virus isolated from the culture at the same passage level had identical oligonucleotide patterns. Because 90 % of sequence changes would have occurred in the non-unique small oligonucleotides, it is clear that at least 50–100 mutations had occurred by 3.5yr, although only a proportion would result in changes in the amino acid composition of the viral proteins. Nevertheless, it was evident that the VSV genome had experienced extensive and progressive mutational change during persistent infection. The genome changes induced by persistent infection seemed to be stable because virus isolated from the carrier culture after 5yr showed no alteration during ten cycles of lytic infection in susceptible cells.

These observations suggest that persistent infections are a major source of genetic variation for viruses like the parainfluenza viruses and rhabdoviruses, which are unable to acquire new variation by recombination. Holland *et al.* also suggest that persistent infection is an important factor in the evolution of influenza A virus. A given strain may show little variation during periods of acute infection, and extensive sequence alterations (with or without reassortment of subunits) may only accumulate in the interval between pandemics when the virus is sequestered in foci of persistent infection in individual hosts.

One of the most interesting properties of these carrier cells was that they could be propagated in nude (athymic) mice, and it was observed that persistent infection by VSV suppressed the normal tumorigenicity of BHK-21 cells (Reid *et al. J. gen. Virol.* **42**, 609; 1979). Analysis of this anti-tumour response may shed light on the difficulties encountered in propagation of certain types of human and other tumours in nude mice. □



A hundred years ago

Intellect in Brutes

The following instance of sagacity in a cat has just been related to me by a friend who knew both the cat and its owner well. The latter, who lived at Ragusa Vecchia, in Dalmatia, was too poor to be able to provide food for the cat; the animal was therefore obliged to cater for himself, and was well known as a thief in the neighbourhood. One day one of the children was being sent off to school without any breakfast; the cat, hearing him sobbing for hunger, immediately went off, and returned with a piece of bread he had stolen from a baker hard by, and brought it to the child. The same thing happened another day, and he came back, dragging along a piece of meat bigger than himself. On crossing the threshold a bit of bone caught in a hole, so puss miawed till some one came to his help. This same cat, who was constantly catching birds on the roof, slept with some pet birds in a cage without attempting to touch them.

Ragusa, Austria, June 18

MARGARET EVANS

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review article

A unifying model for the G1 period in prokaryotes and eukaryotes

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A model to explain the cell division cycle in both prokaryotes and eukaryotes is presented. No specific 'G1 functions' take place during the G1 period, which is merely part of a larger period for the preparation of DNA synthesis which began at the previous initiation of DNA synthesis. A G1 period exists merely because the doubling time of the cells is greater than the sum of the S and G2 periods.

THERE was a time, a few years ago, when the division cycles of prokaryotes and eukaryotes could be clearly distinguished. The division cycle of eukaryotes consisted of three periods—G1, S and G2—whereas prokaryotes had only C and D periods (analogous to the S and G2 periods, respectively) but did not clearly exhibit a G1 period. The S and C periods are analogous when defined as the time of DNA synthesis, and the G2 and D periods are analogous when defined as the time between the termination of DNA synthesis and mitosis or cell division. The analogy between the S and G2 periods and the C and D periods was strengthened by the observations that the lengths of time for these periods were quite invariant with large changes in the growth rate of the various cells[1–10]. More recently, however, it has been shown that prokaryotes can have a G1 period[11] and that there are eukaryotic cells which appear to be devoid of a G1 period[10,12–15]. Is there then any common, unified basis for understanding eukaryotic and prokaryotic division cycles?

There have been two different ways of analysing the division cycle of cells, one based on the classic results with eukaryotes and one based on the results obtained with prokaryotes. I believe that this schism is unnecessary and that there is a simple and encompassing view which allows both types of division cycle to be discussed in the same terms. My main premise is that there is no physical reality to the G1 period which requires that it have any particular function; instead the G1 period is merely part of a larger period involved in the preparation for DNA synthesis. This leads to a common, unified model for the cellular division cycle.

Bacterial division cycle

The bacterial division cycle is illustrated schematically in Fig. 1. The C and D periods, analogous to the S and G2 periods, are 40 and 20 min, respectively. That is, it takes 40 min to replicate the DNA of the cell, and there is a period of 20 min between termination of replication and cell division. Figure 1 illustrates the relative rates of DNA synthesis in the cells (the rate being proportional to the number of DNA growing points). Note that for cells growing with a doubling time greater than 60 min there is a G1 period as well as the S and G2 periods analogous to those in the eukaryotic division cycle. Bacterial cells, however, can grow at rates faster than 60 min per doubling. In these cases Fig. 1 illustrates that there is no G1 period. Instead DNA synthesis occurs during cell division, having started in the previous division cycle. Where the doubling time is less than 40 min DNA synthesis will commence before the completion of previously started rounds of DNA replication. These division cycle patterns are not exhibited by eukaryotes.

The particular pattern of DNA synthesis in the division cycle of a prokaryote therefore is determined solely by the frequency with which DNA replication is initiated. Once initiated, the 'clock' regulating the constant C and D periods determines the subsequent cell division. It is currently assumed that the preparations for DNA synthesis are occurring in the time gap between successive initiations of DNA synthesis. But the preparations for DNA synthesis are occurring continuously and not just in the period designated G1, which will appear when the time for the preparation of initiation of DNA synthesis (equivalent to the doubling time of the cells) is greater than the sum of the C(S) and D(G2) periods.

The nature of the factor(s) responsible for the initiation of DNA synthesis in bacterial cells is not known. Although it is quite reasonable to discuss the phenomenon in terms of a 'hypothetical initiator', circumstantial evidence points to the initiator being synthesised in the same manner as, or in parallel with, the synthesis of cell mass. Therefore, either the cell initiates DNA synthesis when a predetermined cell mass is reached or when some specific initiator, which is a constant fraction of cell mass, is present in the cell. In formal terms, DNA synthesis is initiated when the cell contains a given amount of initiator per origin of DNA to be initiated[16].

Eukaryotic division cycle

In contrast to the bacterial division cycle, the eukaryotic cycle is generally seen to fall into three divisions, a G1 period before DNA synthesis, the S phase defined as the period of DNA synthesis, and the G2 period, defined as the period between DNA synthesis and mitosis plus cell division. (In the following

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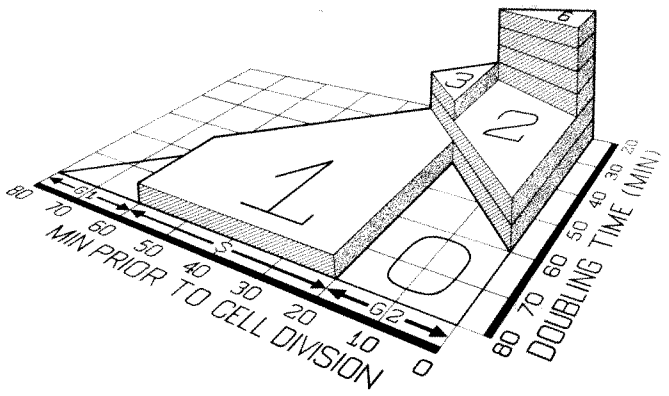


Fig. 1 Theoretical pattern of DNA synthesis during the division cycle of bacteria growing at different growth rates. The vertical axis is the rate of DNA synthesis during the division cycle. Zero DNA synthesis indicates that there is a 'gap' in DNA synthesis and this can be divided into the G1 and G2 periods as indicated. G1 and G2 periods are found when the doubling time is greater than the sum of the S and G2 periods, in this case with doubling time greater than 60 min as the S and G2 periods in *Escherichia coli* B/r are 40 and 20 min, respectively[1]. As the graph extends forward to larger and larger doubling times the G2 and S periods remain constant, and the G1 period grows. This portion of the figure is compatible with both prokaryotic and eukaryotic observations. Bacteria can grow with doubling times less than 60 min, however, and the pattern of DNA synthesis during the cycle is altered so that there is no G1 period and the late gap is shortened. The synthesis of DNA is necessarily initiated before cell division, but the time between the termination of a round of DNA replication is unchanged and remains at 20 min. Thus, between 60 and 40 min doubling times the initiation of DNA synthesis occurs during the period between termination of DNA synthesis and cell division (that is, in the bacterial G2(D) period). At doubling times of less than 40 min DNA synthesis is continuous with no 'gaps' in synthesis, and multiply-forked DNA molecules appear. Eukaryotic cells usually have doubling times which exhibit a G1 and a G2 (as in the cultures with doubling times greater than 60 min in this figure).

discussion it is assumed that there is a very small and negligible mitosis and cell division period, so the cycle is divided into only three parts.) To summarise a large amount of data, it seems that the S and G2 periods are fairly constant time periods and therefore are excellent analogues of the C and D periods of bacteria[10].

A generally current view of eukaryotic cells is that they, in contrast to prokaryotic cells, have a defined G1 period in which occur events preparatory for DNA synthesis[14,17,18]. This view is difficult to reconcile with reports that there are eukaryotic cells which are devoid of a G1 period ("G1-less cells")[14,15]. How can one have cells which have the G1 events but which do not have G1? I will answer this by presenting a simple description of the division cycle, of both prokaryotic and eukaryotic cells, which suggests that the eukaryotic G1 period has no functional significance and can disappear if the cell grows so fast that the S and G2 periods occupy the entire division cycle. In this model, the 'G1 period' is merely part of a larger period of preparation for the initiation of DNA synthesis.

Description of the general model

Consider three reactions which are observed to occur at various times in the G1 period of cells growing at such a growth rate that they have a substantial G1 period. In Fig. 2 they are indicated as a, b and c. The current (eukaryotic) assumption, that G1 is a period of preparation for S, is illustrated in Fig. 2*A*. If these cells are now grown at a rate where G1 grows appreciably smaller, S and G2 being relatively constant, then the rate of sequential appearance of the various G1 functions will increase rapidly as indicated in Fig. 2*B*. In the limiting case where G1 disappears, this model produces a paradox in that there is no G1 period during which the G1 function can take place.

The alternative model, proposed here, is illustrated in Fig. 2*C*. Here the functions a, b and c occur at precisely the same time as in Fig. 2*A* with regard to the observed G1 period but these functions are occurring as part of a longer preparation period which stretches from the initiation of S to the next initiation of S. There is no experimental or operational distinction between Fig. 2*A* and 2*C*. However, if the cells now grow at a faster rate so that G1 is appreciably shortened, then as indicated in Fig. 2*D*, the three functions a, b and c can occur earlier than G1—even occurring in S or G2 as shown in Fig. 2*D*. Where the doubling time is so short that there is no G1, the 'G1 functions' would all take place in either the S or G2 periods.

Illustrative applications of the model

Prescott[10] has summarised the large amount of experimental data on the timing of the G1, S and G2 periods in eukaryotic cells. He has even noted that "These studies lead to the conclusion that the initiation of DNA replication is tied to the attainment by a cell of a crucial mass". He has also noted how this is similar to the bacterial model briefly described above (Fig.

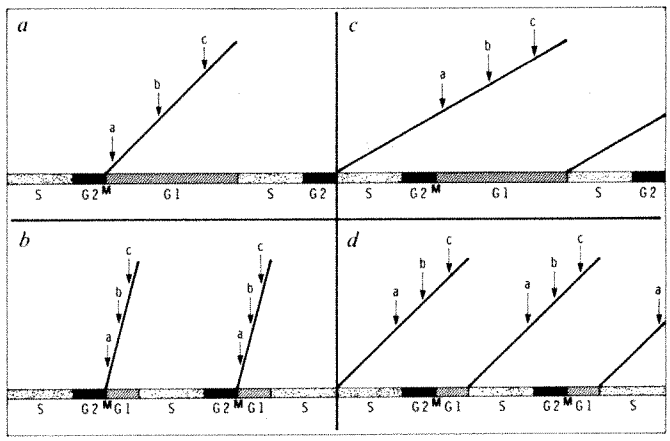


Fig. 2 Comparison of the eukaryotic view of the preparation for DNA synthesis (*A*, *B*) and the prokaryotic view of the preparations for DNA synthesis (*C*, *D*). *A* and *C* are two different interpretations of the observed preparations (a, b, c) for DNA synthesis as experimentally measured during G1. In *A* they are all part of a period of preparation for S which takes place in G1. In *C* they are part of a larger period of preparation for S which takes place from one initiation to the next, but with a, b and c only fortuitously occurring during the G1 period. *B* and *D* show how each of the models visualises the preparatory events when the rate of growth increases and the G1 period decreases in size. Although this figure is drawn as though there are different events occurring during the period of preparation for DNA synthesis, note that there is no firm and compelling evidence that the initiation of DNA synthesis occurs after a sequence of discrete reactions has occurred. The alternative view, that initiation occurs when some unitary factor has accumulated to some crucial level, is also compatible with this figure with the events a, b and c indicating the achievement of a particular level of the 'hypothetical initiator substance'.

1). I do not wish to repeat his analysis, and rather than review the mass of data on the nature, timing and events occurring in the G1 period, I will show with a few examples how the bacterial model serves to rationalise and explain the data on the nature of the G1 period better than the current eukaryotic model.

For example, Tobey *et al.*[19] have described a specific f1 histone phosphorylation event as occurring in G1 and therefore as a G1-specific event. When cells are growing more rapidly, it would be predicted (although it has not been specifically predicted or stated before, presumably because the possibility has not been studied) that the G1 events would merely occur more

rapidly leading to shorter G1 periods as seen in Fig. 2*B*. In contrast, the bacterial view presumes that the G1 events occurring in G1 are found in G1 only because G1 exists as an observable phenomenon when the doubling time of the cells is greater than the sum of the S and G2 periods. At a faster growth rate the events which may be observed previously to occur in the G1 period may now occur in the G2 or even the S period. This is clearly illustrated in Fig. 2*D*. The existence of a G1 event does not support or refute the view proposed here, but study of this phosphorylation reaction in cultures growing at different growth rates might lead to an experimental test of the unified model.

A clearer example is the analysis of eukaryotic cells which do not have a G1 period. Liskay and Prescott[15] have studied such a cell, and have obtained variants from the G1-less cell which contain a G1. They observed that the S and G2 periods did not change and that the appearance of a G1 was entirely accounted for by the increase in the doubling time of the variant cells. I suggest that this is an experimental verification of the idea that the G1 period is produced when the doubling time of a culture is greater than the sum of the S and G2 periods. When Liskay and Prescott selected for cells which had a G1 period, they were actually selecting cells which grew slower and which had less frequent initiations of DNA synthesis. The appearance of the G1 period was not due to any change in the genetic control of the G1 period itself, but merely due to the selection of mutants which had a slower growth rate without any concomitant change in the rate of DNA synthesis (S) or the rate of preparation for cell division following DNA synthesis (G2). Fusion experiments between the slow growing variant and the parental G1-less cell revealed that the variant was a "deficient condition' which is just what one would expect to produce a slow growing cell. Work by Liskay[14] had shown that the G1-less cells, when fused to temperature-sensitive mutants which had defects in their 'G1 functions', were able to complement the mutants. Therefore, the 'G1-less' cells still exhibited the G1 functions. This would normally seem paradoxical unless one subscribes to the model exhibited in Fig. 2*C* and *D* which shows that there is no special G1 period and no special G1 function. Assuming that there are functions which occur sequentially in preparation for DNA synthesis, the model proposed in Fig. 2*C* and *D* explains this apparent paradox since it is merely an accident of the cellular growth rate whether the function appears in the G1 period or not. In contrast to the model proposed here, Liskay and Prescott[15] have interpreted the G1-less cells as having 'full expression' (constitutive expression?) of the G1 functions. This is an *ad hoc* explanation which says that G1 functions can be expressed at other times. But it retains the notion of G1 functions.

Implications of the model

The model proposed here presents a unified view of the prokaryotic and eukaryotic division cycles. The main thesis is that the G1 period is merely an artificial construct of the observation, in slow growing prokaryotes and in most eukaryotic cells, of a period of cell growth and synthesis before the S period. The model described above proposes that it is more profitable to look at G1 as part of a larger period, from one initiation of DNA synthesis to the next initiation of DNA synthesis, during which the preparations for DNA synthesis are taking place.

How do eukaryotes and prokaryotes differ?—With regard to the division cycle only, the only difference between prokaryotes and eukaryotes seems to be the apparent inability of eukaryotes to synthesise DNA, that is, have an S period, at the time of cell division. This is possible for prokaryotes because the two nucleoids of the bacterial cell are self-contained and can function while the cell divides. I suggest that this is not the case for eukaryotes, where the completion of the S period produces a single nucleus with twice the diploid DNA content and which separates into two nuclei at mitosis and cell division. It is very likely that mitosis, and the associated condensation of DNA into chromosomes, is not compatible with continued DNA synthesis, and for this reason there is never any DNA synthesis during cell division in eukaryotes. If eukaryotic cells could produce two 'interphase' nuclei following the S period (for example, if the nuclei underwent an immediate mitosis without cell division), then it would be possible for DNA synthesis to occur in these nuclei while the cells were undergoing cell division. This would be analogous to bacterial cells growing with a doubling time of less than the sum of the C and D periods (60 min in Fig. 1).

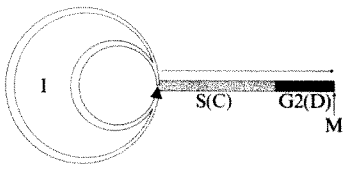


Fig. 3 A schematic representation of the cellular division sequence indicating the period for preparation of initiation, I, which is followed by the S(C) and G2(D) periods finally resulting in M (mitosis). The frequency of initiation is determined by the rate at which preparations for initiation occur, and this is indicated by the two different-sized circles, each representative of a different rate of growth. Note that the preparations for initiation occur continuously and do not start at M.

The cell division sequence—A corollary of my hypothesis is that there is no such thing as the division cycle. The word 'cycle' implies that at cell division something is initiated, the cell cycle. Actually, nothing starts at cell division but it is merely the end of a sequence of events which start with the accumulation of some initiation potential, and which was followed in succession by the initiation of DNA synthesis, the preparations for cell division following termination of DNA synthesis, and the final cell division (Fig. 3). The final cell division is the end of the process and the beginning of nothing. These ideas were expressed in relation to the bacterial cell division a decade ago[20].

The ideas described above were developed about a decade ago in discussions with many colleagues. One who deserves special mention is C. E. Helmstetter of Roswell Park Memorial Institute. At that time it was expected that the analysis of eukaryotic cells would eventually follow the logic of the bacterial analysis and therefore an explicit description of a unified model was unnecessary. This has not been the case, hence this brief article. This work was supported by grant DCM 77-14883 from the NSF.

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articles

Redshift dependence of colour and space density of radio galaxies

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Large deep Westerbork samples of radio sources have been optically identified on Palomar Schmidt and Kitt Peak 4-m plates. The radio galaxy population shows no evolution from $z = 0$ to $z \simeq 0.25$, then rises steeply in proper density beyond that epoch, attaining enhancement factors ~ 30 at $z \gtrsim 0.5$. These distant radio galaxies show a striking blue excess of as much as two magnitudes.

IDENTIFICATION programs for samples of weak radio sources show that the apparent colours of the faint galaxy identifications (presumably at redshifts of about 0.5) are considerably bluer than expected from redshifting the spectrum of nearby giant ellipticals such as M87. From the present data we cannot decide whether these blue colours are caused by young stellar populations or by (central) non-thermal sources, or possibly both. Using the apparent magnitudes of the identified radio galaxies to derive redshifts we have determined the radio luminosity function (RLF, the space density as a function of radio luminosity) of elliptical galaxies as a function of redshift. For moderate and intermediate radio luminosities, the space density does not increase out to redshifts of 0.2–0.3. However, the space density seems to increase strongly towards higher redshifts, by factors of between 10 and 30 at redshifts of about 0.5. This increase is present even for sources of intermediate luminosities, that is just above the 'break' in the RLF. There is qualitative agreement between RLF estimates using blue and red magnitudes. However, there is a quantitative difference which can be explained if one assumes that the distant radio galaxies are intrinsically bluer than local giant ellipticals, consistent with our empirical colour determinations.

Identifications and colours

The present results are based on optical identification programs of samples of weak sources from the Westerbork Background Survey[1] (BGS) and the 3rd Westerbork Survey[2] (3rd Wbk), both defined at 1.4 GHz. These surveys comprise more than 100 fields of about 1° diameter each, distributed in an essentially random manner over the northern sky. The majority of the sources in the BGS was identified on III a-J plates taken with the 1.2-m Palomar Schmidt telescope[3]. When such plates were not available, Palomar Sky Survey prints were used[4]. For about one-tenth of the sources in the BGS, deep III a-J plates made with the Mayall 4-m telescope could be used[3]. The 3rd Wbk sample was identified on III a-J and 127-04 plates taken with the Palomar Schmidt telescope[5].

De Ruiter[6] defined a complete subsample of 785 sources from the BGS. For 155 (that is, 20%) of these sources an optical counterpart was found with $m_b \leq 21.5$ (corrected for galactic absorption). Of the 79 sources for which deep 4-m III a-J plates could be inspected, 14 were identified on these deep plates in addition to those identified on the Palomar Schmidt III a-J plates (a total identification percentage of 47). For 85 of the 238 radio sources in the 3rd Wbk sample (or 36%) an identification was found on the 127-04 and/or III a-J plates. Of the 155 BGS identifications, about 40% are with a stellar object, 45% with a galaxy and 15% with an object for which the type could not be determined. For the 85 identifications in the 3rd Wbk survey these numbers are 20, 55 and 25% respectively.

The criterion for identification was basically one of positional coincidence. From the position uncertainties and the density of objects on the respective plates/prints, we calculate that about 15% of the suggested identifications on Palomar Schmidt plates are spurious. For the identifications on the 4-m plates this fraction is slightly higher, 20–25%. Magnitudes were estimated for the identified objects that were visible on the Palomar Sky Survey prints by measuring image diameters. Magnitude–diameter relationships for galaxies and stellar objects were calibrated using the photoelectric data of Sandage[7,8]. These calibrations were done independently for the BGS sample (H.R. de R.) and the 3rd Wbk sample (P.K.). Magnitude estimates are probably correct to within 0.5–0.7 mag. As the BGS covers a large range in galactic latitude, a correction for galactic absorption was applied to m_b. This was not done for the 3rd Wbk identifications as these are all at fairly high galactic latitude. For identifications visible only on the Palomar Schmidt III a-J plates a blue magnitude was estimated, taking into account the estimated limit of about 22 mag (corrected for absorption).

Figure 1 shows a colour ($m_b - m_r$) versus magnitude (m_r) diagram for all galaxy identifications visible in the red Palomar Sky Survey prints ($m_r \leq 20$ mag) and for which a blue magnitude was available.

The most striking phenomenon in Fig. 1 is the presence of the very blue objects with $m_r \geq 19$ mag, occurring in both samples. In general, the agreement between the distributions of the BSG and 3rd Wbk samples is very good. We therefore conclude that there are no large systematic differences in the two sets of

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magnitude estimates, although we cannot exclude the possibility that both data sets in Fig. 1 contain similar systematic errors. For instance, the very red colours of some galaxies with $m_r \sim 17$ mag are probably due to small (~0.5 mag) systematic errors in m_r. However, it is unlikely that the very blue colours of the galaxies with $m_r \geq 19$ mag are the result of systematic errors. Preliminary analysis of area photometry of some objects in the 3rd Wbk sample also indicates that there are no large systematic errors in the magnitude estimates of the faint galaxy identifications. We hope to obtain accurate photoelectrically calibrated photographic photometry for the objects in the 3rd Wbk sample.

Along the upper horizontal scale of Fig. 1 we have indicated the redshifts at which an elliptical galaxy with $M_r = -22.4$ would have the corresponding apparent magnitude (lower scale) in an $H_0 = 100$ kms s^{-1} Mpc^{-1}, $q_0 = 1$ geometry. The assumption of a constant M_r (independent of redshift) is not unreasonable because the red light is produced by a stellar population which evolves on very long time scales. We used the standard giant elliptical spectrum given by Pence[9] to calculate the red K-correction needed for this conversion. Figure 2 shows this red K-correction together with the blue K-correction calculated for the same spectrum. Figure 2 also contains the blue K-correction given by Code and Welch[10] on the basis of OAO UV-photometry of M87 (dotted line). These data do not seem to conflict with recent IUE spectral information on M87 (ref. 11).

Assuming an intrinsic colour $M_b - M_r = 1.9$ and using the K-corrections of Fig. 2, one predicts the colour–magnitude relationship shown in Fig. 1. Clearly the faint radio galaxies are very much bluer than expected. We may therefore question whether these objects are elliptical radio galaxies. Their classification as galaxy is reasonably certain because they all have extended (fuzzy) images. In principle, they could be subluminous and hence very nearby objects. However, this does not seem very likely since their angular sizes are what would be expected for galaxies at the redshifts that we have inferred. Therefore they are most likely to be elliptical galaxies, especially if one considers the observed ratio between optical and radio flux.

An interesting question is whether essentially all faint (m_r = 19–20 mag) radio galaxies are very blue, or rather: how many radio galaxies populate the inaccessible upper right-hand part of Fig. 1. This question cannot really be answered for the BGS sample, because this was defined on III a-J plates so that faint red galaxies are heavily selected against (if they exist). In the 3rd Wbk sample, which should be complete in m_r, there are two

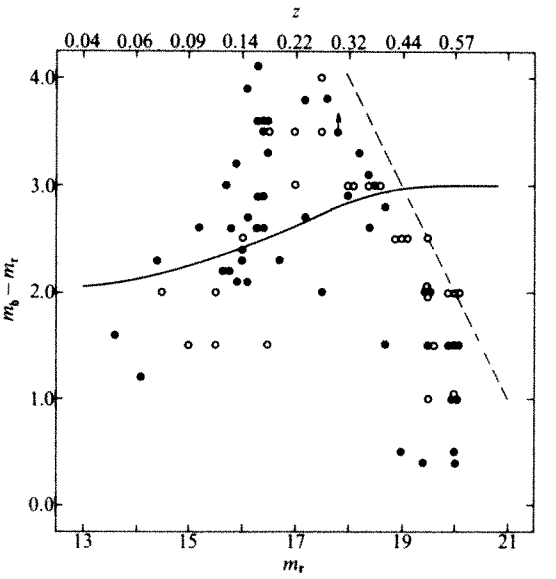


Fig. 1 Colour magnitude diagram of radio galaxies: ●, galaxies from BGS sample; ○, galaxies from 3rd Wbk sample. The solid line is the expected colour-magnitude relationship for a giant elliptical galaxy. The dashed line corresponds to $m_b = 22$ mag, the assumed limit of the III a-J plates.

objects (one galaxy and one object of uncertain type) in the range 19–20 mag that are not visible on the III a–J plate. Comparing this with the 12 objects in the same magnitude range below the $m_b = 22$ mag line, leads to the conclusion that most faint elliptical radio galaxies are much bluer than expected.

Our result only applies, of course, to radio-selected elliptical galaxies at redshifts of about 0.5. This 'colour evolution' may occur only in elliptical galaxies that contain a reasonably strong radio source. This would be consistent with the result of Kristian *et al.*[12], who find that the galaxies associated with the radio sources 3C299 and 3C330 (both brightest members of clusters at redshifts of about 0.5) are significantly bluer than the second brightest galaxy in the cluster. Undoubtedly, quite a few of the faint blue galaxies in Fig. 1 are also in clusters but with the present material we cannot tell whether the radio galaxies are bluer than other cluster members which presumably are even fainter. On the other hand, Butcher and Oemler[13] have obtained photometry of two distant clusters and find that the majority of the galaxies in these clusters are bluer than nearby early-type

Table 1 Magnitude–flux density tables for galaxies

(1) Background Survey identified on IIIa-J 48″ Schmidt plates

$\log(S_{1.4}/\text{mJy})$	1.1	1.5	1.9	2.3	2.7
Ω_{eff}(sr)	7.0×10^{-3}	1.6×10^{-2}	2.2×10^{-2}	2.3×10^{-2}	2.3×10^{-2}
m_b					
17.5–18.5	3(1.2)	1(2.1)	2(2.1)	1(1.0)	0(0.4)
18.5–19.5	2(2.2)	7(3.8)	0(2.8)	1(1.1)	0(0.3)
19.5–20.5	6(3.7)	4(5.3)	2(2.9)	1(1.0)	1(0.3)
20.5–21.5	13(5.1)	11(5.4)	3(2.6)	3(0.8)	1(0.2)

(2) Background Survey identified on IIIa-J 4-m plates

$\log(S_{1.4}/\text{mJy})$	1.1	1.5	1.9	2.3	2.7
Ω_{eff}(sr)	6.4×10^{-4}	1.2×10^{-3}	1.4×10^{-3}	1.4×10^{-3}	1.4×10^{-3}
m_b					
21.5–23.0	2(0.7)	7(0.5)	3(0.2)	2(0.1)	0(0.0)

(3) 3rd Westerbork Survey identified on 127–04 48″ Schmidt plates

$\log(S_{1.4}/\text{mJy})$	1.0	1.4	1.8	2.2	2.6
Ω_{eff}(sr)	1.3×10^{-3}	4.0×10^{-3}	5.9×10^{-3}	6.2×10^{-3}	6.2×10^{-3}
m_r					
14.25–15.75	2(0.3)	1(0.6)	1(0.7)	0(0.5)	0(0.2)
15.75–17.25	1(0.8)	3(1.9)	1(1.6)	0(0.7)	0(0.2)
17.25–18.75	4(1.8)	2(2.9)	0(1.7)	0(0.6)	0(0.2)
18.75–20.1	1(1.6)	9(2.0)	1(0.9)	3(0.3)	0(0.1)
20.1 –21.1	7(1.0)	9(1.0)	4(0.5)	3(0.1)	1(0.0)

The entries in parentheses are predicted galaxy numbers for a bivariate luminosity function identical to the local one for all redshifts.

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galaxies. It is not clear whether this indicates that the mix of early- and late-type galaxies in these distant clusters is different from what it is in nearby clusters, or whether the early-type galaxies in the distant clusters are considerably bluer than the nearby ellipticals. Recent work by Kron[14] shows that the colour distribution of faint field galaxies widens strongly towards the blue at the faintest magnitudes. However, the effect seems to occur at least 2–3 mag below the level where we find the blue radio galaxies.

Redshift dependence of the RLF

Before discussing possible implications of these results we consider the other aspect of the identification statistics, which is the information they contain about the radio luminosity function of elliptical galaxies and its redshift dependence. The identification statistics are summarised in the magnitude–flux density tables shown in Table 1. These tables give the number of radio galaxies in various (Δm, $\Delta \log S$) intervals in three samples. In the case of the BGS samples (1) and (2) we used blue magnitudes, while the table for the 3rd Wbk sample (3) is based on red magnitudes.

Note here that Table 1 not only contains genuine galaxy identifications but also a fraction of the identifications with objects of uncertain type. These latter are generally faint identifications, most of which are expected to be galaxies, but which could not be convincingly classified as such from their appearance on the plate. On average, about two thirds of these objects are included in Table 1. In general their inclusion has a relatively small effect although in one or two bins it almost doubles the number of objects.

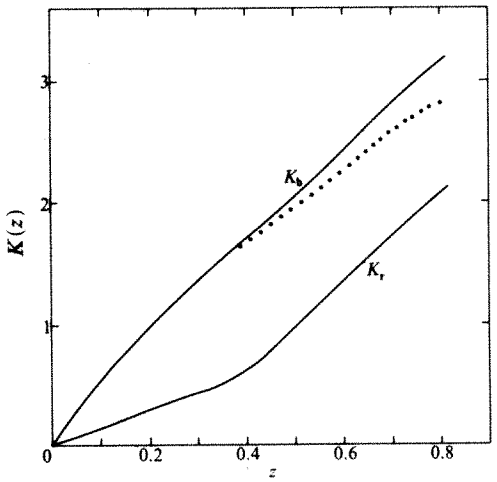


Fig. 2 Red and blue K-corrections based on Pence's standard energy distribution of giant ellipticals (solid lines). The dotted line represents the blue K-correction given by Code and Welch, based on the M87 spectrum.

Table 1 also shows in parentheses the number of radio galaxies expected in each bin if the local bivariate luminosity function of elliptical radio galaxies, as determined by Auriemma *et al.*[15], were valid at all redshifts. This bivariate luminosity function gives the space density of ellipticals as a function of radio luminosity P and absolute optical magnitude M. At any redshift the (Δm, $\Delta \log S$) interval transforms into a (ΔM, $\Delta \log P$)-interval, in which the number of objects per unit volume follows direction from the bivariate luminosity function. Integration over redshift and multiplication of the result by the solid angle covered by the survey in the $\Delta \log S$ interval yields the expected number of galaxies.

Table 1 shows that the observed and predicted numbers are in reasonable agreement for $m_b \leqslant 20.5$ and $m_r \leqslant 18.75$ mag. The average absolute magnitudes of the objects in question can be calculated from the local bivariate luminosity function[15] and are $M_b = -20.5$ and $M_r = -22.4$ mag for objects above the 'break' in the RLF. Using these values and the K-corrections of Fig. 2 the above apparent magnitude limits can be converted into redshift limits of ~ 0.3 and ~ 0.4 respectively. Only at fainter magnitudes (at higher redshifts) do the predictions fall short of the observed numbers, by an average factor of, say, 10 to 20.

The observations in Table 1 can be used to estimate the RLF if we ignore the dispersion in absolute magnitude at a given radio luminosity. Magnitude intervals can then be transformed into redshift intervals and, using the solid angles, into volumes, while flux densities transform into luminosities. The result of such a procedure is shown in Fig. 3 (the full curves shown represent our best estimate of the local RLF of elliptical galaxies, based on work by Colla *et al.*[16] and Meier *et al.*[17]).

Figure 3*b* and *c* give the RLF estimates obtained from samples (1), (2) and (3). The average uncertainty in a single point is shown by the 'error bars'. Estimates based on a single identification are enclosed in parentheses. Different magnitude intervals are indicated by different symbols and nominal average redshifts are given. It is again clear that for redshifts below say 0.3, our estimates do not differ significantly from our best estimate local RLF, which is based on ellipticals with redshifts of $\leqslant 0.1$. This conclusion extends a similar one by Meier *et al.*[17], who found no evidence for changes of the RLF out to redshifts of about 0.1. By a different, indirect method Robertson[18] also found little evidence for 'evolution' of the RLF below redshifts of ~ 0.2–0.3.

Both samples (1)+(2) and (3) show a strong increase of the space density in the high-redshift intervals. The results of the several samples are compared schematically in Fig. 3*d*. Although the three samples indicate the same general dependence of the RLF on redshift, sample (3) implies a much less drastic increase than do samples (1) and (2). We consider these differences significant and have investigated several possible explanations.

Colour and density evolution

Note that to first order an error in a zero-point of a magnitude scale displaces RLF estimates in a direction parallel to the local RLF, and hence cannot explain the differences in Fig. 3*d*. Different alinearities in blue and red magnitude scales could produce an effect like the one observed. For instance, assuming the results of samples (1) and (2) to be correct we find that the limits of the last m_r intervals should be about 19.3 and 20.1 mag, rather than the estimated 20.1 and 21.1 mag. Conversely, the difference in the limits of our III a-J plates from the 48-inch Schmidt and the 4-m telescopes would have to be >2.5 mag to produce consistency between samples (2) and (3). This is out of the question.

A more plausible explanation is, however, that the relationship between m_b and redshift is not what we assumed it to be on the basis of spectra of nearby ellipticals. In particular, if the UV spectra of the elliptical galaxies are more intense at higher redshifts, we would have underestimated their redshifts. In that case, the objects of sample (2) could be at redshifts of 0.7–0.8 rather than 0.5. This explanation produces consistency between the several RLF determinations and predicts a blue excess of the distant radio galaxies. The observed colour distributions discussed above and shown in Fig. 1 indeed show the faint radio galaxies to be markedly bluer than nearby ellipticals.

Note that the observed colours pertain to *bona fide* galaxy identifications, so that identifications of uncertain type are not represented in Fig. 1. The fact that a very small fraction of these galaxy identifications must be spurious does not change the result. Spurious identifications and identifications of uncertain type enter the determinations of the space density to some

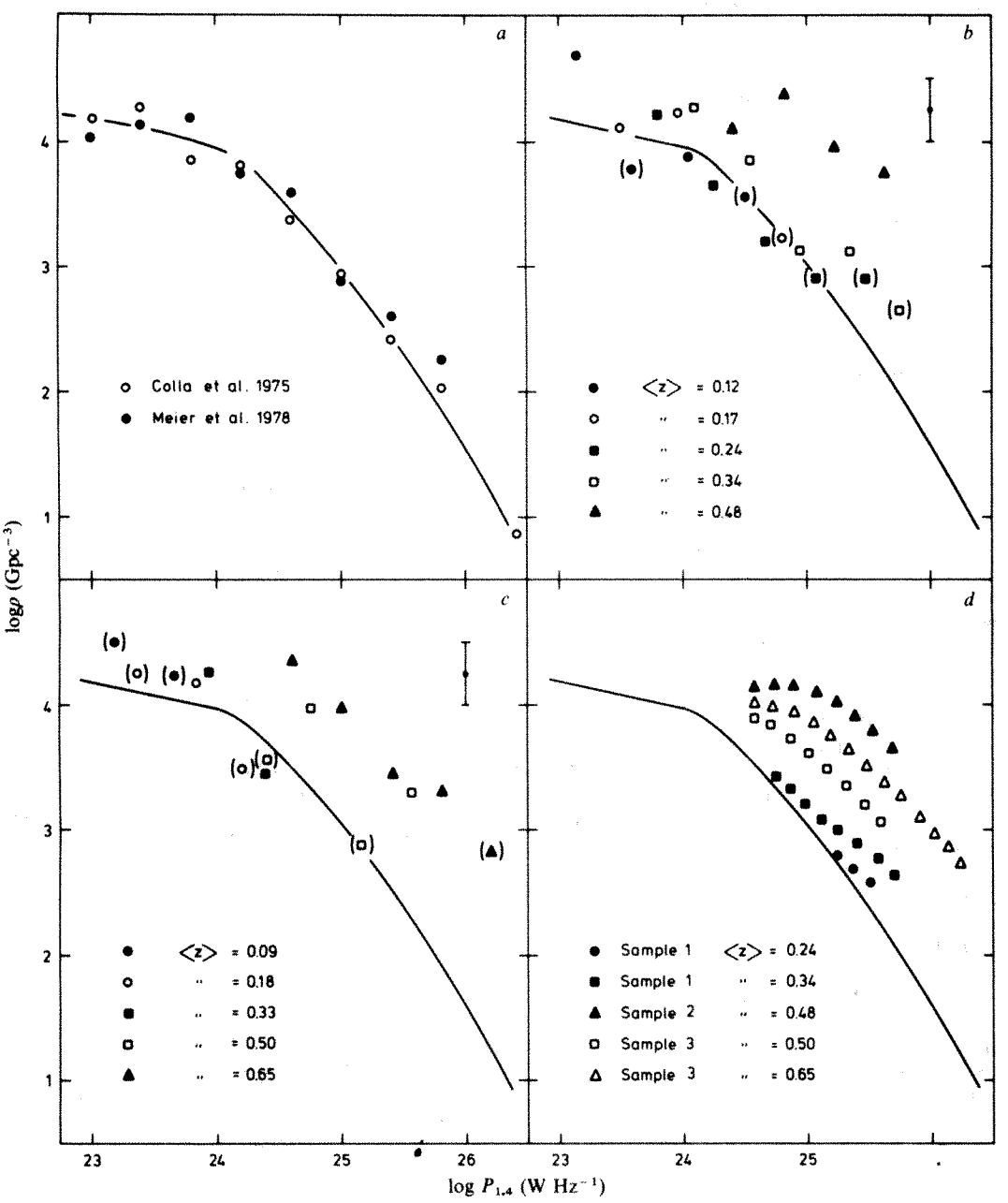


Fig. 3 The radio luminosity function (RLF) of elliptical galaxies: *a*, the local RLF; *b*, RLF estimates from the BGS samples; *c*, RLF estimates from the 3rd Wbk sample; *d*, schematic comparison of *b* and *c*. The uncertainty in the indivdual estimates is shown by the 'error bars'. Nominal average redshifts have been indicated.

extent. The error bars in Fig. 3 include the uncertainties due to these effects, and we therefore think that the differences between the various determinations, which we interpret as evidence for blue colours of the very faint radio galaxies, are significant.

Obviously, the present data should be confirmed and improved. Better magnitudes and colours are required, as well as direct redshift determinations. Nevertheless, the data as they stand indicate that the last 25–30% of the lifetime of the Universe has been relatively quiet. The probability of an elliptical galaxy producing a radio source has decreased drastically. At the same time, the energy output in the UV may have decreased considerably. Of course, the two phenomena may be causally related. If the blue excess has a non-thermal origin, this relationship could be very direct; an active nucleus produces both a blue continuum and a strong radio source. If, on the other hand, the blue excess must be attributed to a very high star-formation rate this relationship would be less direct. In both cases an important question is whether the strong decrease of activity from the $z \sim 0.5$ to the present epoch is caused by evolution of individual galaxies, or is a manifestation of collective processes in clusters.

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Solar luminosity and the sunspot cycle

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Neither sunspots, flares, other solar activity, nor the solar wind, are found to be responsible for the 22-yr solar cycle period found in the [D/H] climate indicator. This period may be due to a periodic variation in solar luminosity induced by a deeply buried magnetic field.

THE discovery of the 11-yr periodicity in the abundance of sunspots by Schwabe[1] in 1843, had initiated the search for solar induced 11-yr periods in meteorological variables of various kinds, and by 1898 many solar–terrestrial relations had been claimed.[2] But many questionable conclusions had been based on inadequate statistics[3] and it was difficult to see how the weak effects expected from solar activity could significantly influence the weather.

Despite these difficulties, it was believed that there might be a connection between solar activity and the weather and the search for a solar–terrestrial relationship apparently led to the establishment of the Laboratory of Tree-Ring Research at Tucson in 1938. But it then took almost 40 yr of hard work to accumulate the tree-ring data which today represent a very substantial statistical base and yield a convincing indication of a drought cycle with a 22-yr period in the western US.[4] Although this 22-yr cycle had been indicated previously[5,6].

The tree-rings show, statistically, a drought cycle with a 22-yr period and not a 11-yr period. Indications of this 22-yr period, and/or the 11-yr period, have been found in several other variables: in the atmospheric pressure at high altitudes[7], in temperatures, rainfall and other phenomena[8–13], but these conclusions have not been uniformly accepted.

The statistically strongest indication of a sharply tuned 22-yr period in a climate indicator seems to be that found by Epstein and Yapp in the [D/H] isotope abundance ratios measured in the cellulose extracted from two bristle cone pine trees which together span 1,000 yr[14,15]. The period found is in excellent agreement with the double sunspot period[16]. The corresponding peak dominates the power spectrum and this peak has substantially the same breadth as that expected from a sinusoidal oscillation of 1,000 yr duration.

The signal strength, its spectral purity and its close agreement with the sunspot period indicate that these data are significant[16]. The suggestion that the 10-yr sampling period of [D/H] data could generate a false periodicity could only be true if successive samples were periodically treated differently in the analysis procedure and this seems unlikely. (Owing to the 10 yr averaging, these data could not provide information about the existence of an 11-yr period.) Assuming that the data are significant, the climate is presumably the source of the 22-yr period in the Epstein–Yapp data, but the details are unclear. Unfortunately, much more information would be needed to obtain a description of a 22-yr variation of the climate.

The advantage of the [D/H] ratio as a climate indicator may be the non-local measure of the climate which it provides. The [D/H] ratio in the precipitated water and incorporated into the plant cellulose depends on the mean surface temperature of the Pacific Ocean where the water evaporates and on the air temperatures where the various precipitations occur.

The 11-and 22-yr periodicities are not the only indications of a solar–terrestrial relationship in the weather. Several independent sources of data suggest that there may be a relationship between mildness of the climate and the average level of solar activity, for example, the sunspot number averaged over the 11-yr cycle. The more sunspots, the milder may be the climate[17–20].

Solar activity and the weather

All the obvious physical means by which the solar activity might influence the weather seem to be insignificant, and the presence of the 22-yr period makes the problem doubly difficult. As measured by number and distribution of sunspots the 11-yr period dominates the sunspot cycle. But when the magnetic polarities of the polar regions and the sunspots are included, the period is 22 yr. On symmetry grounds an alteration of the signs of all magnetic fields should not significantly affect energy balance and energy transport, hence the weather. But the polarity of the Earth's magnetic field does not reverse with the solar cycle and magnetic reversals on the Sun could induce a 22-yr period in the weather, but only if the Earth's magnetic field played a significant part (for example, by affecting the solar wind). The solar wind is estimated by Dessler[21] to carry energy to the Earth at a rate only 6×10^{-7} of the radiation flux from the Sun. This seems too small to be significant.

Other possible physical effects of solar activity seem to be equally weak. At sunspot maximum $\sim3\times10^{-4}$ of the Sun's surface is covered by sunspot umbrae, but a significant change in radiation flux is not implied. First the period is wrong, 11 yr rather than 22 yr, and second, there is not enough heat capacity in the upper part of the convective zone to store the excess heat if the solar radiation is throttled. To obtain an adequate thermal capacity requires a depth of 25,000 km where the pressure ($\sim5\times10^{10}$ dyn cm^{-2}) is $\sim10^5$ times as great as the typical sunspot magnetic pressure. Various effects of solar activity in the upper atmosphere have been reported, for example, on the vorticity[22,23].

Roberts says of the difficulty with solar–terrestrial relations in the weather, "It is hard to imagine a plausible scheme to have this tiny tail wag the huge dog"[6]. But does the "tail wag the dog"? It may be, as the various estimates suggest, that the sunspots, faculae, prominences, flares, solar-wind and all the other external manifestations of the solar cycle have a negligible effect on the weather and that the source of the 22-yr period seen in the [D/H] indicator is a solar luminosity variation generated deep inside the Sun. A significant correlation between A, the solar luminosity, and B, the solar activity, does not imply that B causes A, or vice-versa. Perhaps C causes both A and B. If so, what could C be?

The solar 'chronometer' and the luminosity

It has recently been shown[16] that there is no statistical support for the long-accepted 'eruption-hypothesis' as a description of the sunspot cycle. Under this hypothesis the sunspot cycle undergoes a large random walk in phase, but this random walk is not found statistically. Instead the sunspot cycle seems to be flexibly coupled to some internal 'chronometer', perhaps to a magnetofluid oscillator. The phase of the sunspot cycle correlates strongly with the peak sunspot number. The large phase

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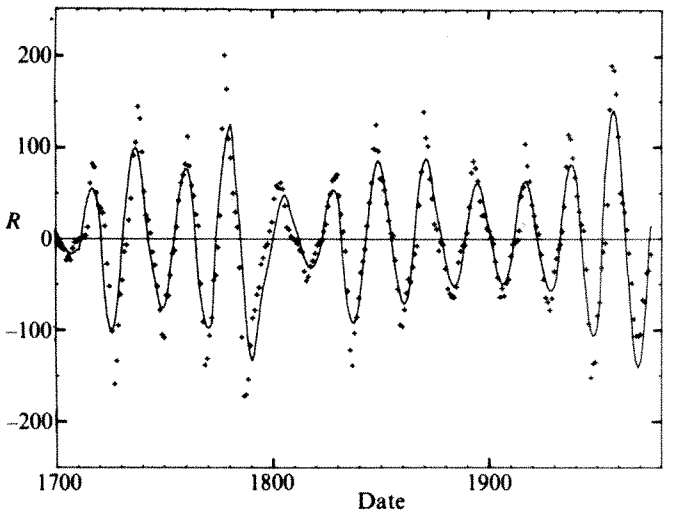


Fig. 1 Sunspot numbers of the (Hale) sunspot cycle and the fitted curve (equation (1)). (See test regarding sunspot numbers.) $\nu = 0.04489\ \mathrm{yr}^{-1}$; $\alpha = 0.015$; $c = 0.8708$.

fluctuation in the cycle may imply a long transit time for the magnetic field responsible for surface activity to float to the surface. If so, the correlation with the peak sunspot number implies that this transit time is less the more active the Sun.

The object C mentioned above might be the 'chronometer'. The Maxwell stress of the magnetic field could affect the energy transport to the surface and modulate the solar luminosity. (A reasonable site for this modulation is near the bottom of the convective zone.) The object C could also be the source of the magnetic field responsible for the sunspot cycle. If so, there are two expectations which might be tested statistically: (i) The solar luminosity should correlate with the sunspot cycle but with a phase delay in the sunspot cycle (the time required for the transport of magnetic flux to the surface). (ii) The correlation of the solar luminosity with the sunspot cycle should be better if the phase fluctuation in the latter is first removed. (The phase shift occurs after the modulation of the solar luminosity by the magnetic field.) These two expectations could be directly tested if one had a measure of the solar luminosity for the past 275 yr.

Solar luminosity, the [D/H] indicator and the sunspot cycle

If the above hypothesis is correct, the 22 yr periodic variation in solar luminosity may be the source of the 22 yr variation in the Epstein–Yapp [D/H] climate indicator[14]. This suggests that, as a working hypothesis, we tentatively interpret the indicator as a 'luminosity indicator' and investigate the correlation of the [D/H] data with the sunspot cycle.

As a first step, a curve is fitted to the yearly sunspot numbers[19,24] for the period 1700–1975 using the previously discussed[16] correlation of the phase of the solar cycle with the strength of the solar activity. According to Eddy[19] the sunspot curve, especially the absolute scale of the curve, is quite unreliable before 1817. Of particular concern is the possible systematic bias of early sunspot numbers through the loss of small sunspots due to poor seeing. From Kiepenheur's 'visibility function' for sunspots[25] an estimated correction factor of ~1.3 is obtained. This factor has been arbitrarily applied to all sunspot numbers before 1810. The correction is not secure and all calculations are also carried out with the correction factor omitted.

Figure 1 shows the yearly means of the sunspot number $R(t)$ for the period 1700–1975, with R plotted negative for alternate cycles starting with the first. The curve is a least square fit of

$$H = c\langle R\rangle \cos[2\pi\nu t + \delta_0 + \alpha\langle R\rangle] \qquad (1)$$

to the sunspot numbers $R(t)$ where

$$\langle R(t)\rangle = [(R^2(t-5) + \cdots R^2(t+5))/5.5]^{1/2} \qquad (2)$$

is an r.m.s. moving mean estimate of the peak sunspot activity. The phase shift $\alpha\langle R\rangle$ represents the previously found correlation of phase with the peak solar activity[16,26]. The parameters, c, ν, δ_0 and α are adjusted by least squares. The values obtained for ν and α are $0.044886 \pm 0.000054\ \mathrm{yr}^{-1}$, and 0.01153 ± 0.0083. The formally calculated error estimates are questionable as the residuals show evidence of unmodelled signal.

The double sunspot period p_s calculated from the above value of ν can be compared with the period previously obtained[16] from the Epstein–Yapp [D/H] data, p_D and with Wittman's[27] value, p_W, derived from a combination of telescopic data and a tabulation of naked-eye sunspot observations. These three values,

$$p_s = 22.279 \pm 0.027\ \mathrm{yr},\ p_D = 22.360 \pm 0.067\ \mathrm{yr},\ \text{and}\ \ p_W = 22.270 \pm 0.014\ \mathrm{yr} \qquad (3)$$

are all in satisfactory agreement. My attempt to obtain a sunspot period from the naked-eye sunspot data floundered in ambiguity[16].

The Epstein–Yapp[14] [D/H] weather indicator, D, is defined as the deviation, measured in parts per thousand, of the [D/H] abundance ratio from a standard oceanic sample. The 1,000 yr interval of time is covered by 100 10 yr samples yielding D_n, centred at t_n, where $n = 1 \ldots 100$. These data are filtered to remove low frequency noise by subtracting an approximately gaussian filtered moving mean to give the filtered data

$$D_n^\dagger = (-D_{n-1} + 2D_n - D_{n+1})/4 \qquad (4)$$

$D_n^\dagger$ is plotted in Fig. 2. The power spectra of both R and $D^\dagger$ (from Figs 1 and 2) are superimposed in Fig. 3 to show the coincidence of the strong 22-yr peaks.

If the fluctuations of the [D/H] ratio samples were induced by some solar function $f(t)$ lagged in time by τ yr, we would expect the decade time average of $f(t)$ defined by

$$f_n(\tau) = 0.1 \int_{t_n+\tau-5}^{t_n+\tau+5} f(t)\,\mathrm{d}t \qquad (5)$$

to correlate with D_n and the corresponding $f_n^\dagger(\tau)$ to correlate with $D_n^\dagger$. But then the least-square fit

$$c_0 + c_1 f_n^\dagger(\tau) \rightarrow D_n^\dagger \qquad (6)$$

to the N values of $D_n^\dagger$ could be used to determine c_0 and c_1 for a best fit to $D_n^\dagger$. The residuals are

$$\delta D_n^\dagger = D_n^\dagger - c_0 - c_1 f_n^\dagger(\tau) \qquad (7)$$

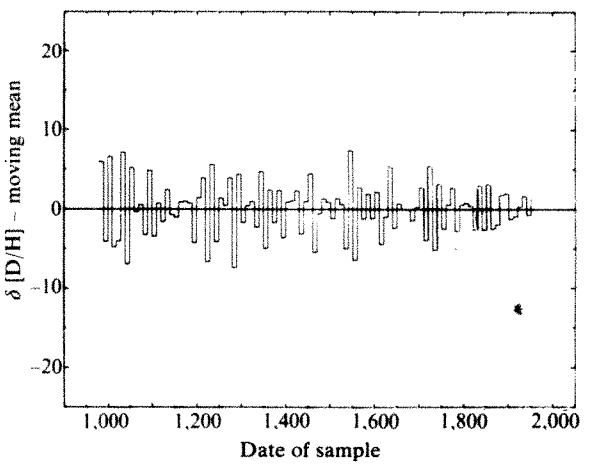


Fig. 2 The Epstein–Yapp Bristle Cone [D/H] data, $D^\dagger$ (filtered). $D_n^\dagger = D_n - 1/4(D_{n-1} + 2D_n + D_{n+1})$.

and the unbiased estimate of the residual variance

$$\sigma^2 = \sum_n (\delta D_n^+)^2/(N-2) \quad (8)$$

provides a measure of the goodness-of-fit, where $(N-2)$ is the number of degrees of freedom remaining after fitting c_0 and c_1. (The equivalent estimated unfiltered white noise variance is greater than in equation (8) by a factor of (8/3). The error of p_D, equation (3), has been corrected for filtering by the factor $(8/3)^{1/2}$.)

A 22-yr sinusoidal variation in solar luminosity might be expected to lead the 'luminosity indicator' D because of heat storage in the Earth. But from the discussion above, a much longer lag, not a lead, of the sunspot number R relative to D would be expected should the hypothetical internal modulation of the luminosity occur.

Adopting the sunspot number $R(t)$ as the function $f(t)$, equations (4)–(8) yield the unbiased estimate of the residual variance plotted as curve (1) of Fig. 4. The least-square fit equation (6) is calculated for the $N = 27$ values of D_n^+ covering the period 1665–1925. This residual variance can be compared with $\sigma_0^2 = 6.275$, the unbiased estimate of the variance of D_n^+ and equivalent to the residual variance obtained when the constraint $c_1 = 0$ is imposed on equation (6).

The above two-least square fits can be considered to represent alternative statistical hypotheses, (1) and (0) and the likelihood ratio of the two fits, with the fit under hypothesis (0) constrained by $c_1 = 0$, is

$$T = (L_0/L_1) = [\sigma^2(N-2)/\sigma_0^2(N-1)]^{N/2} \quad (9)$$

This ratio can be used to test the statistical significance of the various minima of the curves in Fig. 4 using Wilk's method. For large sample, $-2 \ln T$ has a χ^2 distribution with 1 d.f., assuming the truth of hypothesis (0). If a dip of a curve lies below the 1% level the prabability of the dip being a statistical fluke (hypothesis (0) being true) is 1% or less. All three dips of curve (1) are significant at the 1% level.

To see if the correlation is improved by eliminating the phase fluctuation from the sunspot cycle, the sunspot number R is replaced by the fitted function H (equation (1)) modified by replacing the phase term $\alpha\langle R\rangle$ by the constant $\alpha\langle\bar{R}\rangle$, where $\langle\bar{R}\rangle = 91.38$ is the mean value of $\langle R\rangle$ averaged over the period 1700–1975. With R replaced by H (modified), curve (2) is obtained. The two dips, occurring with lags of 2.5 and 13.5 yr, are statistically much stronger than those of curve (1).

The Maxwell stress of the magnetic field, not the field strength, should be responsible for the hypothetical modulation of the solar luminosity. To see if an amplitude modulation proportional to $\langle R\rangle^2$ yields an improved fit to D^+, the H (modified) used to generate curve (2) is further modified, replacing $\langle R\rangle$ by $\langle R\rangle^2$. The resulting curve (3) has dips at $\tau = 2$ and $\tau = 13$ yr which are slightly deeper than those of curve (2). At $\tau = 13$ yr the likelihood ratio between curves (2) and (3) is 17.2 (it is 2.67×10^3 for curves (1) (3)).

The locations of the minima and their depths, in curve (3), depend on two factors, the phase of the sunspot cycle and the amplitude $\langle R\rangle^2$ in H. To eliminate the phase of the sunspot cycle, f^+ in equation (6) is replaced by

$$\mathbf{H}_n^+ = [\langle R\rangle_{n-1} + 2\langle R\rangle_n + \langle R\rangle_{n+1}]^2 \cos[2\pi\nu t + \delta] \quad (10)$$

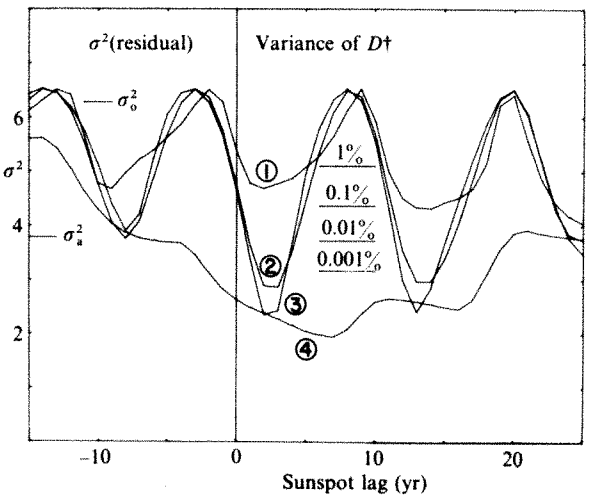


Fig. 4 The residual variance, equation (8), as a function of τ from the fit $c_0 + c_1 f^+ \rightarrow D^+$ with the following choices of f: ① $R(t)$; ② H (equation (1)) with the phase shift $\alpha\langle R\rangle \rightarrow \alpha\langle\bar{R}\rangle$ = constant; ③, same as ② but with amplitude $\langle R\rangle^2$; ④ f^+ from equation (10). The variance of D^+, σ_0^2, and the residual variance, σ_a^2, from equation (11) are also indicated.

with δ adjusted by least-squares. With f^+ replaced by $\mathbf{H}^+$, curve (4) is obtained. The locations of the minima of equations (2) and (3) are little influenced by the amplitude coefficient $\langle R\rangle^2$.

The strongest fit to the data D^+ is derived from equation (10) at the lag $\tau = 7$ yr (see Fig. 5).

Two peripheral questions remain: first, how good a fit to the data D^+ could be obtained by omitting the amplitude coefficient from $\mathbf{H}$ (equation (10))? The resulting function

$$f(t) = \cos[2\pi\nu + \delta] \quad (11)$$

gives a residual variance of $\sigma_a^2 = 3.785$, distinctly greater than the minima of curves (2) and (3) and less than the minimum of (1). Second, how do the above results differ if the bias correction of 1.3 is omitted? The sunspot period, to fit equation (1), is $p_s = 22.423 \pm 0.035$ yr^{-1}. It has a formal error 30% larger. It agrees with p_D but not with p_W (see equation (3)). Figure 4 is qualitatively unchanged. The dips in curve (1) are only a few per cent higher. The minimum of curve (4) is at $\tau = 6$ yr and is 43% higher. This dip is still significant, at a level of 10^{-5}. The conclusion reached is that eliminating the bias correction makes both the fit of equation (1) to the sunspot data and the fit of equation (6) to the D^+ data somewhat worse, but that, except for the 0.7% disagreement between p_s and p_W (a 4 σ discrepancy), the general conclusions reached in this paper are unaffected.

We return now to the expectations (i) and (ii). (i) Curve (1) of Fig. 4 shows a statistically significant correlation of 'luminosity' with R^+ lagged, assuming that D^+ is an indicator for a 22 yr periodicity in the luminosity of the Sun. (ii) Eliminating the variable phase of the sunspot cycle greatly improves the fit to the 'luminosity indicator' D^+. The phase lags (minimum σ^2) are substantially unaffected but the significance levels of the fits are very much better (0.001%) at the lags of $\tau = 2$ and 13 yr. The

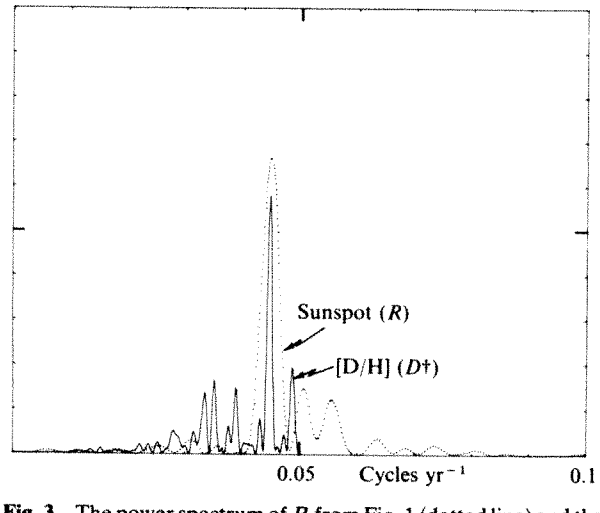


Fig. 3 The power spectrum of R from Fig. 1 (dotted line) and that of D^+ from Fig. 2 (solid line).

inclusion of the amplitude coefficient $\langle R\rangle$ (or $\langle R\rangle^2$) gives a distinctly better fit than is obtained assuming a simple periodic function for $f(t)$.

The phase fluctuation in R is not detectable (at a 2 σ level) in D^+. This is shown by simultaneously fitting H^+ from equation (1) and H^+ modified to eliminate the phase fluctuation to D^+. The regression coefficients are respectively 0.007 ± 0.029 and 0.032 ± 0.008. (The errors are corrected by the factor $(8/3)^{1/2}$ for noise filtering.) The first term is not present in D^+ to a measurable extent.

The two lags, $\tau = 2$ and 13 yr of curves (2) and (3) are equally strong statistically but a delay of 2–3 yr, for the eruption of magnetic field at the solar surface, after the modulation of the luminosity is inadequate to permit the large variation in phase seen in the sunspot cycle. This suggests that ~13 yr is required for the magnetic field to float to the surface.

Discussion

The 22.36 yr period in the [D/H] climate indicator (D) could be a statistical fluke. But if the 22.36 yr periodicity is meaningful, its close correspondence with the period of the sunspot cycle (Fig. 3), strongly suggests a causal relationship with the solar cycle.

If the solar cycle is driving this periodicity in D, neither sunspots, faculae, flares, other solar activity, nor the solar wind can be responsible. Because all of these surface phenomena exhibit the large phase fluctuation discussed above whereas it has been shown that this phase fluctuation is not present (at a 1 σ significance level) in D.

If this 22 yr period in D is meaningful, is due to the Sun, and is not due to any surface or external activity of the Sun, what induces it? A periodicity varying luminosity is a feasible source of variation in the climate, but then the variation must be induced in the deep interior of the Sun, probably towards the bottom of the convective zone and probably by magnetic fields. The work of Epstein and Yapp[14,15,28] has established that D is sensitive to variations in the climate.

We have shown that the Epstein–Yapp indicator D^+ and the sunspot numbers R are consistent with the following assumptions: (1) that the filtered [D/H] data, D^+, monitors a 22-yr luminosity variation of the Sun; (2) that the luminosity is modulated by a deeply buried magneto-fluid dynamic oscillator associated with a 'solar chronometer'[16]; (3) that some part of this magnetic field floats slowly to the surface where, years later, it is responsible for 'solar activity'; and (4) that the magnetic field arrives at the surface approximately

$$13 - \left(\frac{\alpha}{2\pi\nu}\right)(\langle R\rangle - \overline{\langle R\rangle}) = 13 - 0.041\,(\langle R\rangle - 91.4)\ \text{yr} \quad (12)$$

after the modulation of the luminosity, where $\langle R\rangle$ is evaluated at the time of arrival at the surface. The hypothetical luminosity modulation is found to have the phase stability of the 'solar chronometer'[16].

The amplitude of the 22-yr period in D^+ (based on all the data and after correcting for the effect of 10-yr averaging) is 3.8 ± 0.5 (see Table 2 of ref. 16). A crudely estimated temperature equivalent of a variation in D ($\delta T/\delta D \cong -0.2°$) is obtained from a comparison of modern values of D and T with those found in North America at the time of the late Wisconsin glacial maximum[28,29]. Owing to the long time scale of the Wisconsin glaciation and the large magnitude of the variation δD, even the sign of $\delta T/\delta D$ is uncertain when applied to the 22-yr cycle. Nonetheless, the implied times of temperature maximum 1891.0, 1913.3, 1935.7 and 1958.0 (Table 2 of ref. 16) are found to be in rough agreement with the dates of the highplains droughts, 1890, 1913, 1934 and 1954[6].

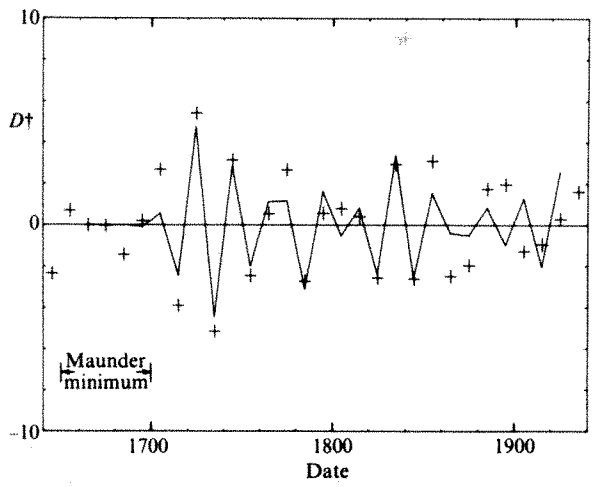


Fig. 5 The fitted function $c_0 + c_1 \mathbf{H}^+$, equation (10), and the data points D^+ where c_0, c_1 and δ are determined for $\tau = 7$ yr. $\nu = 0.04489\ \text{yr}^{-1}$.

For a black-body response of the Earth this estimated temperature amplitude of 0.8 °C corresponds to a 1.1% (amplitude) variation of the solar luminosity. More realistic calculations, including albedo feedback effects yield 0.55%, 0.7% and 0.18% for calculations by Budyko, Manabe and Welterald and Schneider and Gal-Chen respectively[30].

The only long series of observations of the solar constant, that of Abbot, shows a questionable ~23-yr period[31]. A 0.13% amplitude and a maximum at 1937 are obtained from a fit of a 22.3 yr period to the 1926–46 Abbot data. More recently, balloon flight measurements have given a luminosity increase from 1968 to 1978 of 0.4%, corresponding to an amplitude of the 22-yr period of 0.24%[32], assuming the correctness of the D^+ phase.

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$^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ ratios from the Azores and their significance in LIL-element enriched mantle

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Basalts from Sao Miguel are displaced to higher $^{87}Sr/^{86}Sr$ ratios, and have a significantly shallower slope, compared with the main correlation between $^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ for most mantle-derived volcanic rocks. Available data on young magmatic rocks indicate that large ionic lithophile (LIL)-element enrichment in the upper mantle involves two readily distinguishable components which reflect different histories.

MOST oceanic volcanic rocks have higher $^{143}Nd/^{144}Nd$ and lower $^{87}Sr/^{86}Sr$ ratios than the present-day composition for the model bulk Earth[1]. This implies that the oceanic upper mantle has been depleted in Rb and light rare earth elements (LREE) for much of its history. However, there are areas in the ocean basins where volcanic rocks are clearly not derived from a LIL-element depleted source. These are characterised by high concentrations of LIL-elements, high Rb/Sr, Rb/K and Ce/Yb ratios relative to most oceanic lavas, and are referred to as mantle 'hot spots' or 'plumes'[2,3]. $^{143}Nd/^{144}Nd$, $^{87}Sr/^{86}Sr$, and REE analyses from one such 'hot spot', the Azores (38° N 25° W), are compared with those from other LIL-element enriched areas. Constraints on models for the nature and timing of enrichment-depletion events in the upper mantle are then discussed.

Sample analyses

The samples analysed are all from the island of Sao Miguel (Fig. 1). This is the largest of the islands in the Azores and it was selected because it has been the subject of extremely detailed geological[4] and petrochemical work (ref. 5 and Marriner, Norry and Gibson, unpublished data). Its lavas are richer in LIL-elements than those from the other islands of the Azores at the same degree of fractionation; and they were also known to exhibit a larger range in $^{87}Sr/^{86}Sr$ (0.7035–0.7052) (ref. 6).

Eleven samples (Table 1), all alkali basalts, were chosen from the collections made by Gibson and Walker. They are from the stratovolcanoes of Aqua de Pau and Sete Cidades and the fissure eruptions between them, and are associated with large volumes of trachyte and hawaiite. The lavas used in this study contain more than 5% MgO and have olivine, clinopyroxene, and sometimes plagioclase, phenocrysts. K_2O ranges from 1.5 to 2.6% and like the other incompatible elements, its concentration is not a simple function of fractionation.

$^{87}Sr/^{86}Sr$ and $^{143}Nd/^{144}Nd$ were analysed by techniques reported previously[7] and range from 0.7032 to 0.7052 and from

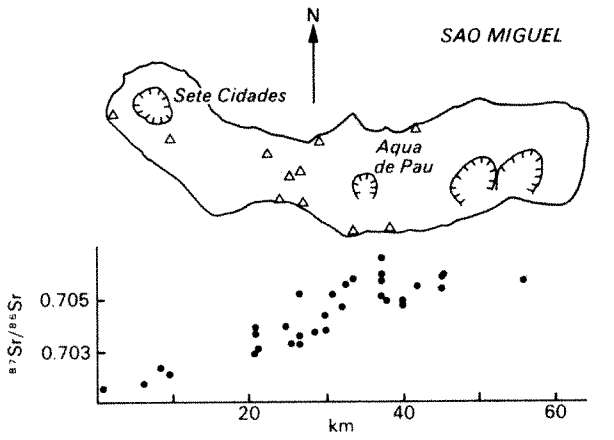


Fig. 1 Sketch map of Sao Miguel in the Azores illustrating the localities of the samples analysed in this study, and the marked increase in $^{87}Sr/^{86}Sr$ ratios from west to east across the island. Data from this work, Marriner, Norry and Gibson (unpublished), and ref. 6.

0.51300 to 0.51276 respectively (Table 1). They exhibit an inverse correlation between $^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ but have a slope which is much shallower than that observed in lavas from MOR and islands such as Iceland, Bouvet, and Tristan da Cunha[8] (Fig. 2). They might, therefore, reflect high level contamination[9] by either seawater or oceanic sediments but for the following reasons we find such an explanation unconvincing.

(1) All the samples were collected from subaerial flows which have not been in contact with seawater. Thus any interaction with seawater (or sediments) presumably takes place within the magma chamber(s).

(2) Assuming that ocean water has $^{87}Sr/^{86}Sr = 0.7092$, $^{143}Nd/^{144}Nd = 0.5124$ (ref. 10 and Hawkesworth and Elderfield,

Table 1 Alkali basalts sample analyses

Sample*	K_2O†	MgO†	Rb†	Sr†	Rb/Sr	$^{87}Sr/^{86}Sr$‡	Sm/Nd§	$^{143}Nd/^{144}Nd$‖
SM 18	1.98	8.54	44	716	0.061	0.70430±4	0.201	0.512886±18
SM 23	1.77	5.57	38	1001	0.038	0.70514±3	0.202	0.512764±20
SM 30	1.93	7.17	43	791	0.054	0.70433±5	0.208	0.512847±16
SM 49	2.65	6.54	59	794	0.074	0.70522±4	0.191	0.512808±18
SM 62	1.43	13.9	37	607	0.061	0.70481±5	0.199	0.512797±30
AZ 1034			48	805	0.060	0.70320±5	0.187	0.513002±20
AZ 1039	1.59	9.17	34	646	0.053	0.70406±5	0.206	0.512910±22
AZ 1322			33	576	0.057	0.70421±3	0.214	0.512872±20
AZ 1324	2.13	5.09	48	906	0.053	0.70391±4	0.202	0.512904±26
AZ 1704			36	612	0.059	0.70348±4	0.200	0.512921±18
AZ 1720	2.51	8.57	55	517	0.106	0.70459±6	0.200	0.512859±20

* All alkali basalts.
† Data from Marriner, Norry and Gibson (unpublished).
‡ Present day $^{87}Sr/^{86}Sr$ ratios normalised to E-A, = 0.70800.
§ From Table 2.
‖ Present day $^{143}Nd/^{144}Nd$ ratios normalised to $^{146}Nd/^{144}Nd = 0.7219$ (BCR-1 = 0.51266±2).

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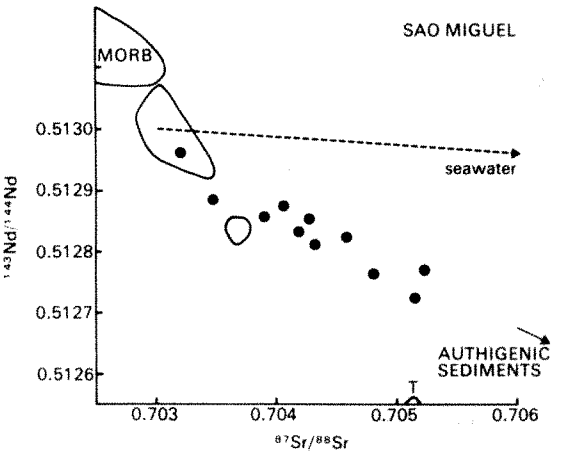


Fig. 2 $^{143}Nd/^{144}Nd$ versus $^{87}Sr/^{86}Sr$ for basalts from Sao Miguel. I, Iceland; B, Bouvet; T, Tristan da Cunha[8]. BCR $^{143}Nd/^{144}Nd = 0.51262$, E–A, $^{87}Sr/^{86}Sr = 0.70800$.

unpublished data), and Sr/Nd = 10^6 (ref. 11), then mixing it[12] with magma of composition 0.705 and 0.5130 results in the almost horizontal trend shown on Fig. 2. This does not seem to be responsible for the slope of the Sao Miguel data.

(3) Given an average of 725 p.p.m. Sr in the basalts and 8 p.p.m. in ocean water, very large water/rock ratios (perhaps 30–50) are implied if the compositions of the basalts are controlled by seawater interaction. Moreover, in contrast to the MOR environment this large volume of water would have to be heated to magmatic temperatures.

(4) Sao Miguel rises some 3,000 m above the floor of the mid-Atlantic Ocean. Moreover, a 1 km drill hole into pillow lavas on the island did not encounter any sediments. Thus for sedimentary material to act as the contaminant it must be dragged down into the magma chamber (presumably along the transform feature), heated to magmatic temperatures, and then mixed in without affecting the primitive nature of the basalts.

It is apparently difficult to attribute the correlation between Nd and Sr isotopes in the Sao Miguel lavas to high level contamination by seawater or oceanic sediments. We believe, therefore, that their isotope geochemistry reflects processes operating within the upper mantle.

There is no simple relationship between the trace element and isotope geochemistry of the lavas studied from Sao Miguel. Eight REE have been determined on each sample using standard isotope dilution techniques[13] (Table 2). They are all highly enriched in LREEs ($Ce_N/Yb_N = 14.2–10.0$) and only three samples contain detectable Eu anomalies which presumably reflect minor accumulation/fractionation of plagioclase phenocrysts. Considering only the samples without Eu anomalies their Rb/Sr ratios fall in the narrow range of 0.053–0.061 while $^{87}Sr/^{86}Sr$ varies from 0.7032 to 0.7048. Similarly $^{143}Nd/^{144}Nd = 0.51300–0.51276$, but Sm/Nd only varies from 0.19 to 0.21. Thus, although we emphasise that there is no clear relationship between isotope composition and trace element ratios, the narrow range of the latter means that they would take 3,000–5,000 Myr to generate the observed range in Nd and Sr isotopes. This is clearly unlikely, and the variation in $^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ in the Sao Miguel basalts, therefore, probably reflects some recent mixing process.

Further support for this conclusion may be found in the striking regional variation in $^{87}Sr/^{86}Sr$ ratios across the island (Fig. 1). It suggests that mixing of magmas or sources took place beneath Sao Miguel and that the high $^{87}Sr/^{86}Sr$ component becomes increasingly important in the centre and east of the island. The low $^{87}Sr/^{86}Sr$ component has Nd and Sr isotope ratios which lie on the main trend of the Atlantic Ocean volcanic rocks (Fig. 2).

Discussion

Combined Nd and Sr studies have shown that lavas from LIL-element enriched source regions often have isotope compositions which indicate that these sources have actually been depleted in LIL elements for much of their history. Thus when discussing the trace element characteristics of a portion of the upper mantle a clear distinction must be made between those deduced from trace element chemistry in basalts and those inferred from isotope composition.

The isotope results presently available suggest that LIL-element enriched volcanic rocks may usefully be divided into two groups (Fig. 3). Group I consists of the main trend of volcanic rocks on which estimates of the present day $^{87}Sr/^{86}Sr$ ratio of the bulk Earth rocks are based[1]. It is characterised by a steep slope on $^{143}Nd/^{144}Nd$ versus $^{87}Sr/^{86}Sr$ and notably, most of

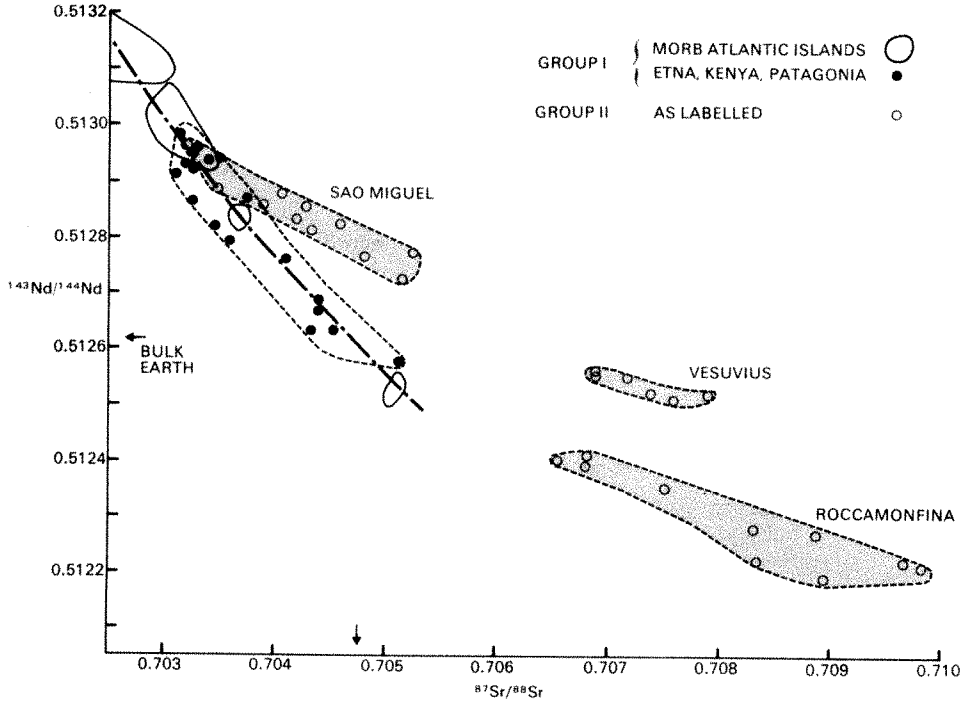


Fig. 3 $^{143}Nd/^{144}Nd$ versus $^{87}Sr/^{86}Sr$ for basalts from oceanic and continental environments. MORB and Atlantic Islands as in Fig. 2. Results from refs 7, 8, 14, 21, 24 and this work. Present day bulk Earth composition after De Paolo and Wasserburg[15] and O'Nions *et al.*[8].

the volcanic rocks from both oceanic and continental environments have present Nd and Sr compositions indicative of LIL-element depletion (relative to the model bulk Earth) for long periods of time.

Group II (Fig. 3) is distinguished because its lavas fall on trends of significantly flatter slope which plot to the right of the main trend of oceanic and continental volcanics (group I) on the $^{143}Nd/^{144}Nd$ versus $^{87}Sr/^{86}Sr$ diagram. Hence they might all reflect contamination; by seawater, sediments, or continental crust. Reasons for rejecting such a hypothesis for Sao Miguel have been outlined above. Hawkesworth and Vollmer[14] argued for Roccamonfina that the positive correlation between $^{87}Sr/^{86}Sr$ (0.7064–0.7096) and Sr content (800–2,000 p.p.m.) in its primitive lavas is most unlikely to have been generated, or affected significantly, by interaction with continental crust. There are much less data from Vesuvius[14] but by analogy with Roccamonfina the geochemistry of its lavas are also thought to have been largely unaffected by crustal contamination. Thus we believe that the flat-lying trends of the group II lavas do not reflect contamination but reflect processes active within the upper mantle.

The variation of the trace element ratios relevant to Nd and Sr isotopes can be simply represented on a diagram of Sm/Nd versus Rb/Sr (Fig. 4). Note that the broad curve of the data does not include the point for the Rb/Sr and Sm/Nd ratios of the bulk Earth deduced from Nd and Sr isotope studies[1,8,15]; as might have been expected if the mantle had a simple history of depletion or enrichment.

Also shown on Fig. 4 are the results of batch melting calculations on garnet lherzolite which have been outlined in more detail elsewhere[16]. They illustrate that for amounts of melting exceeding 5–10%, little displacement occurs between melts and their source rocks when plotted on Fig. 4, and that source rocks in quadrant 2 have had a multi-stage history. The simplest model necessitates depletion of a portion of the mantle by extraction of at least 0.5% of liquid followed by remelting to form a second liquid which could mix with other depleted portions of the mantle[16] (Fig. 4).

The Nd and Sr isotope results also suggest that the source regions of the group I lavas have had a complex history. Hawkesworth *et al.*[16] pointed out that the isotope variations on the main trend of the group I lavas could not be reproduced by two-stage models (see Fig. 5) and concluded that some large-scale mixing process was responsible. O'Nions *et al.*[17] suggested that the main Nd and Sr isotope correlation (group I, Fig. 3) results from fractionation of the crust from the mantle throughout geological time, accompanied by continuous remixing of the mantle reservoir. Such a process, while explaining the remarkably good Nd and Sr isotope correlation observed from a large number of areas (group I, Fig. 3) would imply that most mantle materials should have lower Rb/Sr and higher Sm/Nd ratios than the bulk Earth (see quadrant 1, Fig. 4). This is true for MOR lavas and tholeiites from Iceland but many oceanic island and continental basic lavas do not have these characteristics and plot instead in quadrants 2 or 3 of Fig. 4.

Provided that these lavas are produced by amounts of melting exceeding a few per cent, and have not been subject to large amounts of plagioclase fractionation, their source regions will also plot within these quadrants. Prolonged residence in quadrant 2 will result in Nd and Sr isotope compositions plotting to the left of the main isotope correlation (Fig. 3). Lavas with such compositions are rare and when observed in the British Tertiary Province they have been attributed to assimilation of Rb-poor granulite facies crustal material[18]. In general, however, most lavas in quadrant 2 of Fig. 4 plot on the main (group I) trend of Nd and Sr isotopes (Fig. 3). Thus we conclude that the enrichment of LIL-elements in their source regions must have taken place comparatively recently so that insufficient time has elapsed to affect their isotope ratios significantly.

Table 2 Rare earth elements

Sample	Ce	Nd	Sm	Eu	Gd	Dy	Er	Yb	Eu/Eu*
SM 18	110	55.2	11.1	3.42	9.47	7.25	3.35	2.59	1.00
SM 23	119	61.8	12.5	4.38	10.8	7.48	3.22	2.30	1.12
SM 30	103	51.5	10.7	3.35	9.31	6.88	3.14	2.35	1.00
SM 49	122	54.4	10.4	3.45	8.73	6.45	2.91	2.18	1.08
SM 62	102	51.2	10.2	3.11	8.74	6.44	2.86	2.25	0.99
AZ 1034	122	57.2	10.7	3.31	8.92	6.35	2.91	2.25	1.01
AZ 1039	89.6	45.5	9.37	2.95	8.14	6.10	2.73	2.03	1.01
AZ 1322	73.7	38.3	8.18	2.59	7.38	—	—	—	1.00
AZ 1324	116	65.0	13.1	4.07	11.3	8.38	3.84	2.95	1.00
AZ 1704	85.9	45.2	9.05	2.88	8.01	5.62	2.58	1.94	1.02
AZ 1720	96.8	45.0	8.98	2.60	7.35	6.12	2.85	2.29	0.95

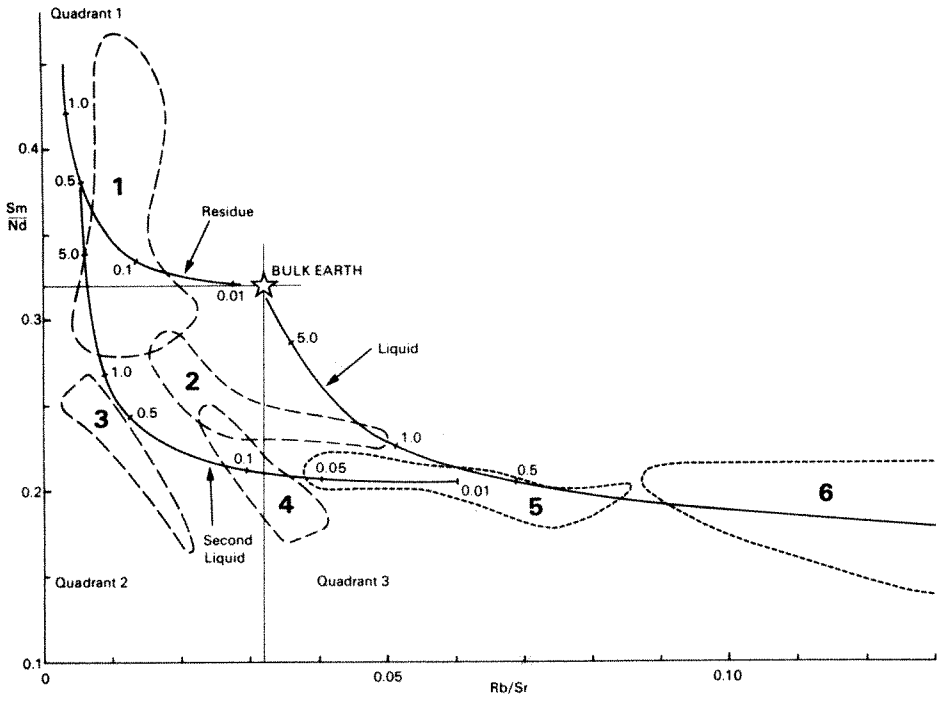


Fig. 4 Plot of Sm/Nd versus Rb/Sr in the more primitive rock types from Iceland (1), Hawaii (2), Etna (3), Patagonia (4), (all group I), and Sao Miguel (5) and Roccamonfina (6) (group II—see Fig. 3). Data from refs 7, 8, 14, 24, 25 and this work. The results of batch melting calculations are from ref. 16 and have the melt fractions in per cent.

Quadrant 3 (Fig. 4) contains lavas derived from source regions with high Rb/Sr and low Sm/Nd ratios; they would develop with sufficient time a flat-lying trend plotting to the right (that is, high $^{87}Sr/^{86}Sr$) of the main (group I) trend on the diagram of $^{143}Nd/^{144}Nd$ versus $^{87}Sr/^{86}Sr$.

The group II lavas plot in Fig. 4 quadrant 3 and have the flat-lying trend between Nd- and Sr-isotopes that is predicted for source regions which have had variable but high Rb/Sr and low Sm/Nd ratios for a significant period of time. Assuming that trace element ratios in the source region can be estimated from those in the lavas, these would seem to be the ideal situations for recognising 'mantle isochrons'[19] which might 'date' old enrichment events in the upper mantle. However, where such lavas have been analysed for more than one isotope system, the simplest interpretation of the data seems to be that they also reflect geologically recent LIL-element enrichment. At Roccamonfina, for example, the primitive rock types exhibit positive correlations between $^{87}Sr/^{86}Sr$ and Rb/Sr, and $^{143}Nd/^{144}Nd$ and Sm/Nd, but the calculated ages are 500 and 2,000 Myr respectively[14]. Since the $^{207}Pb/^{204}Pb$–$^{206}Pb/^{204}Pb$ results correspond to an age of almost 0 Myr (ref. 20) we conclude that these 'mantle correlations' are most unlikely to have any real age significance and probably reflect a recent mixing/enrichment event within the upper mantle.

More speculatively it has been suggested that the Nd and Sr isotope compositions from Sao Miguel also reflect some recent mixing process. The high $^{87}Sr/^{86}Sr$ component in both examples presumably evolved by virtue of its source having had the high Rb/Sr and low Sm/Nd ratios characteristic of quadrant 3 (Fig. 4) for a significant period of time. It is envisaged that at Sao Miguel this component mixed with material that had LIL-element depleted isotope characteristics similar to Iceland, while at Roccamonfina it mixed with material similar in composition to the model bulk Earth (Fig. 3).

Conclusions

The available Nd and Sr isotope results on recent oceanic and continental volcanics may be conveniently considered in two groups (Fig. 3). Group I includes most of the available oceanic data and is therefore much more significant in terms of volume of mantle sampled. It forms a steep, negative correlation on a graph of $^{143}Nd/^{144}Nd$ versus $^{87}Sr/^{86}Sr$, and most of the isotope compositions indicate derivation from source regions which have been depleted in LIL elements relative to the bulk Earth for a considerable time. Recent LIL-element enrichment events are recognised in both oceanic and continental areas, and it is noticeable that (1) the LIL elements have migrated from source regions which also have Nd and Sr isotopes on the trend of the group I samples and (2) that although Rb and Sr abundances are increased during LIL-element enrichment, the Rb/Sr ratios rarely exceed 0.04 (Fig. 4).

Group II samples, in contrast, have shallower slopes and plot to the right of the main correlation (group I) on the graph of $^{143}Nd/^{144}Nd$ versus $^{87}Sr/^{86}Sr$. Volumetrically they are clearly much less significant, but an example is now available from both the oceanic and continental environment and their significance lies in the fact that they clearly involve a different component to those in group I (Fig. 3). We have argued that in the cases studied, this component is most unlikely to be introduced by contamination during magma extraction and that it is therefore present in the upper mantle. It results in high Rb/Sr ratios (>0.04) and correspondingly high $^{87}Sr/^{86}Sr$ ratios relative to $^{143}Nd/^{144}Nd$. Moreover, since these are often taken to be crustal characteristics, one possible explanation is that this group II component might reflect subducted crustal material which has retained its identity in the upper mantle (O'Nions *et al.* unpublished).

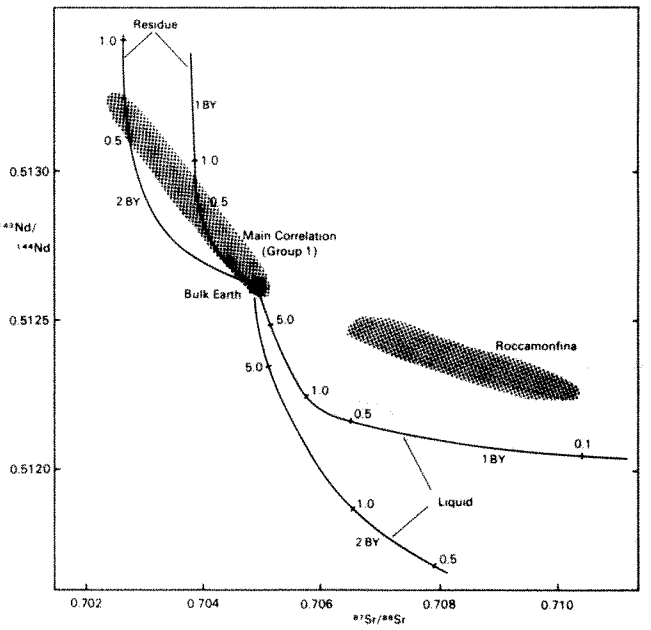


Fig. 5 Nd- and Sr-isotope evolution for two-stage models in which melting occurred 1,000 and 2,000 Myr ago[16]. Melt proportions in per cent.

Alternatively mantle metasomatism, as documented from mantle xenoliths in kimberlite by Erlank and coworkers[22,23], can result in LIL-element enrichment which will with time generate the relatively high $^{87}Sr/^{86}Sr$ and low $^{143}Nd/^{144}Nd$ ratios apparently characteristic of the group II end members. It is difficult to determine how long such material needs to be isolated, but note that the data from nodules of metasomatised mantle indicate that $^{87}Sr/^{86}Sr$ ratios of 0.705–0.710 can be generated in only 150 Myr (ref. 23).

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An exceptional intraplate earthquake sequence in Meløy, Northern Norway

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A unique earthquake sequence began in Meløy, Northern Norway, in November 1978, and 10 weeks later about 10,000 shocks had been recorded from a volume not larger than $10 \times 8 \times 6$ km^3. The largest earthquake occurred on 28 November, 1978, with an ML magnitude of 3.2 and a maximum MM intensity of VI.

ALTHOUGH Norway is far away from plate boundaries—the nearest ones being the Mohn's and Knipovich Ridges in the Norwegian Sea—the region exhibits a relatively high seismic activity in the context of intraplate earthquake occurrence. Husebye *et al.*[1-2] have scrutinised all available evidence on the seismicity of this region, including historical records of 400 yr ago. The largest occurring earthquakes were found to have had magnitudes of the order of 6.0–6.5, and broad seismic zones could be delineated, usually in agreement with major tectonic features. In November 1978 a remarkable earthquake sequence began in Northern Norway, where about 10,000 shocks were recorded during the first 10 weeks of instrumental coverage. This unique phenomenon, which is still taking place at a reduced rate is described here.

Background and instrumentation

It is uncertain when the Meløy earthquake sequence started, but the first earthquake positively recorded at NORSAR (the Norwegian Seismic Array, almost 700 km away) occurred on 3 November 1978 and was measured at $ML = 2.4$. *ML* is the local magnitude calculated from the Sg phase and based on wave attenuation characteristics representative for Scandinavia[3]. Between 12 and 19 November, ground shakings were often felt, particularly at night time, and some cracks in chimneys and walls were observed. The shocks were accompanied by relatively strong bangs and rumblings. On 16 November NTNF/NORSAR were asked to monitor the area, and two days later the first seismographs (type Sprengnether MEQ-800) were installed. A maximum of five stations were in operation at one time while seven different sites were used (for details, see Fig. 1 and Table 1). Three of these sites—ENG (Enga), NEV (Neverdal) and ORN (Ørnes)—were chosen as permanent locations. Figure 2 shows the microearthquake activity on one of the most active days. Accelerometers, hydrophones and analog and digital tape recording of three-component seismometers were also installed on an experimental basis and operated for short time intervals. (Field work in the rugged Meløy area is very difficult in winter so more extensive monitoring experiments are planned for the summer assuming the earthquake sequence continues until then.) Besides the NORSAR array, several other seismograph installations in Scandinavia have recorded the largest Meløy earthquakes. Whenever possible, the NORSAR recordings have been used to measure the local *ML* magnitude.

Earthquake sequence in time and space

Minor tremors are not uncommon in the Meløy area, and the first reported rumblings that may be associated with the present sequence date back to around 20 October 1978. While it is difficult to determine the exact starting date, it seems that the main activity started around 10 November 1978.

The timing of the earthquake sequence between 11 November 1978 and 26 January 1979 is given in Fig. 3 for station NEV and shows the total number of events per 4-hourly time interval, the cumulative number of events (ending at about 7,500), and all events with an *ML* magnitude of at least 2.0. The main concentration of larger shocks was between 11 and 18 November, and there is also an interesting predominance of night-time shocks during this interval. The activity was high and fairly constant (although with large short-term variations) up to 5 December, then decreased to a minimum around 20 December, whereafter new outbursts of activity occurred on 27–28 December, 3–4, 8–10 and 18–20 January. Comparing the occurrence of the larger events with the total activity we see a repeated pattern of 'main' shocks followed by aftershocks lasting sometimes a few hours, sometimes a few days.

A detailed study of the sequence shows only one example of foreshock activity: the $ML = 2.9$ event on 27 December which was preceded by nine small shocks starting 90 s before the main one. Apart from that event, the main shocks were always preceded by a period of relative quiescence. There are also examples of large sub-sequences not preceded by a main shock,

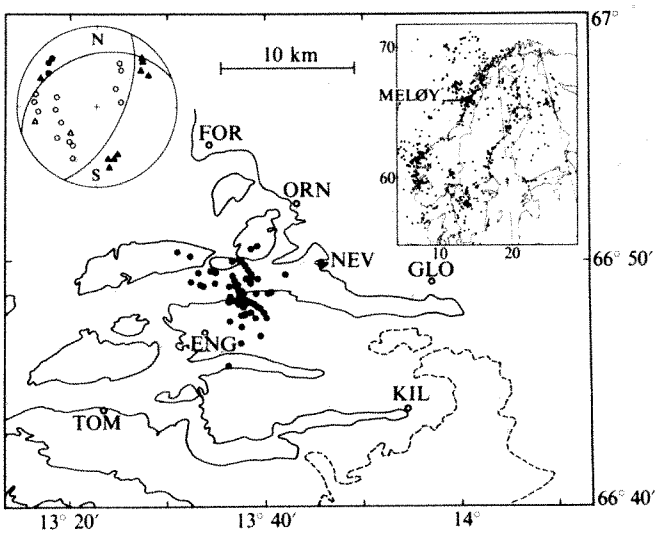


Fig. 1 Map of the Meløy area with the locations of seven seismic stations whose times of operation are given in Table 1. The dotted line to the south-east indicates a glacier (the whole area is very mountainous). ●, Computed epicentres for 66 earthquakes for which from 5 to 10 readings (phases) were available (see text for details) and the corresponding depth range is 3–9 km. The upper right insert shows Fennoscandia with instrumentally located earthquakes for the time period 1951–78, and the location of Meløy is also given. The upper left insert is a composite focal mechanism solution for the Meløy earthquakes, where compressions (solid symbols) and dilatations (open symbols) are plotted in a lower hemisphere stereographic projection. See text for explanation of symbols. The two nodal planes have strikes/dips of 25°/63° E and 250°/35° N, respectively.

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Table 1 The Meløy microearthquake stations with recording time intervals and the respective numbers of recorded events

Station	Code	Start	Stop	Recorded events
Fore	FOR	18.11.78	22.11.78	95
Neverdal	NEV	18.11.78	26.01.79*	7,494
Tømmervik	TOM	19.11.78	23.11.78	34
Kilvik	KIL	21.11.78	27.11.78	175
Enga	ENG	23.11.78	26.01.79*	7,628
Glomfjord	GLO	24.11.78	29.11.78	41
Ørnes	ORN	26.11.78	26.01.79*	2,970

* Station still in operation, March 1979.
The total number of individual events recorded up to 26 January 1979 was 9,954. See Fig. 1 for station locations.

such as on 4 January when 103 microearthquakes were recorded at ENG within an hour with no event magnitude exceeding $ML = 2.0$. The largest number of events recorded in one single day occurred on 2 December with 820 at ENG, 750 at NEV and 270 at ORN.

The hypocentre was located as follows: an initial epicentre estimate was obtained on the basis of S–P time differences using a maximum likelihood technique[4,5] and then refining this estimate iteratively using absolute P/S arrival times when such data were available. The associated crustal model was the simplest possible, with a P_g velocity of 6.25 km s^{-1} (P_b or P_n were never observed), and a P/S velocity ratio of 1.78. Depth of focus was obtained by looping over a specified number of depths, choosing the one which resulted in the smallest time r.m.s. value. This procedure has proved to be reliable even with unfavourable conditions such as non-symmetric station configuration, few observations and unreliable station timing corrections.

We have computed hypocentres for 255 events evenly distributed in time throughout the recording period. These were all intermediate magnitude events, as the largest ones could not be read reliably because of large deflections (saturations) on the paper recordings. Between three and five stations and between six and 10 phases (S–P relative time counts as one phase) have been used. Out of 87 hypocentral solutions with five or more phases, 85% have r.m.s. values <0.20 s and 45% have r.m.s. values <0.10 s. For the same events the largest semi-axis of the 95% uncertainty ellipse has a length of ~1 km (assuming a reading error of 0.1 s on both P and S), and for the 'poorer' other events usually not more than 2 km. In Fig. 1 66 events with at least five readings (phases), and r.m.s. values <0.15 s are plotted. The epicentres are confined within an area of roughly 10 km N/S, 8 km E/W, with a concentration around 66.81 °N, 13.63° E. In view of the small location uncertainty the epicentral scatter in Fig. 1 is clearly significant, and is not an artefact of recording or data analysis. The computed depths for the 66 events are in the range 3–9 km, with 19 within 3–5 km, 27 within 5–7 and 20 within 7–9 km. Based on the computations of r.m.s. values against depth for each event, we find that the uncertainty in depth is of the same order as for the epicentral coordinates—usually within ±1 km. There is one important exception here, however, when only stations ORN, NEV and ENG are used, there is poorer resolution between shallow events to the NW and deeper events near the centre of the activated area. Finally, as the epicentres are roughly surrounded by stations, possible small errors in the crustal model are considered unlikely to adversely affect the hypocentre locations.

A study of the hypocentral 'time development' shows that essentially the whole $10 \times 8 \times 6$ km^3 volume has been activated for all the time. However, there are indications of some migrations in the earthquake activity, predominantly in a N–S direction. The southernmost and easternmost events have rather shallow depths (3–6 km), while events nearer the centre of the area cover the whole depth range of 3–9 km. The magnitude–frequency relationship for the largest events in the sequence is plotted in Fig. 4, with a non-anomalous slope of 1.13.

We have measured first-motion directions for a large number of events—not an easy task due to a 60 mm min^{-1} time resolution in our seismograms. For KIL, GLO and ORN all readings were unidirectional, while for the four other stations (see Table 1) there was one predominant direction but also observations to the contrary. At this stage it is difficult to decide whether these changes in first-motion directions are caused by either genuine differences in focal mechanisms or in hypocentral locations, or possibly also by reading errors. Notwithstanding these problems we have obtained a composite focal mechanism solution for the Meløy earthquakes as presented in the upper left corner of Fig. 1. The solution is based primarily on four events with locations along a line striking about N 10° E, each one separated by about 2 km. Two of the events were recorded by five stations, one by four stations and one by three stations, while the first-motion directions for the rest of the seven recording sites (▲) are inferred from other events with nearby locations. The solution indicates normal faulting, where the plane striking N 25° E and dipping 63° E is the one which gives the best fit to hypocentral solutions, therefore also being the probable faulting plane.

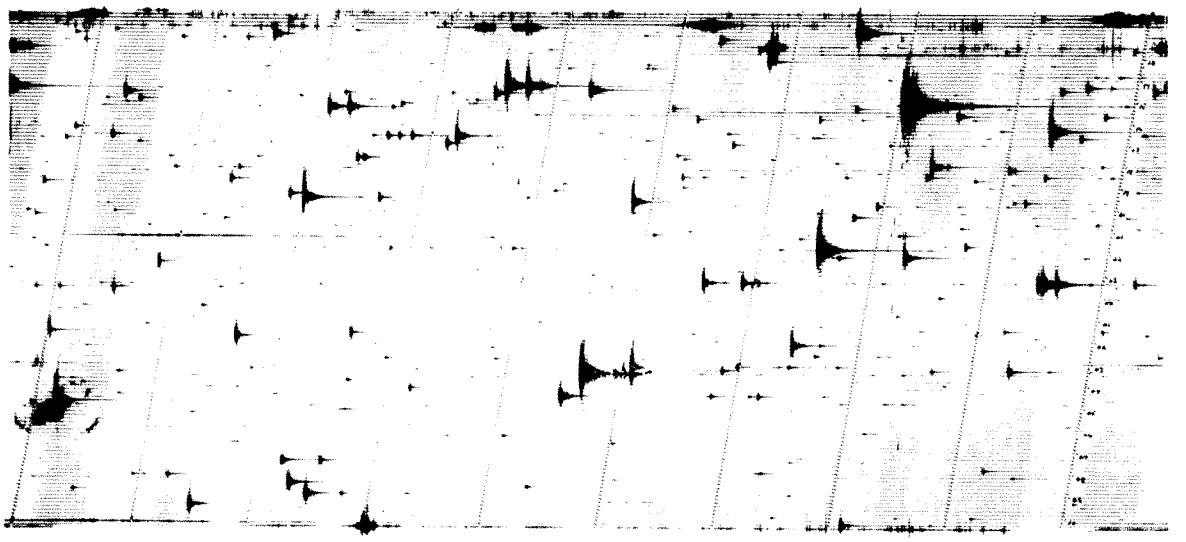


Fig. 2 Reproduction of the seismogram from station ENG covering about 24 h starting at 10.40 GMT on 2 December 1978. The earthquake swarm on the seismogram is an aftershock sequence following a magnitude (*ML*) 2.7 event at 02.04 GMT the same day, about 540 events were then recorded during the next 9 h until the onset of the reproduced seismogram, from which about 400 new events have been identified. The largest of the earthquakes on the seismogram (at 14.57 GMT) had a magnitude of $ML = 2.1$.

Macroseismic observations

Newspaper ads, instead of conventional macroseismic questionnaires[7], published during the main earthquake activity in the Meløy area (about 2,000 households along the shore lines in Fig. 1), one on 18 November covering the activity up to then and one on 30 November covering the large events on 28 and 29 November, produced 33 and 63 answers, respectively, from which we have derived a maximum radius of perception of 50 km and a maximum intensity of VI for the largest earthquakes in the sequence. This intensity was assigned to 15 of the 96 answers, all of them <10 km from the focus. In 10 of these cases damages were reported on houses, such as minor cracks in walls and chimneys.

Most of the events above *ML* = 2.0 were felt, and many more were heard. The sounds reported can be classified in three groups: (1) sounds without feeling tremor (on one occasion this was reported as a more or less continuous phenomenon over a period of several hours); (2) sounds associated with tremors, that is, simultaneous with P/S body wave arrivals; and (3) sounds associated with propagation of pressure waves through the air and from the epicentral area. The latter sounds (described as sounding like a load of snow falling off a roof) were quite different from group (2) and for the largest of the earthquakes in the sequence (28 November, *ML* = 3.2) these sounds were also observed/heard (with sufficient timing accuracy) by one of us (B.K.H.).

Discussion

The Meløy earthquake sequence is an outstanding example of intraplate seismic activity[8]. Earthquake swarm activity is not an exceptional geophysical phenomenon, as this type of process is frequently observed in volcanic areas[9,10] and also at interplate boundaries like the San Andreas fault[11]. We have not, however, been able to find a clear published counterpart to the Meløy sequence, though there are some similarities to earthquake activities reported in the Mississippi valley[12], Caucasus[13] and the Garm[14] areas.

The seismicity map for Fennoscandia in Fig. 1 shows that, although the area is not very active on the global scale, it is still far from being aseismic. Moreover, Meløy is located in the middle of a distinct seismicity zone along the coastal area between 65° and 70° N. Within this zone one of the largest events in Fennoscandia so far took place in 1819, the so-called Lurøy earthquake, with a presumed location of about 50 km south-west of Meløy. Its magnitude is estimated to 6.0–6.5m_b units, and was followed by an extensive aftershock sequence. Historical records, however, give no indications of foreshock activity.

Further west of the Meløy area (and south-west of the Lofoten Islands) there is another seismicity zone[2] which incidentally coincides with a passive continental margin (escarpment) dating back to the opening of the Norwegian Sea some 58 Myr ago[15]. The Lofoten area where the oldest rock ages are of the order of 3,500 Myr are generally considered a larger Baltic Shield fragment left mainly undeformed during the Caledonian orogeny[16], resulting in the closing of a proto-Atlantic ocean some 400 Myr BP. The seas between the two seismicity zones discussed above are part of an epicontinental basin with maximum sedimentary thicknesses of the order of 8–9 km (ref. 17). In the northern Vestfjorden sub-basin seismic profiling surveys have given clear evidence of block faulting[18]. Sometimes a causal relationship has been suggested between the Norwegian Sea opening and the contemporaneous early Eocene uplift of western Fennoscandia which amounted to a maximum of 2 km. This uplift was strongest in the central parts of Norway, tapering off rapidly westwards and gently eastwards.

A spectacular neotectonic feature is found some 200 km to the north-east in Swedish Lappland, namely, the so-called post-glacial Pärve fault[19]. The length of this fault, which is 'facing' westwards, is approximately 150 km and the maximum height is 25–30 m. Another neotectonic feature is the presumed glacial rebound of Fennoscandia, where a controversial suggestion is that this rebound has a significant tectonic component[20].

The main problem is how to fit the Meløy intraplate earthquake sequence into the tectonic framework of the area[21]. A strike direction of 20°–30° (as inferred from the epicentre distribution as well as the focal mechanism solution) is coincident with both the Caledonian folding axis, the sedimentary basin axes and that of the Pärve fault. Second, the normal faulting with an eastward dipping angle of 63° seems to rule out a causal connection both to the Eocene uplift and the present glacial rebound, having positive gradients for onshore areas. Note that the present uplift with its centre in the Bothnian Bay does not seem to correlate with Fennoscandian seismicity as discussed recently by Husebye *et al.*[2]; nor do we see a direct relationship to the off-coast sedimentary basin block tectonics

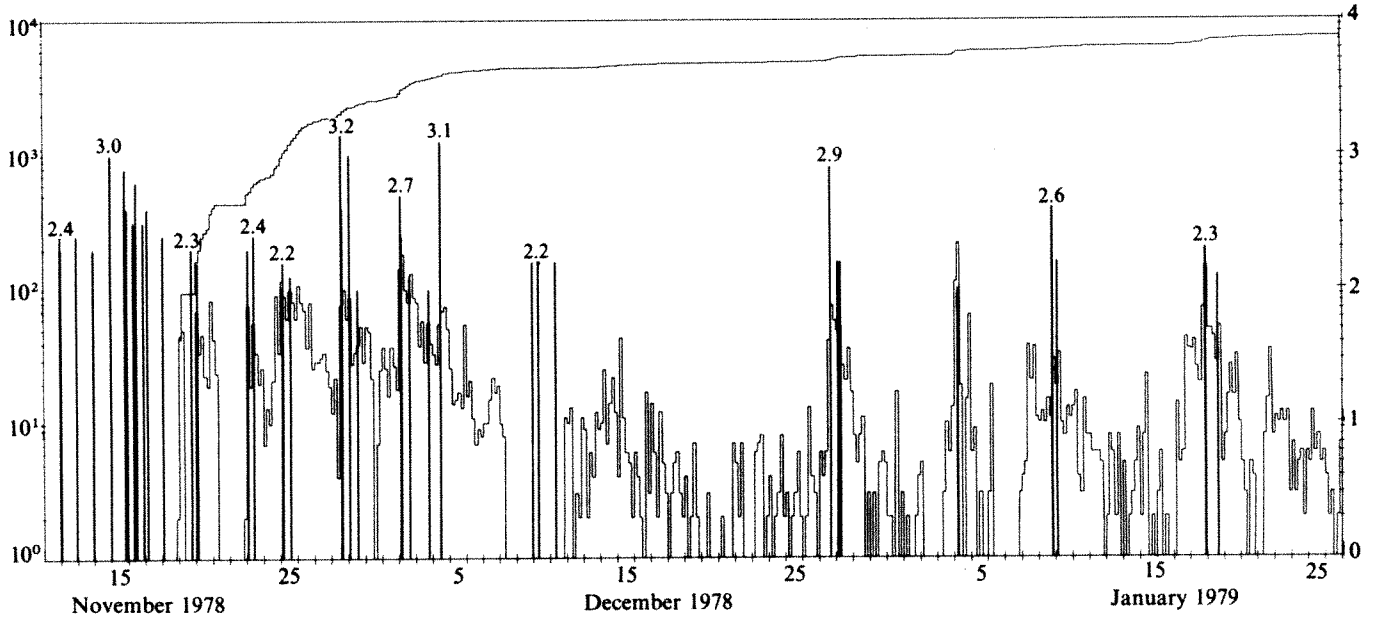


Fig. 3 Time development of the Meløy earthquake sequence as recorded at station NEV (see Fig. 1). The histogram shows the number of events on a 4-hourly basis (non-operational gaps on 21–22 November, 8–11 December 1978 and 2–3 January 1979), including the cumulative number of events and with reference to the left side scale. All events with an *ML* magnitude at 2.0 or above are plotted separately as vertical lines as referenced to the scale on the right side. For some of these larger earthquakes the magnitude is also given directly.

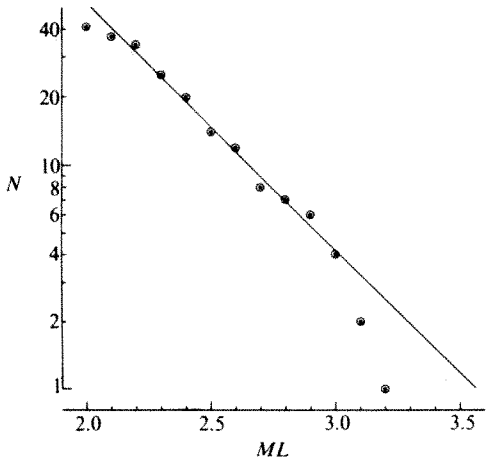


Fig. 4 Cumulative magnitude–frequency distribution for the largest events of the Meløy sequence (plotted individually in Fig. 3). The straight line is fitted by eye and satisfies the Gutenberg–Richter[6] recurrence formula ($\log N = a - b \cdot ML$) with a slope $b = 1.13$.

where the fault walls nearest to the coast face westward. However, a more subtle relationship to the off-coast sedimentary basins is possible if recent hypotheses on their formation are approximately valid[22]. The arguments here are as follows: after an initial rifting/graben formation process the sedimentary depository potential of the area in question is maintained due to continuous subsidence of the area as part of an isostatic compensation mechanism on a lithosphere whose loading response is either elastic or visco-elastic. Such a process could be associated with an 'overshoot' effect on the flanks, and in our particular case normal faulting facing eastwards. Another very generalised explanation is that in Meløy we have an exceptional example of release of remnant stresses associated with the Caledonian orogeny.

One active seismological research area is that of reanalysis of large earthquakes to investigate whether there was evidence of a precursory phenomena. In the case of Meløy the problems are somewhat different, as earthquake activity of the type reported here intuitively will be suspected as being precursory for a significantly larger event. However, in view of the exceptional nature of this intraplate earthquake sequence, it is difficult to argue convincingly either for or against such a hypothesis. If the magnitude–frequency relationship (see Fig. 4) should be used in a predictive context, we would expect a few somewhat larger earthquakes ($ML \sim 3.4$–3.6) to occur in the Meløy area. Also most processes in nature are multiplicative (exhibit a log normal distribution) so we would expect that a few events should be significantly larger than others.

We know from historical records that the general Meløy area has the potential of large earthquakes[2]. We have no firm evidence, however, of whether or not the present microearthquake activity is precursory to somewhat larger earthquake(s) to take place there in the near future. The large Lurøy earthquake of 1819 is not considered conclusive in this context as the lack of foreshocks in this case may simply reflect poor reporting practices at that time. The Meløy earthquake sequence is not only very interesting but is also puzzling, and hopefully the planned close monitoring of the area in the time to come will shed some further light on the still remaining problems.

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Recognition and initiation site for four late promoters of phage T7 is a 22-base pair DNA sequence

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T7 late promoters have a 22-base pair sequence in common. The 22 base pairs are necessary and sufficient for recognition and initiation by T7 RNA polymerase.

T7 RNA POLYMERASE, the product of viral gene 1, is a single subunit protein of molecular weight 107,000 (refs 1, 2). This enzyme exhibits almost absolute specificity for the transcription of the late T7 genes, both *in vivo* and *in vitro*[3,4]. The early promoters for the *Escherichia coli* RNA polymerase are not recognised by the T7 enzyme and even the genes of the closely related phage T3 are transcribed only poorly[5].

Transcription of intact T7 DNA with purified T7 RNA polymerase produces seven discrete transcripts initiated from promoter sites located in the 45–95% region[3,4]. Additional promoter sites at 14.2, 14.6, 18.9, 22.6, 27.9 and, possibly, at

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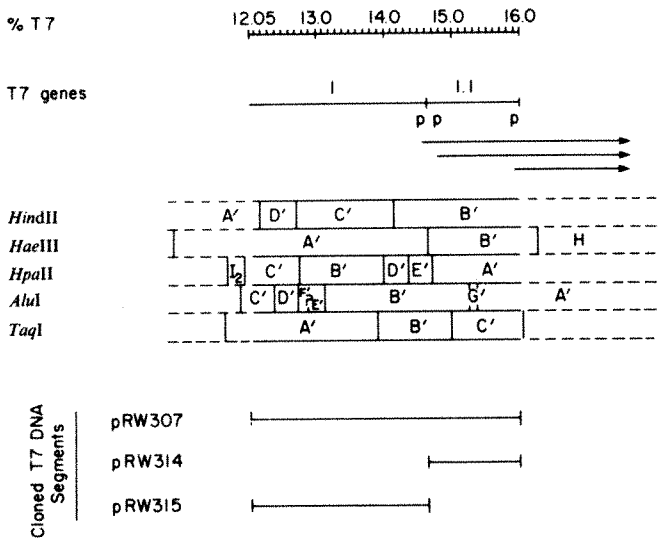


Fig. 1 Genetic and restriction map of a T7 DNA segment cloned in pRW307 (ref. 12). The restriction fragments inserted in the recombinant plasmids pRW314 and pRW315 are also indicated. The exact boundaries of the insertion in pRW307 have been determined with respect to the T7 map at 12.05% (less than 20 base pairs to the right of a *Hpa*I site at 12.03%[12]) and at 16.00% (in the middle of the *Hae*III site, as determined herein). The positions of the late promoters in this region at 14.6, 14.8 and 15.9% are indicated (p) and were identified in previous studies[8,11]. The horizontal arrows point in the direction of transcription from each promoter. The restriction fragments of the vector which flank the insertion in pRW307 are designated by unprimed letters. The junctions between the solid and dashed lines represent the boundaries of the insertion. A more detailed map is presented elsewhere[11,12].

13.5% have been recognised from transcription studies on T7 DNA restriction fragments[6,7]. The sequence of two restriction fragments containing the promoters at 14.6 and ~70% has been reported[6,8].

Recently, the presence of late promoters in cloned segments of the 12.05–18.19% region was investigated[9–11]. The several hundred-fold stimulation of T7 RNA synthesis by some of the recombinant plasmids and the lack of stimulation by others narrowed the possible location of late promoters to three small regions[10,11]. Subsequent determination of the number and size of the late transcripts synthesised off a variety of restriction fragments allowed the localisation of three late promoters inside these regions, at 14.6, 14.8 and 15.9% (ref. 11). These studies also demonstrated that late promoters were not located at 13.5 and 14.2%, in contrast with previous reports[6,7].

This article describes the sequence and template properties of two restriction fragments containing the promoters at 14.8 and 15.9%.

Isolation and sequencing of cloned promoter fragments

The T7 RNA polymerase promoters at 14.8 and 15.9% are located within *Hae*III fragment B' which spans the 14.65–16.00% region cloned in pRW307 (refs 1, 2: Fig. 1). The promoter at 14.8% is also contained within a 112 base pair *Hpa*II–*Taq*I fragment, whereas the promoter at 15.9% is contained within a 260 base pair *Alu*I–*Taq*I fragment (Fig. 1). These smaller fragments were isolated and sequenced as described in Fig. 2. Autoradiograms of typical sequencing gels of the 112 and 260 base pair fragments are shown in Fig. 2*a*, *b*, and Fig. 2*c*, *d*, respectively. The complete sequence of the 112 base pair fragment is shown in Fig. 3*a*. The first 85 base pairs from the *Taq*I site are shown for the 260 base pair fragment in Fig. 3*b*.

On the basis of the restriction mapping data obtained for the 12.05–18.19% region of T7 DNA[11,12], the sequence of the 260 base pair fragment shown in Fig. 3*b* was expected to span the region 15.8–16.0% of T7 DNA and extend past the insertion–vector junction in pRW307. (The exact end of the insertion was not known because the T7 DNA fragment had suffered a deletion during cloning[12].) The location of this junction was deduced from a comparison of the sequence in Fig. 3*b* with the published sequence of a 29-nucleotide RNA fragment transcribed from the 16.0% region of T7 DNA[13]. The sequence of the first 22 nucleotides from the 5' end of this RNA was identical with the sequence between nucleotides 50–71 of Fig. 3*b*. In contrast, the homology was lost over the next seven nucleotides. This abrupt loss of homology indicated that position 71 corresponded to the last base pair of the T7 DNA insertion before the vector junction in pRW307. The deletion of T7 DNA beyond nucleotide 71 caused the loss of the right half of a *Hae*III site (GG ↓ CC) that is evident in the sequence of the RNA and has been mapped by F. W. Studier (personal communication) at 16.00% of the T7 genome. Thus, the restriction map of T7 DNA and the homology with the sequence of the short RNA are both consistent with the assignment of the end of the insertion at 16.00%.

The T7 fragment in pRW307 was inserted into the *Hpa*I site of the pRT29 vector[12]. The sequence of the vector-half of this site corresponds to nucleotides 72–74, indicating that the vector remained intact at this site, in agreement with the conclusions drawn from the restriction analysis data[12].

A recognition–initiation site for T7 RNA polymerase

Our previous studies have shown that two late promoters are located at 14.8 and 15.9% of the T7 genome[11], that is, within the sequences of the 112 and 260 base pair fragments shown in Fig. 3. Measurements of the lengths of the transcripts initiated at these promoters and terminated at various restriction sites downstream, placed the initiation points at ~93 and ~35 nucleotides to the left (upstream) of the *Taq*I sites of the 112 and 260 base pair fragments, respectively[11]. The initiation points for these transcripts were more accurately determined from the data shown in Fig. 5 (lane *a*). The lengths of the transcripts shown in this figure were determined to be 93±4 and 47±2 nucleotides. These sizes place the initiation point at a corresponding number of base pairs upstream from the *Taq*I sites, within positions 16–24 of the 112 base pair fragment (Fig. 3*a*) and positions 37–41 of the 260 base pair fragment (Fig. 3*b*).

Inspection of the sequences around these two regions immediately revealed the presence of a nearly identical sequence of 23 contiguous nucleotides TAATACGACTCACTATAGGPuPuNA between positions 5–27 and 22–44 in Fig. 3*a* and *b*, respectively.

Comparison of this sequence with the published sequences of the promoters at 14.6 and ~70% (refs 6, 8) revealed almost complete homology, for the region around and immediately upstream from the transcription initiation site (Fig. 4). On the basis of these four structures, the following DNA sequence is proposed for the recognition–initiation site for T7 RNA polymerase:

5' AATACGACTCACTATAGPuPuPuNA 3'

In proposing this sequence, only nucleotides identical in all four promoters were considered, with the following two exceptions: (1) Position −5 of the promoter at 15.9% is a G rather than a C. We have assumed this transversion to be a feature of this particular promoter, and believe it to be responsible for the approximately fivefold lower activity observed relative to the promoters at 14.6 and 14.8% (ref. 11). (2) Position −16 of the promoter at ~70% reads C rather than A. Because the other three sequences have an A at this position, we have assumed that the A→C transversion is a deviation from the general structure. It may be of interest to determine the strength of this promoter relative to the others.

For consistency, we have chosen to include in the general structure the adenosine at position +6, although the continuity of the homology is interrupted at position +5. The preservation

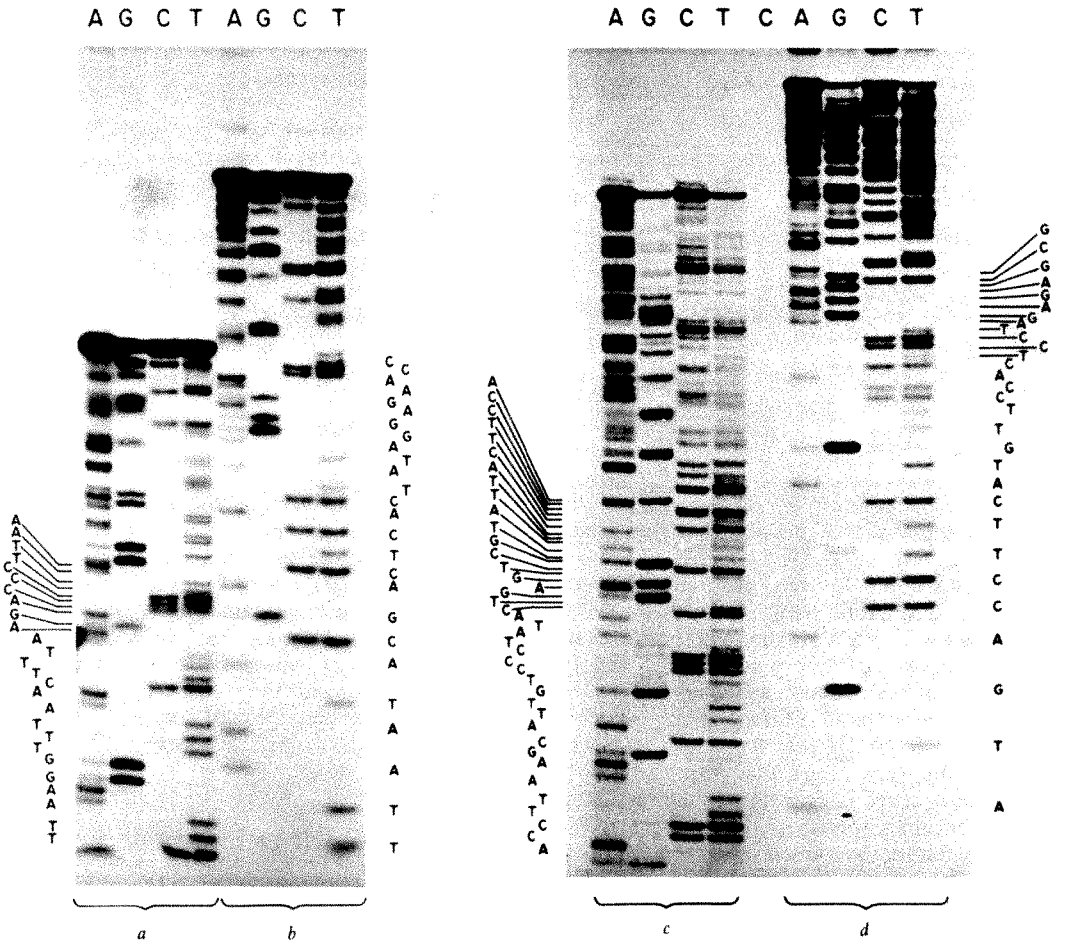


Fig. 2 Autoradiograms of sequencing gels of two promoter-containing fragments of T7 DNA cloned in pRW307. *Hae*III fragment B′ (Fig. 1) was purified from a *Hae*III + *Eco*RI digest of pRW307 by RPC-5 column chromatography[14]. This fragment was digested with *Hpa*II, labelled at the 5′ ends with T4 polynucleotide kinase and redigested with *Taq*I. These reactions produced a 23 base pair *Hae*III–*Hpa*II fragment, a 112 base pair *Hpa*II–*Taq*I fragment containing the promoter at 14.8%, a 409 base pair *Taq*I fragment C′ and a 99 base pair *Taq*I–*Hae*III fragment. These fragments were fractionated on 8% polyacrylamide gels and visualised by autoradiography and ethidium staining. The 112 base pair fragment labelled at the 5′ end of the *Hpa*II site was recovered from the gel and sequenced. Lanes *a* and *b* show an autoradiogram of a typical sequencing gel for this fragment. The complete sequence of the *Hpa*II–*Taq*I fragment which spans the region 14.70–15.00% of T7 DNA is shown in Fig. 3*a*. The promoter at 15.9% is contained within a 260 base pair *Alu*I–*Taq*I fragment which was isolated and sequenced as follows. The purified *Hae*III fragment B′ was digested with *Taq*I, labelled at the 5′ ends with T4 polynucleotide kinase and redigested with *Alu*I. These reactions produced a 135 base pair *Hae*III–*Taq*I fragment, a 104 base pair *Taq*I–*Alu*I fragment a 45 base pair *Alu*I fragment G′, a 260 base pair *Alu*I–*Taq*I fragment containing the promoter at 15.9%, and a 99 base pair *Taq*I–*Hae*III fragment. These fragments were fractionated on 8% polyacrylamide gels and visualised by autoradiography and ethidium staining. The 260 base pair fragment (labelled at the *Taq*I site) was eluted from the gel and sequenced. An autoradiogram of a typical sequencing gel for this fragment is shown in lanes *c* and *d*. The sequence of the first 88 nucleotides from the *Taq*I site is shown in Fig. 3*b*. The sources of the enzymes and the procedures for the restriction reactions, end-labelling, gel elution and sequencing gel electrophoresis were based on published procedures[15] and have been described[11,12].

a

Hpa II *Hinf* I *Taq* I

```
5' CGGTTAATACGACTCACTATAGGAGAACCTTAAGGTTTAACTTTAAGACCCTTAAGTGTTAATTAGAGATTTAATTAAGAATTACAAGAGAGGACTTTAAGTATGCGTAACT
   CAATTATGCTGAGTGATATCCTCTTGGAATTCCAAATTGAAATTCTGGGAATTCACAATTAATCTCTAAATTAATTCTTAATGTTCTCTCCTGAAATTCATACGCATTGAGC 5'
         10        20        30        40        50        60        70        80        90       100       110
14.70 %                                    % T7                                                          15.00 %
```

b

Hinf I *Taq* I

```
5' GACCAGTTGAAGGACTGGAAGTAATACGACTCAGTATAGGGACAATGCTTAAGGTCGCTCTCTAGGAGTGGAACATGAAGGTCAT
   CTGGTCAACTTCCTGACCTTCATTATGCTGAGTCATATCCCTGTTACGAATTCCAGCGAGAGATCCTCACCTTGTACTTCCAGTAGC 5'
         10        20        30        40        50        60        70        80
                                                                     | vector
15.82 %                                    % T7                    16.00 %
```

Fig. 3 DNA sequence of promoter-containing fragments. *a*, Nucleotide sequence of the 112 base pair *Hpa*II–*Taq*I fragment spanning the region 14.70–15.00% of T7 DNA. This fragment was isolated from a larger cloned segment of T7 DNA and sequenced as described in the legend to Fig. 2. *b*, Nucleotide sequence of part of the 260 base pair *Alu*I–*Taq*I fragment showing the region 15.82–16.00% of T7 DNA and 17 base pair of vector DNA at the *Taq*I end. This fragment was isolated from a longer, cloned segment of T7 DNA and sequenced as described in Fig. 2. Positions 70–71 correspond to the vector–insertion junction at 16.00% of T7 DNA. Positions 69–73 cover half of the *Hae*III site of T7 and half of the *Hpa*I site of the vector (see text).

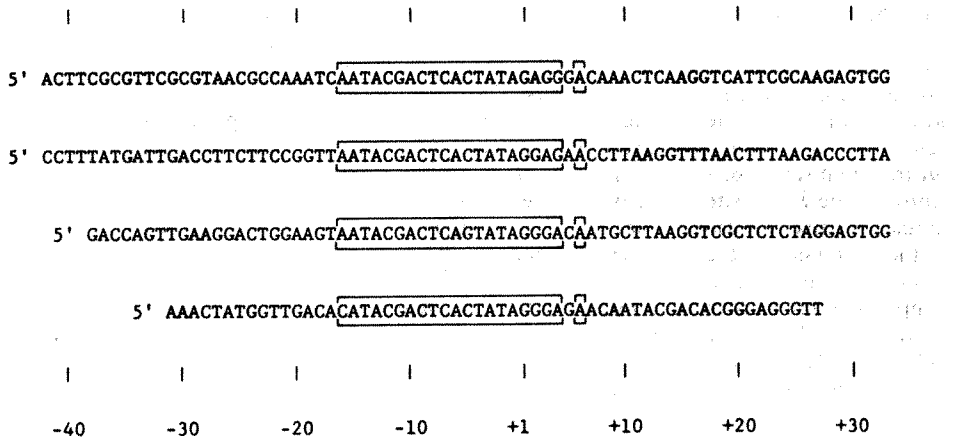
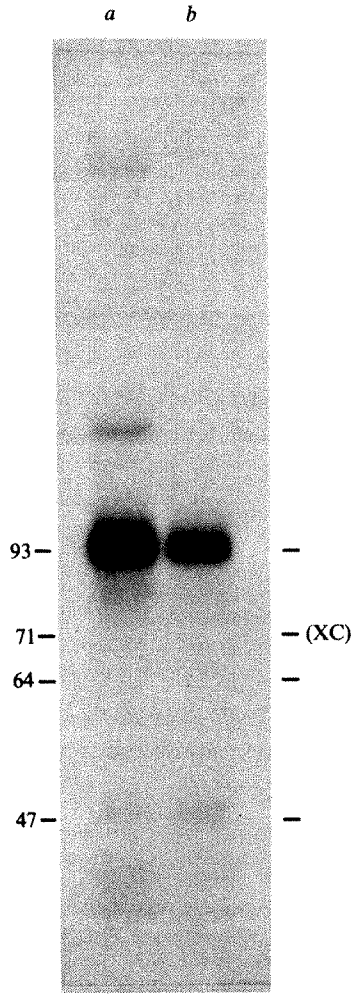


Fig. 4 DNA sequence around the T7 late promoters located at 14.6 (ref. 6), 14.8 (this report), 15.9 (this report) and ~70% (ref. 8) of the genome. The highly preserved sequence which constitutes the recognition–initiation site is indicated by the solid lines. Positions are marked from the initiation point at +1. The complete DNA sequence between the promoters at 14.6 and 14.8% is shown on the top two lines, interrupted at the *Hae*III site (at 14.65%) for clarity.

of adenosine at position +6 may not be fortuitous but may be due to an important contact of the polymerase with this nucleotide.

The DNA region between positions −16 and +6 around the transcription initiation point is proposed to constitute a recognition site for T7 RNA polymerase because of its highly preserved sequence in the four promoters examined thus far. Random occurrence of the common sequence in T7 DNA is extremely unlikely. (The probability for a 16 base pair sequence to occur 4 times in T7 DNA (40,000 base pairs) is 8×10^{-16} (W. Fitch, personal communication). The probability for the 22 base pair common sequence is even lower but calculation of the exact number becomes complicated by the possible variation in the PuPuPuN part of the sequence.) However, this region might not contain all pertinent structural elements. If nucleotides outside the common sequence also contributed structural information necessary for recognition, their positions should also have been preserved among the four sequences. Inspection of the nucleotides outside the common sequence (Fig. 4) shows that the same nucleotide does not appear at a given position more than twice, with the exception of the adenosine at −19 and the thymidine at +21, which appear three times. This complete lack of homology outside the −16 to +6 region, as opposed to the almost complete preservation of homology inside, suggests that positions −16 to +6 constitute a necessary and sufficient recognition–initiation site.



Further identification of the promoter

Direct evidence to support the previous conclusion comes from the results of cloning experiments. In pRW315, the T7 DNA region beyond the *Hae*III site at position +33 of the 14.6% promoter was substituted by vector DNA. In pRW314, the T7 DNA region ahead of the *Hae*III site at position −42 of the 14.8% promoter was also substituted by vector DNA (Figs 1 and 4). Despite the substitution of the natural viral sequences by vector DNA, the activity of these promoters in pRW314 and pRW315 was not significantly reduced relative to their activity in pRW307 (ref. 11).

Similar conclusions were reached when purified *Hae*III fragments A′ and B′ were assayed[11]. In both cases, discrete transcripts were obtained at comparable yields from the promoters at 14.6 and 14.8%, indicating that the absence of DNA upstream from position −42 and downstream from position +33 from the initiation point was not required by T7 RNA polymerase.

Fig. 5 Autoradiogram of ^{32}P-labelled transcripts fractionated on a 8% polyacrylamide–7 M urea gel. RNA was synthesised with highly purified T7 RNA polymerase (0.15 μg of fraction V; the gift of G. Kassavetis and M. Chamberlin[7]) as described previously[11]; 0.75 μg of either *Hae*III fragment B′ digested with *Taq*I (lane *a*) or *Hae*III fragment B′ digested with *Taq*I + *Hpa*II (lane *b*) was used as the template. The heaviest bands in both lanes *a* and *b* correspond to 93 ± 4 base transcripts[11] initiated at the 14.8% promoter and terminated at the *Taq*I site. The weak bands near the bottom of the gel correspond to 47 ± 2 base long transcripts initiated at the 15.9% promoter and terminated at a *Taq*I site. The ~5 × weaker expression of the 15.9% promoter relative to the 14.8% promoter (as judged by band intensity) is consistent with previous observations[11] and is attributed to a variation in the structure of the 15.9% promoter (see text). The weak band in the upper half of the gel in lane *a* apparently represents an end-to-end transcript synthesised off the 135 base pair *Hae*III-*Taq*I fragment. When this fragment is cleaved by *Hpa*II 23 base pairs from the *Hae*III site upstream from the 14.8% promoter, this band disappears (lane *b*). A 64 base transcript synthesised by *E. coli* RNA polymerase off a 203 base pair *Hae*III fragment containing the *lac* UV5 promoter was also used as a marker[20]. Its position, as well as the position of xylene cyanol which migrates as a 71-base long transcript on this gel, is indicated.

Further evidence on the boundaries of the sequence that defines a T7 late promoter was obtained from transcription studies (Fig. 5) of the 112 base pair *Hpa*II–*Taq*I fragment (at 14.70–15.00% in Fig. 1) in which the DNA sequence ends just 4 base pairs upstream from the recognition site (Fig. 3*a*). The activity of the 14.8% promoter in this fragment was determined relative to the 135 base pair *Hae*III–*Taq*I fragment (at 14.65–15.00%) in which the T7 DNA extends through the *Hpa*II site upstream to the *Hae*III site (Fig. 1). Each reaction mixture also contained *Taq*I fragment C' (Fig. 1); thus, the expression of the 15.9% promoter in the latter fragment served as an internal control. In identical assay conditions, comparable amounts of discrete transcripts (~93 bases long) were synthesised from the 14.8% promoter in either the *Hpa*II–*Taq*I or the *Hae*III–*Taq*I fragments. In both cases, the 47-base transcript from the 15.9% promoter control was expressed to a similar extent. This result demonstrated that elimination of the DNA as close as 4 base pairs upstream from the common promoter sequence had no effect on the efficiency or selectivity of transcription.

In summary, the lack of homology outside the 22 base pair common sequence indicates that the neighbouring DNA structure did not contribute directly to recognition. The results of experiments in which the DNA upstream or downstream from the common sequence was either replaced by foreign DNA or removed are consistent with this interpretation. By these criteria, the 22 base pair contiguous sequence constitutes a fully defined recognition–initiation site for T7 RNA polymerase.

Comparison with other promoters

The sequence of the recognition site contains very little two-fold symmetry. Only the 6 base pair sequence between positions −5 and +1 is symmetrical and additional symmetry around the same axis depends on the particular nature of the nucleotides in the –PuPuPuN– part of the sequence. In this regard, the 14.6% promoter contains much more symmetry than the 14.8% promoter, but this feature does not seem to affect the relative expression of these two promoters. The lack of extensive two-fold symmetry is also observed with bacterial promoters[16].

In promoters for *E. coli* RNA polymerase, a 7 base pair site of partially preserved sequence (the 'Pribnow box') is located 7 base pairs upstream from the transcription initiation point[17]. A second region of partially preserved sequence also exists approximately 30 base pairs further upstream and mutations in this region affect promoter function[16,18]. In contrast, T7 promoters consist of one contiguous recognition site, which includes the initiation point and no other sequence further upstream, seems to be required for complete function. In this respect, T7 promoters are quite distinct from their bacterial counterparts. The physical separation of the two recognition sites and the initiation site in the *E. coli* promoters, as opposed to the condensation of these elements into one contiguous site in T7 promoters, may reflect the size difference between the two enzymes (the *E. coli* polymerase is about five times larger than the T7 RNA polymerase).

The possibility has been raised that bacterial promoters may be located at easily denatured, A+T-rich regions of the DNA template[19]. Such a correlation was also proposed for T7 late promoters[5]. The unusually high (69%) A+T content of the 112 base pair fragment containing the promoter at 14.8% agrees with this model. However, neither the promoter at 14.6% nor the promoter at 15.9% are located in unusually A+T rich regions. (A+T = 52% for both *Hpa*II fragment E' and the 71 base pair T7 segment around 15.9% in Fig. 3*b*.) Thus, A+T richness does not seem to be a general property of T7 promoter sites. The unusually high A+T content of the 14.7–15.00% region may be important for some other regulatory site in the vicinity of this region, such as the origin of T7 DNA replication[12].

Noted added in proof: M. Rosa[21] has recently reported that four class *3* T7 late promoters share the same sequence.

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letters

On the detection of large-scale inhomogeneities in the Universe

THE detection of large-scale inhomogeneities in the Universe has proceeded along a number of fronts. The observed spatial distribution of galaxies indicates clumping of matter on length scales up to ~30 Mpc (ref. 1). Limits on fine-scale temperature fluctuations in the microwave background[2] constrain mass concentrations at the epoch of recombination, and later[3,4]. Neither of these methods is particularly sensitive to large-scale low-density contrast ($\delta\rho/\rho \ll 1$), perturbations at the current epoch (that is, masses $\gg 10^{19} M_\odot$ on a scale $\geq$1,000 Mpc), although in principle the distribution of discrete radio sources on the sky may be investigated for such effects (see for example ref. 5). We show here that measurements of the isotropy of the X-ray background provide a sensitive means of detecting such large-scale inhomogeneities within $z \sim 5$.

A matter perturbation, with excess mass δM, retards the motion of our Galaxy relative to the overall expansion of the Universe with a peculiar velocity δv_G given, from energy considerations, by

$$v_G \delta v_G \simeq \frac{G\delta M}{r} \qquad (1)$$

Here the perturbation is at a distance r from our Galaxy and v_G is the Hubble flow velocity over that distance. We then obtain

$$\delta v_G \simeq \frac{1}{2}\left(\frac{\delta\rho}{\rho}\right)\Omega H_0 \frac{l^3}{r^2} \qquad (2)$$

where Ω is the cosmological density parameter ($8\pi G\rho/3H_0^2$),

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$\delta\rho$ is the excess matter density and l is the size of the perturbation.

Other cosmological effects are small, and can be neglected, provided that $(\delta\rho/\rho) \ll 1$ and r corresponds to a redshift $\leqslant 1$. The peculiar velocity of the Galaxy then induces a 24-h variation[6] in the microwave background through the Compton–Getting effect;

$$I = (2-\alpha)\left(1+\frac{\delta v_G}{c}\cos\theta\right)I_0 \qquad (3)$$

where θ is measured from the direction towards δM, and α is the photon number spectral index of the radiation. ($\alpha = 1$ for the Rayleigh–Jeans part of the microwave spectrum and ~ -1.4 for the X-ray spectrum; $2 < E < 20$ keV.) Twenty-four-hour anisotropy in the microwave background, implying a peculiar velocity of 600 km s^{-1}, has been reported by Smoot *et al.*[7] after correcting for the rotation of the Galaxy.

The microwave background originates at $z \sim 10^3$, whereas the X-ray background probably originates from within $z \leqslant 3$. Only weak evolution of the known classes of X-ray source[8] (particularly quasars and active galaxies) is needed to explain the observed intensity. If $r \ll cH_0^{-1}$ (that is if the redshift of the matter perturbation $z_M \ll 1$), then a similar 24-h variation may be expected in both the X-ray and microwave backgrounds. On the other hand, if $r \sim cH_0^{-1}$, δM generates large-scale shear across the Universe out to $z \sim 1$ and the X-ray background varies in a significantly different manner. A 12-h variation, peaking at $\theta \sim 90°$, is predicted as shown in Fig. 1. The sources of the X-ray background between ourselves and the mass are decelerating more rapidly than we are and are thus seen to be receding. In the opposite direction we are decelerating more than the background, which also gives a recession. Only in intermediate directions is our velocity and that of the background comparable. The 12-h effect reduces if r is much greater than cH_0^{-1}. The peculiar velocity δv_r of the Galaxy relative to a shell of X-ray emitting matter at distance d is given, from (2) as

$$\delta v_r \simeq \delta_{vG}\left(\cos\theta + \frac{(d/r - \cos\theta)}{(1+(d/r)^2 - 2(d/r)\cos\theta)^{3/2}}\right) \qquad (4)$$

This gives a 12-h variation through equation (3) with $\delta v_G \cos\theta$ replaced by δv_r. The overall effect on the X-ray background may be further complicated by any X-ray emission from δM itself, and by the rotation of the Galaxy together with any other local peculiar velocities.

An oblate 12-h variation in the X-ray background can thus, in principle, measure large-scale shear over distances out to $z \sim 1$. The net differential velocities between different directions need not exceed more than a few hundred km s^{-1} before giving a detectable X-ray signal. This is a consequence of the fact that the X-ray background provides an absolute measurement of integrated quantities out to $z \sim 1$. This has been noted by Wolfe[9] in constraining world models with shear using the 1% limit to 12- and 24-h variations in the X-ray background placed by Schwartz[10]. The Leicester Sky Survey Instrument on Ariel V can detect such variations at the level of ~0.5% (ref. 11).

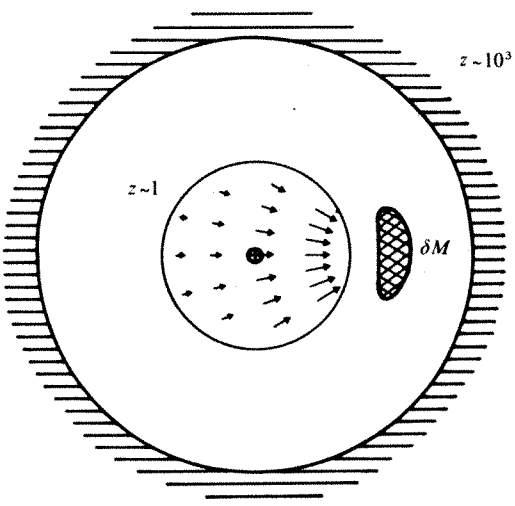


Fig. 1 Schematic representation of tidal gravitational influence of density inhomogeneity, mass δM, on the velocity shear of the X-ray background.

The 12-h isotropy of the X-ray background is most sensitive to masses situated at $z_M \sim 1$;

$$\delta M \simeq 10^{20} z_M^2 H_{50}^{-1} \delta v_6 \qquad M_\odot$$

with size

$$l \simeq 1{,}000\left(\frac{\delta\rho}{\rho}\right)^{-1/3} z_M^{2/3} H_{50}^{-1} \delta v_6^{1/3} \Omega^{-1/3} \qquad \text{Mpc}$$

where $\delta v_G = 600\,\delta v_6$ km s^{-1}, $H_0 = 50 H_{50}$ km s^{-1} Mpc^{-1}.

A large-scale density inhomogeneity, with $(\delta\rho/\rho) \sim 0.01$ now, corresponds to $(\delta\rho/\rho) \sim 10^{-5}$ at the epoch of recombination. We conclude that the combination of microwave and X-ray background anisotropy observations will enable us to study the very large scale structure of the Universe at the current epoch.

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The possibility of accretion onto radio pulsars

AS an explanation for the observed long-term variations in pulsar emission[1–3], an accretion model is proposed here which permits interstellar neutral hydrogen to infiltrate the light-cylinder and, following ionisation, to modulate the pulsed emission. This would suggest that pulars have an eventful passage through the interstellar medium, and do not always remain as pulsars.

The widely accepted picture of a pulsar is that of a magnetised neutron star rotating in a vacuum[4]. The intense electric fields thereby induced are supposed to remove charged particles from the surface near the magnetic poles and accelerate them to high energies[5]. The radiation generated by these particles then interacts with the local magnetic field to produce cascades of electron–positron pairs[6,7], whose emission we observe. The site of the emission is still unclear. Around the light-cylinder the rotating magnetic field produces a strong electromagnetic wave whose pressure prevents the interstellar medium from moving into the magnetosphere. In all emission models based on this general picture it is vital that the region inside the light-cylinder be maintained as a near-vacuum.

Theoretical attention is usually concentrated on explaining emission features which have time scales of the order of the neutron star rotation period or less[6,8] (microstructure[9], for example). Instabilities of the flow between the magnetic poles

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and the light-cylinder are seen as a natural explanation for such short time-scale modulations. It has been observed, however, that both individual and integrated pulse intensities are also strongly modulated on every time scale from hours to years[1,2]. Recently, substantial day-to-day changes in the spectra of nine pulsars have been reported[3]. It is difficult to see how such long-term variability can be achieved by any mechanism whose fundamental time scale is the period of rotation. On the other hand, the shape of the integrated profiles and their polarisation are generally stable[10] (although even here occasional 'mode-changing' occurs[11]). The overall impression is that a basic emission pattern is being modulated by some external influence.

The Solar System was once thought to be insulated from the interstellar medium by the shielding effect of the solar wind[12]. Later work has shown that the Solar System is in fact penetrated by interstellar hydrogen atoms[13]. This is made possible by the relative motion of the Sun and the interstellar medium (about 15 km s^{-1}). One is led to speculate whether pulsars, whose velocities are often well in excess of 100 km s^{-1}, may be subject to such a process.

The radius, R_w, of a pulsar's wave-zone is given by the condition

$$\frac{\dot{E}}{4\pi R_w^2 c} \sim \frac{B_0^2}{8\pi}$$

where $\dot{E}$ is the spin-down energy loss-rate and B_0 is the ambient interstellar magnetic field. Taking $\dot{E} \sim 10^{31}$ erg s^{-1} and $B_0 \sim 5\times10^{-6}$ G, we obtain $R_w \sim 5\times10^{15}$ cm. By comparison, the mean free path of a hydrogen atom with respect to ion collisions is $\lambda \sim 1/(n_i\sigma)$, where n_i is the ion density and σ the collisional cross-section. Taking $n_i \sim 0.03$ cm^{-3} and $\sigma \sim 3\times10^{-15}$ cm^2 we obtain[14,17] $\lambda \sim 10^{16}$ cm. Thus $\lambda > R_w$ at least for weaker (low period change $\dot{P}$) pulsars and, although ions will be deflected by the strong wave, neutral hydrogen atoms can pass insensibly through the interface between the interstellar medium and the wave and move on hyperbolic orbits towards the neutron star.

The highly energetic particles emitted by the pulsar present no threat to the oncoming atoms, both because of their extremely low cross-sections ($\sim10^{-18}$ cm^2) and low density ($\ll 1$ cm^{-3}) outside the light-cylinder. But thermal UV radiation from the star's surface may be more dangerous. It can be shown that an atom travelling at 100 km s^{-1} will survive against photoionisation until it enters the light-cylinder radius ($\sim5\times10^9$ cm) only if the surface temperature has fallen below 10^5 K. On favourable assumptions, this temperature may be reached within 0.1 Myr of the formation of the neutron star[15]. Similar conclusions have been reached independently by Tsygan[16].

If an atom is ionised before reaching the light-cylinder, it will be driven out of the magnetosphere by the pulsar's strong wave. But if ionisation takes place within the light-cylinder (through interaction either with the thermal or the beamed radiation) it can play a role similar to that ascribed in some models[6,7] to pair-creation: one of the two resulting charged particles will, if on an open field-line, be pulled to the polar cap by the electric field, whilst the other will be accelerated outwards and expelled. If charged particles can be supplied in sufficient numbers, the electric field parallel to the magnetic field-lines will be 'short-circuited'. This would result in the breakdown of any emission mechanism which requires particles to be removed from the surface.

For a pulsar of period P moving with a velocity V through a medium of density n_H the rate of accretion of neutral particles into the light-cylinder radius, R_L, is $\pi R_L R_A V n_H$, where R_A is the 'accretion radius' of the neutron star defined by $R_A = 2GM/V^2$. This is the appropriate formula when $\lambda \gg R_A$. Taking $M \sim 1.4\,M_\odot$, $V \sim 100\,V_{100}$ km s^{-1}, we obtain the accretion rate

$$\dot{N}_A \sim 5.6\times10^{29}\frac{Pn_H}{V_{100}} \quad \text{s}^{-1}$$

To eliminate the electric field along the magnetic fieldlines we require a charged particle flux of $(\pi R_P^2)(B/\text{Pec})c$, where B is the polar magnetic field strength and R_P the cap radius (whose circumference is defined by the fieldlines which just touch the light-cylinder). With $R_P \sim 1.4\times10^4/P^{1/2}$ cm and $B \sim 10^{12}$ G the necessary particle flux is

$$\dot{N}_p \sim \frac{1.3\times10^{30}}{P^2} \quad \text{s}^{-1}$$

For the typical values $P \sim 1$ s and $n_H \sim 1$ cm^{-3}, $\dot{N}_A$ is a significant fraction of $\dot{N}_P$ and disturbance of the emission pattern can be expected. Comparing the expressions for $\dot{N}_A$ and $\dot{N}_P$, we see that this effect is more likely to be pronounced in high-period, low-velocity pulsars. A further consequence is that fast-moving pulsars (through a lower accretion rate) may actually 'live' longer than slow-moving pulsars.

Long-term variations in emission can be interpreted, through variations in n_H, as the result of inhomogeneities in the interstellar medium. It is therefore possible to regard pulsars as monitors of the detailed structure of the gas in their neighbourhood. Furthermore, one might question the usual anthropomorphic assumption of a single uninterrupted lifespan for each pulsar. It is believed[17] that the Solar System has passed through many neutral hydrogen clouds in the 5×10^3 Myr since its formation. In its much shorter life ($\sim$2 Myr) a fast-moving pulsar will also have led a chequered existence, passing through clouds of various densities. The calculations here suggest that even a 'standard' H I cloud[18] ($n_H \geq 10$ cm^{-3}) can switch a pulsar off. A pulsar moving at 100 km s^{-1} covers 200 pc in 2 Myr. On current estimates[17], 12 pc of this (or 6%) will be spent in clouds with $n_H \geq 10$ cm^{-3}. There are now over 300 known pulsars, so there might be 20 single neutron stars which would otherwise have been detectable as pulsars at present immersed in dense H I regions. Pulsars are probably born in or near OB associations, so they are more likely to be surrounded by gas in their early lives and it may be significant that no pulsar has yet been found in a supernova remnant shell.

Accretion onto neutron stars suggests affinities between the model given here and the so-called X-ray 'bursters' (especially those which have yet to reveal evidence of being in a binary system[19]). These are often of a transient nature, inviting comparison with the 'nulling' phenomenon of radio pulsars, whereby emission ceases abruptly at all wavelengths. The time spent in the null state varies from pulsar to pulsar, but values of over 50% have been given for some pulsars[20]. There is evidence[20] that nulling is more common in long-period pulsars, as would be expected from the above accretion rate estimates ('drifting subpulses'[21], which are often associated with nulling, may therefore also be related to accretion). The time spent in a null sequence might sometimes be related to the energy of the following or preceding stretches of emission (as in MXB1730−335)[19].

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The size of Jupiter's electrically conducting fluid core

WHEN the magnetic field of a planet is due to hydromagnetic dynamo action in an electrically conducting fluid core surrounded by a poorly conducting mantle, Hide's[1] method can, in principle, be used to determine the radius r_c of the core from determinations of secular changes in the magnetic field **B** in the accessible region above the surface of the planet, mean radius r_s ($\geq r_c$). It will be possible to apply this method to Jupiter (and other planets) when magnetic measurements of sufficient accuracy and detail become available. We describe here a preliminary study in which we have analysed the magnetic measurements made in December 1973 and December 1974 when the Pioneer 10 and Pioneer 11 fly-by space-probes encountered Jupiter[2]. We expected that over such a short time interval any true secular changes would be masked by errors and the corresponding estimate of r_c/r_s highly implausible or even physically impossible, but this turns out not to be the case. Taken at their face value the apparent secular changes in the dipole and quadrupole components of Jupiter's magnetic field imply that r_c/r_s is close to 0.7. Somewhat higher values of r_c/r_s are found when contributions from the octupole component are also included.

Jupiter is the largest known planet. It has a mean radius r_s of 6.97×10^7 m, more than 10 times that of the Earth, and a mean density of 1,330 kg m^{-3}, close to that of the Sun and about a quarter of that of the Earth. At about 5×10^{12} Pa (50 Mbar), Jupiter's central pressure is more than 10 times that of the Earth and this ensures that Jupiter's main chemical constituent, hydrogen, is metallic throughout most of the planet. Only above the level $r=r_m$ (r being the distance from the centre of the planet), in the 'mantle' and the 'atmosphere' where the prevailing pressures are less than a certain pressure of a few megabars, does hydrogen take its more familiar molecular form. This transition pressure is not yet known to much better than a factor of two and the corresponding estimates of r_m/r_s given in the recent literature range from 0.7 to 0.8. The planet is generally considered to be fluid throughout[2-4].

The existence of a strong jovian magnetic field of internal origin and nearly dipolar form, with a dipole moment about 10^4 times the magnitude of and opposite in sign to that of the Earth's present field, was first inferred in the 1950s from radio-astronomical observations at decametre and decimetre wavelengths[2]. Important details of the field were obtained by Smith, Davis and Jones, and by Acuña and Ness from *in situ* magnetometer measurements on board the Pioneer 10 and Pioneer 11 space probes[2] and further details are expected from measurements on board Voyager 1 spacecraft, which passed close to Jupiter very recently (1–5 March 1979) and Voyager 2, which will encounter Jupiter later this year (2–9 July).

The only serious suggestion as to the origin of Jupiter's magnetic field closely parallels ideas developed by geophysicists over the past 30 years towards an explanation of the Earth's magnetism[2,5]. Thus, we can suppose that the jovian field is due to ordinary electrical currents circulating in the conducting regions within the planet and that these currents are produced by the hydromagnetic dynamo process, involving inductive interactions between fluid motions and the magnetic field. Whether dynamo action is possible depends *inter alia* on the value of the so-called magnetic Reynolds number

$$R\equiv\sigma\mu LU \qquad (1)$$

where R is the ratio between the time scale of free ohmic decay $\sigma\mu L^2$ and an advective time scale L/U; L is a typical length scale; U is a typical flow speed associated with the fluid motions, and σ and μ are average values of the electrical conductivity and magnetic permeability of the electrically conducting core of outer radius r_c within which dynamo action is hypothesised to occur. Such action is impossible when R is very small for then motional induction cannot overcome the effects of ohmic dissipation, and also when R is infinite as the magnetic flux linkage of a perfect electrical conductor cannot change. The most efficient dynamos occur when R has some optimum value $\bar{R}$ where typical values of $\bar{R}$ range between 10 and 100.

It is tempting to assume that r_c is equal to r_m, the radius of the metallic core, as it would be if the mantle of Jupiter were a very good insulator. But the value of σ required to make $R=\bar{R}$ might be a good deal less than the high values (typically greater than $10^6\ \Omega^{-1}\ m^{-1}$) found in the metallic core, and it is conceivable that impurities and high pressure and temperature render the lower reaches (at least) of the molecular hydrogen mantle sufficiently conducting to support dynamo action there, in which case r_c would be significantly greater than r_m (refs 2, 4, 5).

The new method[1,6] which estimates r_c with a typical error of the order of but greater than $r_c/\bar{R}$, has been applied to the Earth, using the best determination of the geomagnetic secular variation for the period 1955–75 expressed in terms of a spherical harmonic series truncated at degree $n=n^*=8$. Values of r_c were obtained differing by typically 10% or 15% from the radius of the liquid metallic core as determined from seismological data. (The agreement to within 2% for the first epoch considered has been checked and confirmed but the subsequent analysis of data from other epochs suggests that 2% is atypically low[7].) When sufficiently accurate measurements of the magnetic fields of other planets (for which seismological data are not available) have been made, it should be possible to use the method in the investigation of their interiors. The Pioneer 10 and Pioneer 11 measurements of Jupiter's magnetic field[2] are not, however, considered to be sufficiently accurate and detailed to give reliable information on secular changes in **B** during the short interval of only one year between the times $t=t_{10}$ and $t=t_{11}$ (say) of the two encounters[2]. We expected that these uncertainties would manifest themselves as highly improbable or even physically impossible values of r_c based on these limited measurements but surprisingly we found this was not the case.

Smith *et al.*[2] have constructed several spherical harmonic models of the jovian magnetic field for $t=t_{10}$ and $t=t_{11}$. These are based on the vector helium magnetometer observations on board Pioneer 10 and Pioneer 11 made within $8r_s$ of the centre of Jupiter, in the region of the inner magnetosphere where the contribution to **B** made by local currents should be negligible. For the inner magnetosphere **B** consists of an 'internal' part originating within the planet and a smaller 'external' part due to electrical currents flowing in the middle and outer magnetosphere or in the magnetopause. Smith *et al.*[2] give for each of the Pioneer 10 and Pioneer 11 data sets four spherical harmonic models, each comprising sets of coefficients for the field of internal origin up to degree and order n_i and for the field of external origin coefficients up to degree and order n_e, as follows: (a) $n_i=3$, $n_e=2$; (b) $n_i=3$, $n_e=1$; (c) $n_i=3$, $n_e=0$; (d) $n_i=2$, $n_e=2$. For each of the four pairs of models we have calculated the total 'unsigned' flux through spheres of various radii $r=r_s(1-0.05p)$, $p=0, 1, 2, 3, 4$, and so on, by numerically approximating the integral

$$N(t;r)=\int_0^{2\pi}\int_0^{\pi}|B_r(r,\theta,\lambda,t)|r^2\sin\theta\,d\theta\,d\lambda \qquad (2)$$

from values of B_r synthesised on a 10° by 10° zenographic θ (co-latitude) and λ (longitude) grid. (Here, r, θ, λ are spherical polar coordinates, t denotes time and B_r the radial component of **B**.) For each pair we have also calculated the secular change in N, namely

$$\Delta N(t_{11}-t_{10};r)=N(t_{11};r)-N(t_{10};r). \qquad (3)$$

The effective vanishing of ΔN at $r=r_c$ provides the theoretical basis of Hide's method for estimating r_c (refs 1, 6).

The computed values of B_r and hence of N and ΔN will depend on n^*, the maximum degree and order of terms of internal origin included in the spherical harmonic expansion, so

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Table 1 The r-dependence of N and ΔN

r/r_s	N (10^{12} Wb)				ΔN (10^9 Wb)			
	$n^*=2$, dipole plus quadrupole only							
	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)
1.00	25.9	25.6	24.8	26.0	−329	−30	+1573	+70
0.95	27.2	27.0	26.2	27.3	−341	−31	+1441	+65
0.90	28.8	28.5	27.7	28.9	−356	−30	+1234	+60
0.85	30.5	30.3	29.4	30.6	−362	−16	+935	+52
0.80	32.5	32.2	31.4	32.5	−353	+7	+490	+42
0.75	34.7	34.4	33.7	34.7	−331	+27	−117	+23
0.70	37.3	37.0	36.5	37.2	−279	+62	−988	0
0.65	40.3	40.1	39.7	40.2	−191			−49
0.60	43.8	43.7	43.7	43.6	−23			−129
0.55	48.1	48.1	48.6	47.7	+275			
0.50	53.4	53.6	55.0	52.7	+825			
	$n^*=3$, dipole, quadrupole and octupole							
	(a)	(b)	(c)		(a)	(b)	(c)	
1.00	25.0	24.8	25.2		+568	+962	+56	
0.95	26.3	26.2	26.8		+431	+1007	−716	
0.90	27.8	27.7	28.7		+158	+888	−1761	
0.85	29.6	29.3	31.0		−339	+559		
0.80	31.7	31.3	33.7		−1200	−122		
0.75	34.2	33.6	37.0			−1200		

See equations (2) (3). Based on the spherical harmonic models (a), (b), (c) and (d) in terms of which Smith, Davis and Jones[2] analysed Pioneer 10 and Pioneer 11 observations of Jupiter's magnetic field for epochs December 1973 and December 1974.

we have calculated N for $n^*=2$ and $n^*=3$. (We omitted the most highly truncated case of all, $n^*=1$, because both N and ΔN then vary as r_s/r and r_c is indeterminate.) We also investigated the effect of omitting the external contribution to B_r, and found that this made negligible changes to the values of N and ΔN. Table 1 lists the mean value of N and the difference ΔN for each pair of models at $r=r_s(1-0.05p)$, with the integer p increasing from zero to at least two steps beyond the point at which ΔN changes sign. The values of N from the various models evidently agree well, even for the smallest values of r, but the values of ΔN are much more varied, and even at $r=r_s$ there is little agreement between the models. Nevertheless, not only do all the models show a change of sign of ΔN for both $n^*=2$ and $n^*=3$, but all of these sign changes occur over a surprisingly small range of r_c, between $0.6r_s$ and $1.0r_s$.

If, by taking $n^*=2$, only the dipole and quadrupole terms are included, the four models give $r_c=0.60r_s$, $0.82r_s$, $0.76r_s$ and $0.70r_s$ (see Table 1), the mean of which, $0.72r_s$, lies within the range of recent estimates of the radius r_m of Jupiter's metallic core, $0.7r_s$ to $0.8r_s$ (see above). When, in addition to the dipole and quadrupole terms, the less reliable octupole contribution is included (by taking $n^*=3$), only the first three models can be used. These give $r_c=0.88r_s$, $0.81r_s$ and $0.99r_s$ respectively, mean value $0.89r_s$, corresponding to levels well above the metallic core. (These estimates of r_c should also be compared with the white noise level $0.54r_s$ and the terrestrial analogue level $0.69r_s$, as determined by Elphic and Russell on the basis of considerations of the n-dependence of the quantity

$$(n+1)\left(\frac{r_s}{r}\right)^{2n+4}\sum_{m=0}^{n}[(g_n^m)^2+(h_n^m)^2] \qquad (4)$$

where g_n^m and h_n^m are the usual spherical harmonic coefficients of order m and degree n ref. 8).)

It is surprising that the new method[1], when applied to models based on data that are so poorly distributed in space and (more importantly) time, should give any value of r_c/r_s at all, let alone values which are both plausible and fairly consistent. While the present results must be regarded as very preliminary, they at least suggest that the method is appropriate and practical for the investigation of the interiors of planets with magnetic fields. It might be possible fairly soon to obtain better estimates of r_c for Jupiter by combining the Pioneer 10 and Pioneer 11 data with new data from Voyager 1 and Voyager 2, and it might also be possible to use the Pioneer and Voyager measurements of the magnetic field of Saturn to obtain a rough estimate of the radius of the electrically conducting fluid core of that planet.

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A relationship between solar activity and planetary albedos

WE have observed a significant anti-correlation between solar activity and the brightnesses of two Solar System objects. Both the planet Neptune and Saturn's satellite Titan increased in brightness by several per cent between 1972 and 1976[1] and subsequently became fainter by comparable amounts[2]. This period corresponds to the decline of solar activity at the end of solar cycle 20 (1972–76), followed by the rapid increase of activity at the beginning of cycle 21. Solar minimum and the maximum observed brightness of Titan and Neptune both occurred in 1976. (The maximum of cycle 21 is forecast for late 1979 or early 1980, with a sunspot number ~150[3].) We hypothesise that what we have observed are changes in planetary albedos induced by solar activity. Such changes may have an important bearing on the energy balances of the outer planets and their satellites.

The observed solar–planetary relationship is shown in Fig. 1*a* for Titan and Fig. 1*b* for Neptune. Two measures of solar activity were chosen from the several available in the literature: the commonly used Zürich sunspot number, R_z, and an *ad hoc* index, F, the number of observed flares per month of importance 1 or greater. They are shown on the right-hand ordinate scales. The left-hand scales give the brightnesses of Titan and Neptune in the blue (b) and yellow (y) spectral regions in units of stellar magnitudes. The range of brightness variation was ~0.08 mag for Titan and 0.03 mag for Neptune, while the range of sunspot number was 0–130.

Figure 1 shows that there are usually two data points per observing season; they represent the mean magnitudes obtained from observations on a number of nights, typically 5–10, preceding and following opposition. The observations are normalised to zero solar phase angle and to mean heliocentric distances of 9.539 and 30.071 AU for Titan and Neptune, respectively. During a single season, each object is measured differentially with respect to two nearby comparison stars whose colour approximates that of the Sun, and subsequent observations determine the relative magnitudes of the comparison stars. A detailed error budget for these observations and a rationale for the method by which they were obtained have been given elsewhere[1].

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All the plotted quantities in Fig. 1 are obviously highly correlated with one another. The correlation of the *y* magnitudes of both objects with the sunspot number is significant at better than the 99% level, while the *b* magnitude correlations with sunspot number are significant at the 95% level. The correlations of magnitude with the flare index are significant at the ~90% level.

Many directly observed indicators of solar activity (such as 10.7 cm radio flux, coronal line spectrum, number of flares and prominences), as well as solar–terrestrial effects (such as geomagnetic field strength, and cosmic ray flux at the Earth) rise and fall during the 11-yr sunspot cycle, just as the sunspot number does, and would therefore also show a good statistical correlation with the magnitudes of Titan and Neptune. We chose to illustrate the correlation using the sunspot number and flare index.

The observations were made using the 0.5-m reflecting telescope of the Lowell Observatory, a photoelectric photometer equipped with a thermoelectrically cooled EMI 6256 photomultiplier, pulse-counting electronics, and DEC PDP-11 computer. There were no changes in the auxiliary instrumentation during the entire course of the programme. Observations were obtained through two intermediate-band interference filters of the Strömgren photometric system; *y* at 551 nm (HPBW = 23 nm) and *b* at 472 nm (HPBW = 20 nm). All magnitudes are expressed on the natural instrumental scale (that is, not transformed to the 'standard' Strömgren scale) to preserve their inherent high internal accuracy which derived from an extremely stable instrumental configuration.

The change in aspect of a Solar System body as seen from the Earth can cause photometric variations. In the present case these seem to be ruled out. The maximum apparent inclination of Titan's orbital plane (27° with respect to the ecliptic) occurred in May 1974, while maximum brightness was reached in early 1976 when the inclination was 22°. Assuming rough symmetry of the northern and southern hemispheres of Titan (a plausible assumption in view of Titan's thick atmosphere), a seasonal variation would have a period of 15 yr and an amplitude of ~0.2 mag, based on the variations observed by us during one-fifth of a Saturn year. Historically[4], variations of Titan do not exceed 0.1 mag. Moreover, many configurations of surface markings, if any should be visible through the atmosphere, would lead to substantial variations during Titan's 16-d orbital period. These variations, if they occur at all, are very small (<1%)[5]. The case against seasonal effects on Neptune is even stronger. Since 1972, both the change in aspect as seen from the Earth and the fractional change in solar irradiation were completely negligible.

How the 'solar constant' changes over long periods remains uncertain: the best available Nimbus satellite data suggest that

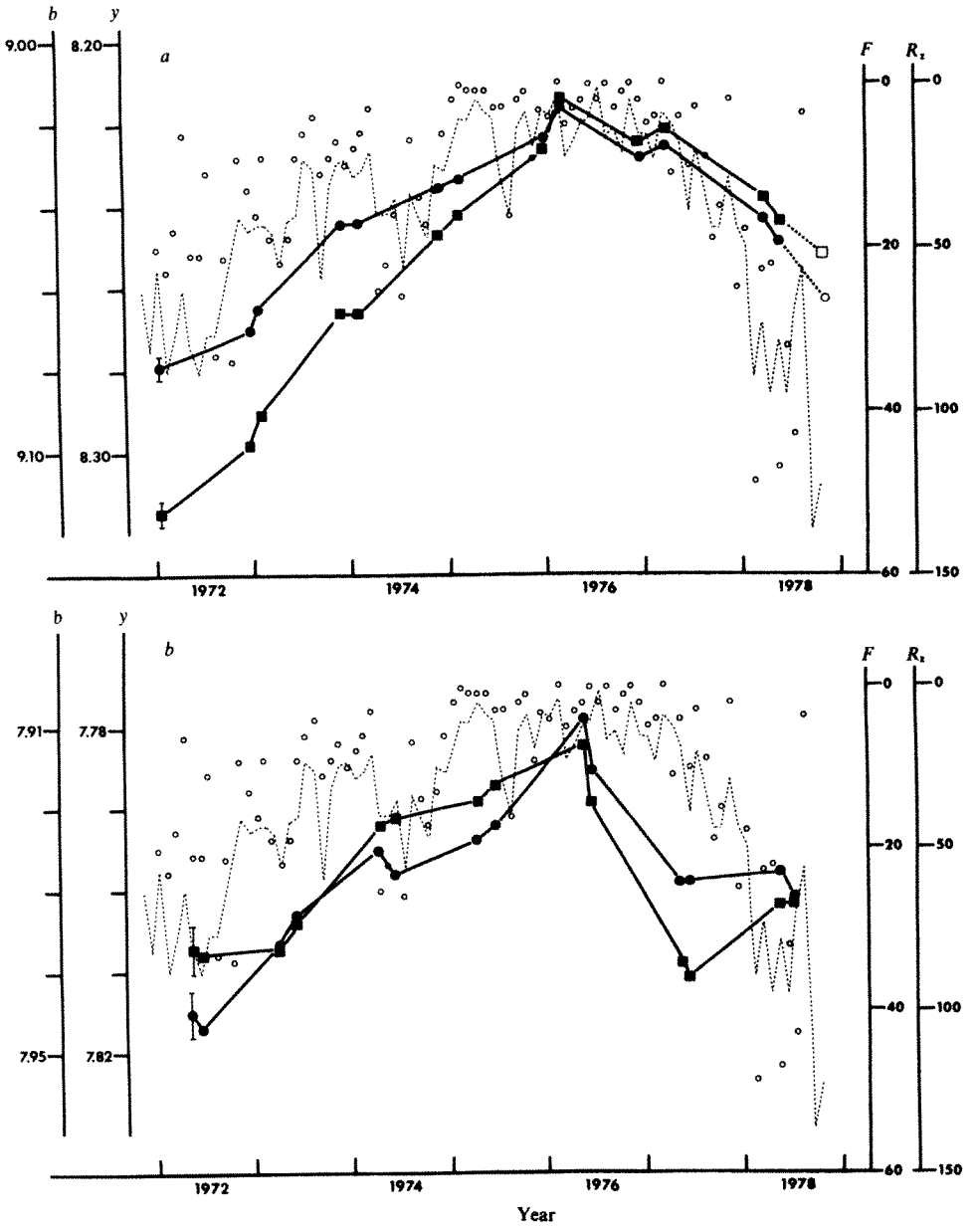


Fig. 1 *a*, Titan and *b*, Neptune. Planetary brightness as a function of time in units of stellar magnitudes (0.01 mag ~1%) plotted with brightness increasing upward. Note that the magnitude scales for *a* and *b* are not the same. Typical error bars are shown on the left-most data points. The monthly mean sunspot number R_z (dashed line), and the 'flare index', *F* (○), are plotted increasing downward. The solar data are from the *IAU Quarterly Bulletin on Solar Activity* and *Solar-Geophysical Data*. The flare index was made by counting the number of separate flares per month of importance ⩾1 tabulated in these publications. ●, yellow values; ■, blue values.

any changes on a time scale of months are smaller than ~0.2%[7], far too small to account for the effect we have observed.

The apparent synchronisation of solar activity with the observed variations of two separate and independent planetary bodies led us to hypothesise that one or more components of the solar output which vary during the solar cycle could cause changes in the albedos of planets and satellites.

While the thermal structure of Titan's atmosphere remains doubtful, its low albedo in the UV and visible regions implies, according to current models, the existence of a dust layer or photochemical 'smog'. Recent work has explored the possibility of chemical reactions in Titan's atmosphere intiated by cosmic rays, the 'Saturn wind', and the solar wind[8], as well as photochemical processes leading to the conversion of the abundant CH_4 molecule to heavier hydrocarbons in the atmospheres of the major planets[9]. Such reactions, if they occur, must surely be sensitive to variations of the incident particle or radiative flux; and, therefore, variations in atmospheric composition and structure could also occur, leading to variations in albedo.

Variations of the solar radiative flux below 300 nm during a solar cycle, according to the best best available data, range from ~1% at 300 nm to several orders of magnitude below 2 nm[6]. The solar wind and interplanetary magnetic field have recently been sampled in the outer Solar System and have been found to vary in complex ways during the solar cycle[10,11].

However, to our knowledge, no studies have been made to consider the consequences of variations of the solar radiative and particle output on planetary atmospheres; and therefore our meagre interpretation of the observed planetary brightness variations is rather speculative. We emphasise that the atmospheric dynamics and chemistry of the major planets and Titan are incredibly complicated and much theoretical work remains to be done before an adequate interpretation will be possible.

In addition to the recent data shown on Fig. 1 we have analysed[12] the more extensive but less accurate Lowell Observatory photoelectric photometry of Uranus and Neptune dating from 1953 to 1966[13,14] to look for evidence of transient effects such as a planetary response to solar flares, for short-term (month-to-month) correlations with daily and mean monthly sunspot numbers, and for a periodicity in observed brightness at or near the solar rotation frequency. Using correlation techniques, we have looked for time-delayed effects lagging by a few hours (corresponding to propagation at the speed of light), by a few weeks to several months (corresponding to the average speed of the solar wind), and by longer intervals up to one year. None has yet been found at a level of 0.003 mag or greater for Neptune or Uranus, which suggests that transient events may be relatively unimportant.

Thus it seems more likely that it is the long-term envelope of solar activity that drives planetary albedo changes and that these changes derive from relatively slow structural and chemical changes in planetary atmospheres.

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Drag reduction in fibre suspensions: transitional behaviour due to fibre degradation

IT is known that the addition of small amounts of certain polymers to a fluid can produce spectacular reductions in turbulent friction[1,2]. It is also possible to reduce friction by suspending macroscopic fibres in a fluid[3]. Superficially there are many similarities between the two effects but they are believed to involve different mechanisms because, for example, using polymers and fibres together can produce much larger reductions in drag than would be the case with either additive on its own[4]. We report here measurements of turbulent velocities in a fibre suspension, using laser–Doppler anemometry. We found that the effect of fibres on turbulent structure was not the same as that of polymers. However, when the fibre was degraded by passing it through the system several times, there was a transition to the type of structural change found in polymer solutions.

The fibres used were chrysotile asbestos and are known to be very effective drag reducers. A nominal fibre concentration of 300 p.p.m. by weight was used throughout. Following the usual practice, the fibres were dispersed in 0.5% aqueous solution of Aerosol OT.

A 'blow-down' flow rig was used to minimise degradation of the fibres. Suspensions were prepared in a header tank and then allowed to flow under gravity into a pressure tank. Air pressure was used to propel the suspensions through a horizontal pipe of 2 cm nominal bore and then through a return line to the header tank. Flow rates were controlled by a constant-flow valve. The actual flow rate was determined by timing the change in levels on a sight-glass in the pressure tank. The test section of the pipe was

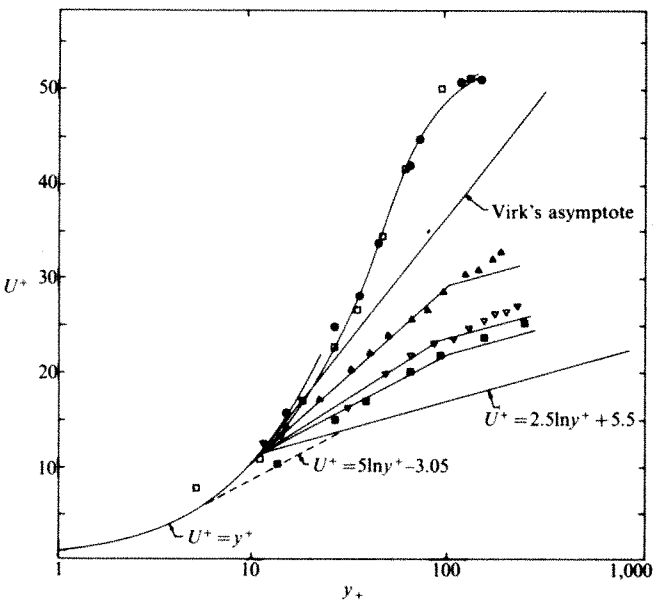


Fig. 1 Mean velocity profiles in a 300 p.p.m. asbestos fibre suspension at various levels of drag reduction for $R_e = 9 \times 10^3$. ● Run 1, drag reduction (DR) = 63%. □ Run 2, DR = 63%. ▲ Run 4, DR = 44%. ▽ Run 10, DR = 22%. ■ Run 17, DR = 17%.

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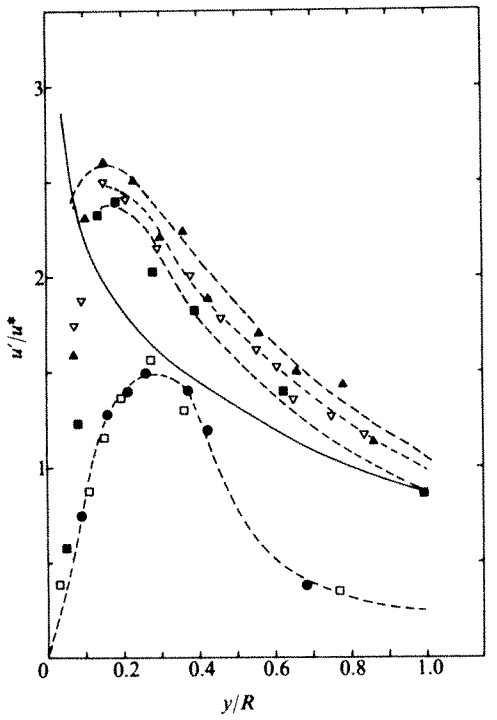


Fig. 2 Streamwise turbulent intensity profiles in a 300 p.p.m. asbestos fibre suspension at various levels of drag reduction, for $R_e = 9 \times 10^3$: continuous line denotes results for water. ● Run 1, DR = 63%. □ Run 2, DR = 63%. ▲ Run 4, DR = 44%. ▽ Run 10, DR = 22%. ■ Run 17, DR = 17%.

situated at 154 diameters from the inlet to ensure well developed flow. Pressure drops were measured using pressure taps and a liquid-in-glass manometer.

Streamwise velocity profiles were measured over the cross-section of the pipe, using a laser–Doppler anemometer (LDA). The optical and signal-processing arrangements were as reported previously[5].

An experimental 'run' consisted of one pass of the whole amount of working fluid through the apparatus at a fixed Reynolds number. Further 'runs' were further passes of the same fluid at the same Reynolds number. Experimental results were always taken in steady conditions, avoiding the beginning and end of each run. Reynolds numbers were calculated from the pipe diameter, the bulk mean velocity and the kinematic viscosity of water.

These particular fibres are known to be susceptible to degradation under shear. Our results confirmed this and showed a decline in drag-reducing effectiveness with increasing run number. We decided to make a virtue of necessity and used this as an experimental parameter. The results were interesting.

Figure 1 shows mean velocity U^+ (scaled) plotted against distance from the pipe wall y^+ (scaled), in the usual 'wall-region' coordinates, for a Reynolds number of 9×10^3. The amount of drag reduction appears as a parameter. It can be seen that the first two runs gave a drag reduction of 63% and that the corresponding mean velocity profiles lay beyond the Virk asymptote for polymer solutions. In contrast, the results for later runs in the degraded suspensions were in the polymeric regime. Like results for polymers, they show a trend to the newtonian result, as the amount of drag reduction decreases.

Figure 2 shows the r.m.s. velocity u' (divided by the friction velocity u^*) plotted against distance from the wall y (divided by the pipe radius R). Here the differences between high and low drag reduction are seen to be no longer a matter of degree only. Runs 1 and 2 showed a marked suppression of the streamwise fluctuating velocity, compared with the newtonian result. However, in later runs the intensity over most of the cross-section was increased above the newtonian result. Again, these results in later runs were similar to those previously found in polymer solutions[1].

This behaviour has also been found in further experiments at a higher Reynolds number, 1.4×10^4. Thus it seems that we are observing a transition between two different drag-reduction mechanisms. When the fibre is at maximum effectiveness, it behaves quite differently from polymers which produce comparable amounts of drag reduction. In particular, it suppresses the streamwise turbulence. After degradation, when the fibre is (presumably) smaller it seems to behave rather like a polymer of the same effectiveness. In particular, it enhances the streamwise turbulence.

It might be tempting to conclude from Figs 1 and 2 that the undegraded fibres reduce drag by suppressing turbulence and making the flow 'more laminar'. Such a conclusion would be wrong. In our more recent experiments we have used a second LDA to measure the spanwise turbulent intensity. During runs 1 and 2, the spanwise turbulent intensity was actually larger than in water alone. In the later runs, the spanwise intensity was less than that for water alone. Rudd has previously reported[6] reduced spanwise intensities in polymer solutions.

We already know that the turbulent structure is changed during drag reduction in polymer solutions[1,6]. Our present results indicate that in undegraded fibre suspensions the change in turbulent structure during drag reduction is of a different kind. This effect was previously predicted on theoretical grounds[7].

We thank Turner Brothers Asbestos Co. Ltd for drag-reducing chrysotile asbestos suspensions.

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Ferromagnetism in ancient copper-based coinage

WHEN extensive forgery of silver coinage has taken place in the past, a small permanent magnet was a well known technique for distinguishing between counterfeit nickel and genuine silver coins. The accidental discovery of ferromagnetism in undisputably genuine ancient Kushan copper coins by Nisbet during a routine check with a permanent magnet was, however, totally unexpected. Although we have found very few coins possessed a magnetic moment sufficiently large for the coin to be lifted by the permanent magnet, subsequent examination has revealed that several coins possess a spontaneous remanence magnetisation which can be detected with a simple deflection magnetometer. Quantitative measurements of the room temperature magnetisation have been performed in fields up to 5×10^5 A m^{-1} using a Faraday balance magnetometer. To our knowledge they form the first systematic study of the magnetic properties of ancient coinage.

The magnetometer sensitivity was about 10^{-2} J T^{-1} kg^{-1} and the Pauli paramagnetism of the conduction electrons could not be detected. As Fig. 1 shows, the magnetisation of an orichalcum semis of the Roman Emperor Tiberius remained below the noise level up to the maximum fields available. Similar null results

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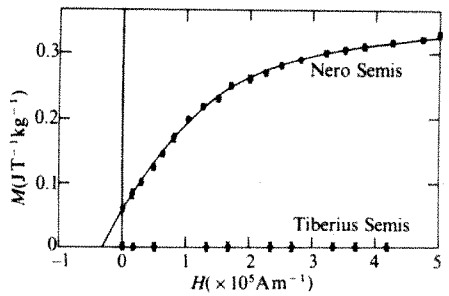


Fig. 1 Magnetisation as a function of field for two Roman orichalcum coins. The magnetisation of the Tiberius semis was at all times well below the instrumental sensitivity.

were obtained with modern bronze coinage, entirely consistent with the expected paramagnetic behaviour. In marked contrast, an orichalcum semis of the Emperor Nero (dated to 63–64 AD) not only showed significant remanence magnetisation but also a marked increase with increasing magnetising field. Although the susceptibility fell as the field was raised, no tendency towards saturation was observed. When the field was reduced, the remanence magnetisation returned to its initial value. The coercive field H_c, (that is, the reverse field required to reduce the magnetisation to zero) was found to be 3.2×10^4 A m^{-1} (400 Oe) and the remanence magnetisation M_r to be 5.5×10^{-2} J T^{-1} kg^{-1}.

Orichalcum, first analysed in 1798 by Klaproth[1], is an alloy of copper and zinc and is generally believed to have been produced by a cementation process, in which copper bars were heated in a crucible containing a zinc ore and charcoal[2]. Such an alloy should not possess a spontaneous magnetic moment. Recent analyses have shown, however, that some Roman orichalcum coins may contain up to 1% iron impurity[3], and with this in mind magnetisation measurements were performed on a series of coins exhibiting ferromagnetism which had been previously analysed by Thompson using spark emission spectroscopy.

Although neglected in secondary school teaching in the West, the Kushan Empire was the third of the great empires of antiquity controlling the caravan routes between Rome and Cathay. At its height it covered what is now Afghanistan, Pakistan, North-west India and Southern Russia.

Magnetisation measurements were made on 12 copper Kushan coins of known chemical composition. A typical magnetisation curve is shown in Fig. 2*a*. All coins showed ferromagnetic behaviour, and saturated in fields of about 1.5×10^5 A m^{-1} in marked contrast to the Roman orichalcum coin. The coercive field was low, having a mean value of 3.9×10^3 Am^{-1} (with a 1σ error of 1.2×10^3 A m^{-1}). Although both the shapes of the magnetisation curves and the values of the coercive fields were all very similar, two distinct groups of coins were identified. Coins of the late Kushan period (mid-third to mid-fourth century AD) displayed a very low saturation magnetisation M_s and remanence so small as to be undetectable with a deflection magnetometer. Coins produced during the period of great expansion of the Kushan Empire under Kanishka (second quarter of second century AD) showed saturation and remanence magnetisation two to three orders of magnitude greater than those of the late Kushan period. These remanence magnetisations were easily measured with a deflection magnetometer. The strong ferromagnetism was thus confined to coins of a restricted historical period.

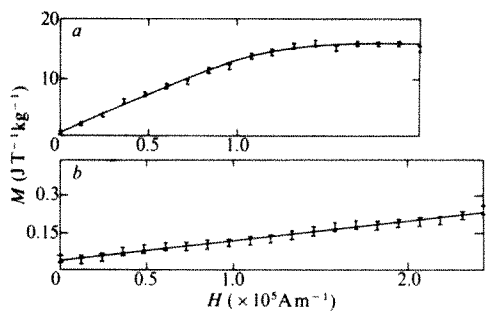


Fig. 2 *a*, Magnetisation curve for a copper drachm of Kanishka (Kushan Empire). Note the saturation, small remanence magnetisation and low coercive field. *b*, Magnetisation curve for a similar Kashmiri coin. Note the high coercive field, and lack of saturation.

In contrast, early Hindu copper coins from Kashmir, showed markedly different magnetisation curves. Nine coins whose dates ranged over a wide period were examined. All showed ferromagnetic behaviour but, as with the orichalcum coins, no saturation occurred up to the highest fields available (Fig. 2*c*). A large spread occurred in the coercive fields but all were significantly larger than that of the Kushan coinage. The mean value of 2.9×10^4 A m^{-1} (with 1σ error of 1.8×10^4 A m^{-1}) is nearly an order of magnitude greater than the mean value for Kushan coins and is comparable with that of the Roman orichalcum. No correlation has yet been established between strong ferromagnetism in the Kashmiri coins and the historical period.

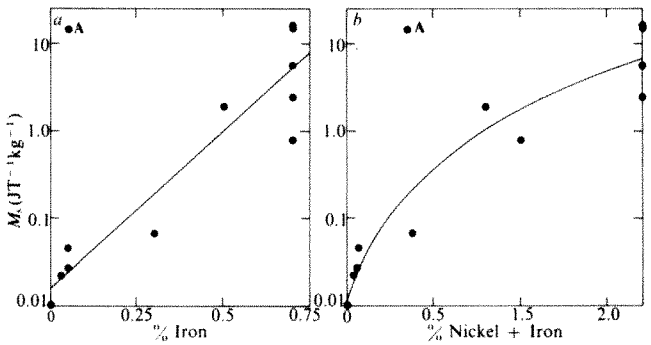


Fig. 3 *a*, Log-linear plot of saturation magnetisation versus iron content in Kushan coinage. The straight line is a least squares fit to the data. The point A was rejected in the fit on the grounds of unreliability of the analysis. *b*, Log-linear plot of M_s versus total iron and nickel content. The line is a guide for the eye.

A correlation does exist between concentration of iron and the saturation magnetisation M_s in the Kushan coinage. The chemical analyses show the lead, nickel and iron content of the Kanishka coins to be of the order of 1%, significantly above that of the late Kushan coins. Nickel, though ferromagnetic itself, forms a complete solid solution in copper and a 99% Cu–1% Ni alloy is not ferromagnetic at room temperature. However, iron is almost insoluble in copper and a dispersed-phase alloy of 99% Cu–1% Fe would have a magnetisation comparable with the largest values measured. Figure 3*a* shows a log-linear plot of M_s versus iron content. Clearly, the saturation magnetisation is directly related to the iron content. Fitting a straight line to the data by the least squares technique yielded a correlation coefficient of 0.94. However, a correlation still persisted when the total ferromagnetic impurity concentration (Fe + Ni) was plotted against M_s (Fig. 3*b*). As iron and nickel form a range of ferromagnetic alloys it is unclear what role the nickel plays and until more information on the structure of the material is available care should be exercised in interpreting these data. As yet it is unknown whether the iron is present as the element or as an oxide.

The ratio of saturation to remanence magnetisation (M_s/M_r) versus iron content is shown in Fig. 4. M_s/M_r is approximately the same for all the coins and there is no significant dependence on iron (or nickel) content.

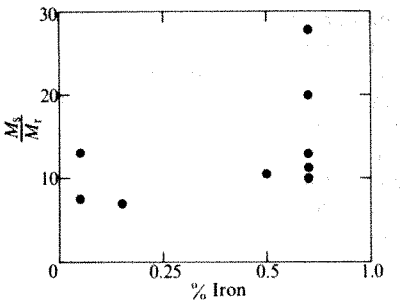


Fig. 4 Ratio of saturation to remanence magnetisation versus iron content in Kushan coinage. (Note that this is a linear plot, unlike Fig. 3.)

We may summarise the results of these initial investigations as follows. (1) Ferromagnetism in ancient coinage arises from the presence of ferromagnetic impurity in the copper-based matrix. (2) The occurrence of ferromagnetism may well have archaeological significance. Very high values of saturation magnetisation in the Kushan coinage are confined to a relatively short period during which time the empire was rapidly expanding. There is some numismatic and historical evidence to suggest that these coins may have been minted from freshly mined copper. (3) The magnetic characteristics (for example, the coercive field) are very different in the otherwise similar Kushan and Kashmiri coins. The magnetic properties may well give a clue to the origin of the ore. High coercive field and high saturation field could be strong indicators of re-usage of old coinage in the minting process. (4) Within a factor of three, the ratio of saturation to remanence magnetisation of the Kushan coinage is constant. Thus M_r can be used to give an order of magnitude estimate of M_s. As M_r can be measured with a simple deflection magnetomerer, useful physical measurements can be performed on confined collections such as that in the British Museum.

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Confirmation of the Suess wiggles: 3200–3700 BC

USING dendrochronologically dated tree rings de Vries[1] showed that the atmospheric ^{14}C concentration has not been entirely constant during the past few hundred years. By extending the North American bristlecone pine tree-ring chronology[2] to nearly 8,000 yr ago, several radiocarbon laboratories succeeded in measuring deviations in the ^{14}C concentration from the mid-nineteenth century natural ^{14}C level to a maximum of about 10% around 4000 BC (refs 3–7). Superimposed on this general trend, Suess drew with 'cosmic schwung', medium-term variations through his data. These so-called 'wiggles' should correspond with variations in the abundance of radiocarbon in atmospheric carbon dioxide on a time scale between one decade and a few centuries. The resulting irregular shape of Suess' calibration curve has been questioned during the past few years[8,9]. The overall result, however, is that no generally accepted ^{14}C calibration curve is yet available. It would be most convenient to measure ^{14}C concentrations in single tree rings with much higher precision than the usual 4–6‰. For this, however, much larger samples are required than are available from the bristlecone pine tree. An excellent opportunity was presented by the sub-fossil oak trunks discovered in the river valley sediments of southern Germany[9]; our measurements of these trunks are reported here.

Our first measurements were carried out on a 500-yr section of the 1,235-yr floating South German Neolithic tree-ring chronology. The section used was derived from well preserved sub-fossil oaks, which grew along the River Danube (49°25'N, 10°5'E). To avoid recycling due to soil CO_2 during an early stage of tree growth no rings from near the centre of a log were analysed. Because recent results[11] have shown that if year to year ^{14}C variations are small then radial movement of ^{14}C across ring boundaries in an oak is negligible, the measured ^{14}C concentration in a single tree ring nicely represents the atmospheric ^{14}C concentration in the growth season of a tree ring. Although wood, after pretreatment with successive solutions of 4% hydrochloric acid, 4% sodium hydroxide and 4% hydrochloric acid again (AAA) probably do not exactly originate from exactly the same time of the year as the cellulose fraction, the AAA pretreatment seems to be acceptable for monitoring past ^{14}C levels[11]. The final sample prepared for analysis was 50–55 l of CO_2 gas at STP. The special high-precision proportional gas counting system will be described elsewhere[12].

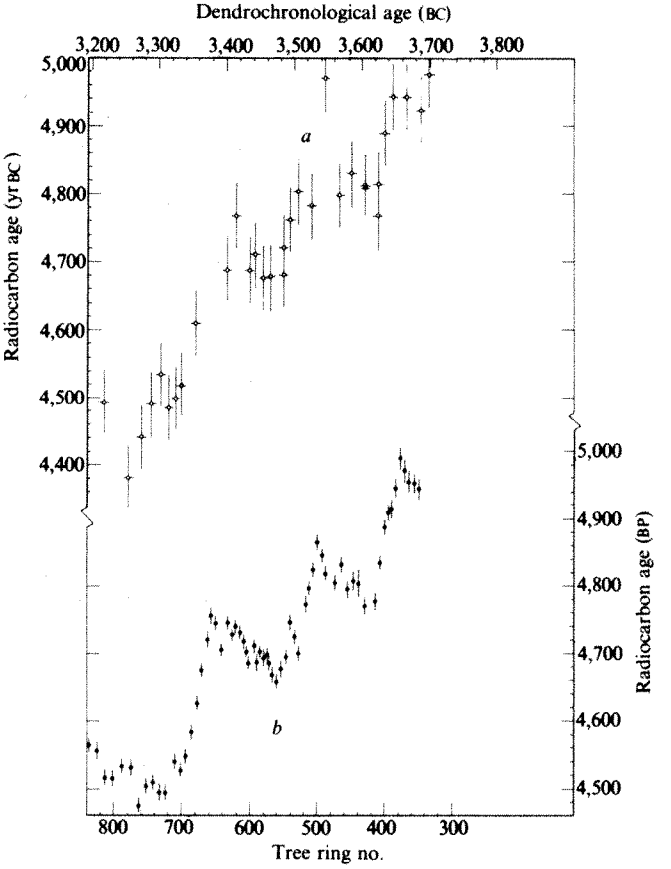


Fig. 1 ^{14}C analyses on dendrochronologically dated wood (*b*). The relation between radiocarbon age and the tree-ring number from the floating South German Neolithic master chronology. For comparison, *a* shows the bristlecone dates, corrected to the proper standard activity (0.95 × NBS-oxalic acid)[6].

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The results are presented as conventional ^{14}C ages in Fig. 1*b*, related to the tree-ring number from the floating South German Neolithic master chronology.

Figure 1 confirms quantitatively the existence of pronounced wiggles between the thirty-third and thirty-seventh centuries BC. The fact that the upward slopes from right to left are less steep than the downward slopes suggests that we are faced with secular variations in the atmospheric ^{14}C level consisting of relatively fast rises followed by a restoration of the stationary state. This conclusion is supported by the rather regular periodic behaviour of the 'wiggles' with a period of approximately 180 yr. Calculations based on geophysical and geochemical models might provide a more definite answer to the question of the origin of the variations.

A comparison with the bristlecone dates of Suess[6] corrected to the proper standard activity (0.95 NBS oxalic acid), which makes the dates approximately 50 yr older than listed, leads to the absolute time scale for our floating chronology (Fig. 1*a*). The South German Neolithic master chronology seems to start at 4035 ± 3 BC. Wiggle matching Suess' results on the German material with the bristlecone sequence gave a comparable result (4057 BC)[10,13].

For archaeological samples which are 'short lived' (of the order of a few tens of years) these medium-term variations add an extra uncertainty to the purely statistical error of a ^{14}C-age determination.

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Sulphur uptake from the atmosphere by forest and farmland

MEASUREMENTS of sulphur uptake by plants are needed to aid our understanding of the long-range transport of sulphur dioxide and the role of sulphur uptake in acidification and the nutrient balance of forest ecosystems[1]. The traditional profile method of analysing sulphur uptake has proved inadequate for forest measurements because the vertical concentration gradients above forests are small and difficult to measure with current techniques[2]; the best estimates available are based on extrapolation of measurements from individual shoots to the whole forest[3]. We report here the first direct measurements of the uptake of sulphur from the atmosphere by a forest in the absence of precipitation (that is, dry deposition). These measurements were possible because of the development of a fast response sulphur detector which allowed, for the first time, use of the eddy correlation method[4] for sulphur uptake measurements. The sulphur uptake ranged from 0–0.1 $\mu g\ m^{-2}\ s^{-1}$ (as SO_2) for atmospheric sulphur concentrations up to 35 $\mu g\ m^{-3}$ (expressed as SO_2). On average, ~75% of the atmospheric sulphur was in gaseous form. These uptake rates support those derived from use of the recent hypothesis that sulphur dioxide uptake by vegetation takes place through the stomatal opening for a dry canopy[3]. Some measurements of dry uptake of sulphur over farmland were also made and are in close agreement with published measurements made over similar surfaces using the profile and other methods.

The eddy correlation method can give a direct measurement of the vertical flux of a constituent within the air. If the concentration of the constituent is fairly constant (that is, storage is negligible), the vertical flux within the atmosphere is equal to the uptake rate at the underlying surface. At a fixed point above the surface, continuous fast response measurements are made of the vertical wind velocity, w, and the concentration (ambient density) of the constituent, c. The vertical flux of the constituent, F, is given by the covariance $F = \overline{wc}$ where the overbar indicates the time average. We assume that the time averaged vertical velocity is zero, $\bar{w} = 0$, (ref. 5), therefore $F = \overline{w'c'}$ where the prime indicates the instantaneous departure from the long-term mean. This uptake of the constituent from the atmosphere is conveniently expressed in terms of a deposition velocity, v, and the air concentration of the constituent, c, by an equation $F = vc$ (refs 1–3, 6).

A Gill propeller anemometer was used to measure the vertical velocity. While the response time τ of a Gill propeller (that time to reach $1 - 1/e$ of the final speed following a step change in wind speed) depends on mean wind speed U, a response length $L = U\tau$ should depend only on the turbulent intensity and L was estimated to be 2 and 3 m for the Crowthorne (forest) and Blewbury (farmland) measurements respectively[7,8]. The flame photometric sulphur detector[9], with a 4-m gas inlet tube, had a response time measured in the field of 0.3 s. This response time was determined mainly by the inlet tube. There was also a time delay of 1.8 s in the gas-inlet system of the sulphur detector. This was corrected for in the covariance calculation by displacing digital records from the sensors by this time interval. Ambient air temperature was measured using a fast-response bead thermistor to determine the sensible heat flux. The sensors were sampled at 5 Hz by a digital computer for the covariance calculations. No low frequency filter was used in the system but fluxes were computed for individual 10- or 12-min periods.

The flux values were corrected[7,8] due to inadequate speed of response of the anemometer and to smoothing from the sulphur detector inlet system to allow for flux losses. The corrections were 10 and 20% for the Crowthorne and Blewbury data respectively. Low frequency flux losses, probably of the order of 5–20% (ref. 10) have not been corrected for in the flux estimates. The ratio of height of sampling to length of uniform fetch upwind of the sensors was such that even in the worst case the flux should be 90%, adjusted to the underlying forest surface. Adjustment was often complete[11].

Measured non-zero values of $\bar{w}$ indicated slight misalignment of the vertical propeller amounting to ~1°. This misalignment introduces an error into the mass flux measurement of the order of 4% per degree[5,12]. Considering these factors we estimate the absolute accuracy of the average flux measurements to be of the order of ±50%. The short sample period will cause a scatter in individual flux values of ±25% (refs 13, 14).

Measurements in the field suggest that the standard deviation in a single 10-min flux measurement due to instrument noise alone would be ±0.02 to ±0.03 $\mu g\ m^{-2}\ s^{-1}$ and that the standard error in average values from hours of observations as in Table 1 from instrument noise alone would be perhaps ±0.006 $\mu g\ m^{-2}\ s^{-1}$. A description of the full system along with a complete analysis of the data will be published elsewhere.

Measurements were made at two sites (see legend to Table 1). At the farmland site the equipment was located at the boundary between two fields. In an easterly wind the fetch was entirely over a grass crop, and in a westerly wind, over a pea crop. Table 1 shows the average uptake rate for each range of concentration and is composed from all the individual flux measurements made in the concentration range. The data suggest a deposition

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Table 1 Sulphur uptake measurements

Site and times of measurements	Period of measurement (h)	Air concentration ($\mu g\ m^{-3}$)	Gaseous fraction (%)	Mean uptake ± s.e.m. ($\mu g\ m^{-2}\ s^{-1}$)	Wind ($m\ s^{-1}$)
Forest*					
1030–2000 h	16	6–11	60	<0.01	3.2–4.8
1200–1700 h	7	12–35	84	0.04 ± 0.02	0.8–4.2
Farmland†					
1200–1730 h	3	3–8	50	0.03 ± 0.02	2.2–5.7 (westerly; peas)
1100–1600 h	5	16–50	75	0.19 ± 0.02	2.2–4.4 (easterly; rye grass)

* Forest: Crowthorne, Berkshire. 30-yr-old Scots Pine 14 m high unthinned since planting. Flux measurement 3 m above tops. Measurements are for 9 days between 1 and 20 May 1977.

† Farmland: Blewbury, Berkshire. Easterly fetch; Italian rye grass 75 cm; westerly fetch, peas 40 cm. Flux measurement 4 m above soil surface. Measurements are for six days between 21 June and 5 July 1977.

velocity of about 0.2 $cm\ s^{-1}$ for these forest measurements and about 0.6 $cm\ s^{-1}$ for the farmland measurements. All measurements were made in a turbulent atmosphere.

The sulphur detector responds to gaseous and particulate compounds. Particulate sulphur contributes only a fraction of the fluxes reported here, as the sulphate concentration at the field sites, assumed to be similar to that measured at Harwell, was only 20% of the total sulphur at the higher concentrations and the deposition velocity for small particles such as sulphate is much smaller than, or comparable with, that for sulphur dioxide[2,15].

Garland[2] summarises measurements of sulphur dioxide deposition over grass, crops and bare soil made by the profile and radioactive methods that give deposition velocities in the range 0.3–1.2 $cm\ s^{-1}$ with a median value 0.8 $cm\ s^{-1}$, in close agreement with that measured by the eddy correlation method here. Profile measurements in a forest[2] suggested an upper limit of the deposition velocity of 2 $cm\ s^{-1}$. Estimates based on measurements of individual shoots and extrapolation suggest a deposition velocity varying from a daytime maximum value of 0.6 $cm\ s^{-1}$ in the morning to 0.2 $cm\ s^{-1}$ in late afternoon to a night-time value of 0.1 $cm\ s^{-1}$, depending on stomatal resistance (closure) for a dry forest canopy[3]. The measurements presented here for the higher sulphur concentrations suggest a similar low deposition velocity for sulphur on the forest canopy during the afternoon. (We found no evidence of a diurnal variation in surface resistance during the period of our measurements.) Forest measurements were made when the new growth on the forest was just emerging and most of the exposed needles would have been from previous year's growth and are known to display lower uptake rates[3]. Furthermore, the sensible heat flux from the forest was frequently about 50% of the global total incoming solar radiation. This indicates that little evapotranspiration was taking place. The stomatal resistance to gaseous transfer would most probably be high in these conditions. Indeed, stomatal resistance is generally higher during the afternoon[3,16], and the measurements presented here may be for a forest in conditions of maximum resistance to sulphur uptake. The observed fluxes over forest and farmland can be entirely accounted for in terms of sulphur dioxide deposition. However this does not preclude a large deposition velocity for particulate sulphur.

The dry deposition to a dry forest canopy at a mean sulphur dioxide concentration of 10 $\mu g\ m^{-3}$, typical of many rural locations, would be perhaps 2–3 kg sulphur $ha^{-1}\ yr^{-1}$, if the representative deposition velocity is as low as that measured here. This is a significant fraction of the estimated total sulphur deposition in English and Scottish upland sites of 10–20 kg sulphur $ha^{-1}\ yr^{-1}$ (ref. 1). If as suggested[3] gaseous and particulate uptake onto a wet canopy could account for a much larger fraction of the total deposition, then measurements with the method presented here can resolve this question.

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Magnetic measurements used to assess sediment influx at Llyn Goddionduon

THE preliminary results of an attempt to estimate sediment influx in a small upland lake using magnetic susceptibility measurements on whole unextruded sediment cores obtained from a grid covering almost the whole area of open water are reported here. The susceptibility variations, reinforced by a wider range of magnetic measurements on individual samples, provide a basis for core correlation over the whole lake. The speed with which such studies can be accomplished makes possible a much more secure empirical basis for calculations of total sediment input versus time than do conventional methods of core correlation and analysis.

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Increasing attention is being devoted to using lake sediments as sources of information about changing ecological processes in the recent past[1,2]. In many cases a large proportion of the lake sediment can be shown to have been derived from the lake watershed. The palaeoenvironmental record may, therefore, often be interpreted largely in terms of changes in the terrestrial ecosystems draining into the lake[3,4]. A prerequisite of fully quantitative studies designed to relate the sedimentary record in the lake to changing material output from its catchment is some procedure for calculating or estimating sediment influx to the whole lake for defined time intervals. Without this procedure all calculations of influx may be biased by the unrepresentative nature of a small number of coring sites.

Empirical attempts to overcome this problem[5] are limited largely by the time-consuming nature of the techniques normally available for core correlation in the absence of synchronous visual stratigraphic markers. Theoretical models developed to allow calculation of total sediment input from a single central core[6], though valuable are unconvincing in view of the numerous unverified assumptions on which they depend.

Thompson *et al.*[7] have shown that magnetic susceptibility measurements of whole, unextruded sediment cores can provide a rapid method of core correlation. The technique leaves the material unaltered, precludes no subsequent study of any kind and can be followed up by a range of related simple, rapid and non-destructive magnetic measurements[8] which often indicate sediment source[9,10] and are directly related to changes in human activity[7,8,11].

Llyn Goddionduon (Fig. 1*a*) lies 244 m above OD in the Gwydyr Forest area of North Wales, ~4 km north-west of Bettws y Coed (G.R. SH 753 586). It covers 6.2 hectares and receives drainage from a catchment of ~25 hectares. Maximum water depth is 6 m, in a small 'trench' occupying <5% of the area of the lake bed. Of the present sediment surface, 50% is between 0.75 m and 4 m deep. A small mire ~0.6 hectares in extent at the southern, outflow, end of the lake represents overgrowth of peat lying partly on marginal lake sediments. Some 25% of the lake is colonised by macrophytes among which *Schoenoplectus lacustris, Phragmites communis, Eleocharis palustris, Nymphaea alba, Potamogeton natans* and *Equisetum fluviatile* are the most abundant species[12].

An accurately surveyed grid of 130 minicores[13] was sampled during a 2-week period in July 1977, using a research team varying from five to nine people. Cores were taken at 20 m × 20 m intersections. Where impenetrable substrate precluded this, intermediate cores were taken. In the small trench area an extremely high density of sampling was achieved (27 cores in 950 m^2). Whole core susceptibility measurements were carried out on all cores during the same period of field work using a low-field susceptibility bridge[14] temporarily installed in the Drapers Field Study Centre 3.5 km from the lake. Volume susceptibility (κ) versus depth graph plots were produced

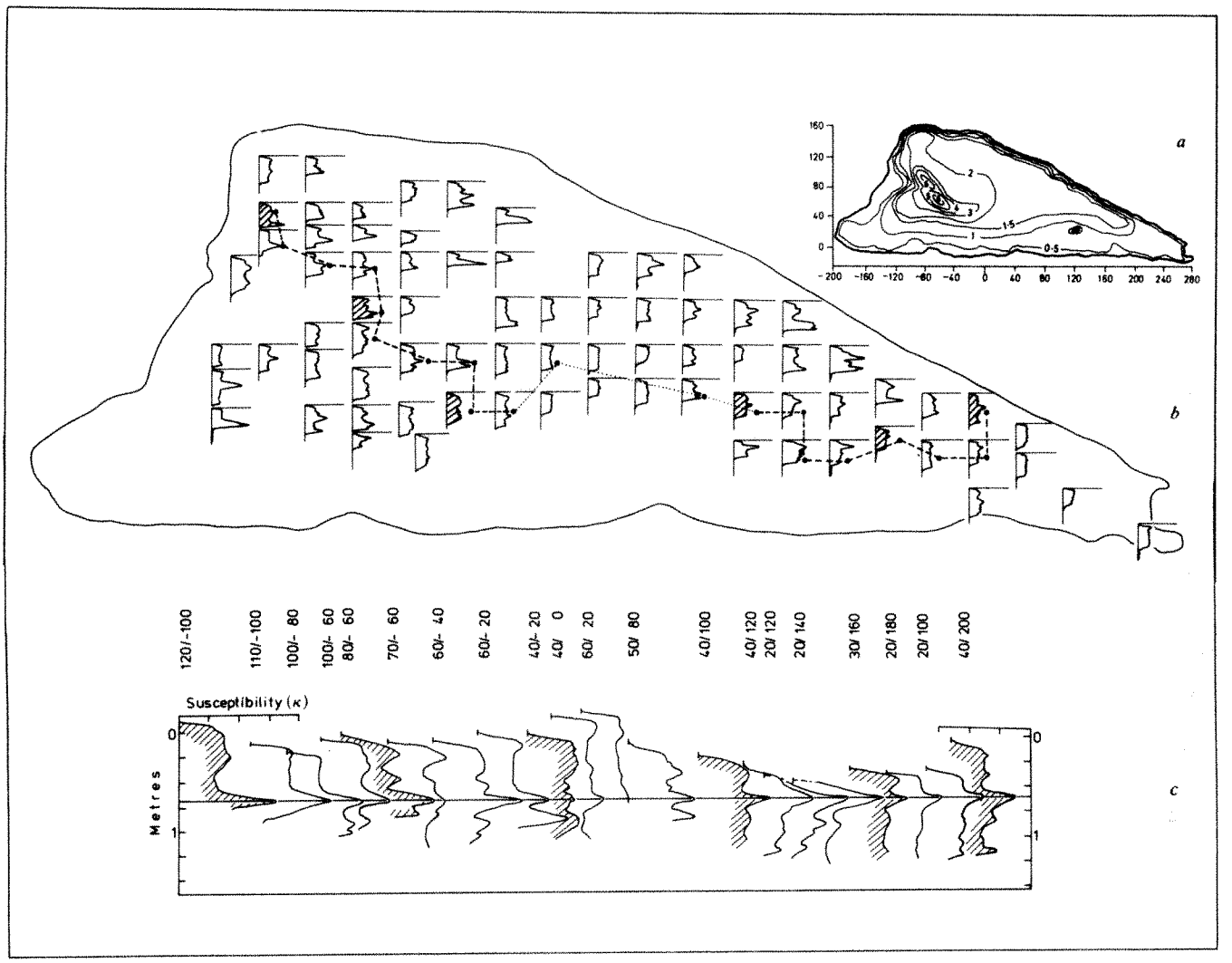


Fig. 1 Llyn Goddionduon: *a*, Bathymetric map and coring grid coordinates; *b*, whole-core susceptibility traces from the main coring grid. The 'tie-line' joins the cores from which the linked and correlated traces shown below in *c* were obtained. The cores for which single sample χ, SIRM and χ/SIRM ratios are plotted in Fig. 2 are identified by shaded traces.

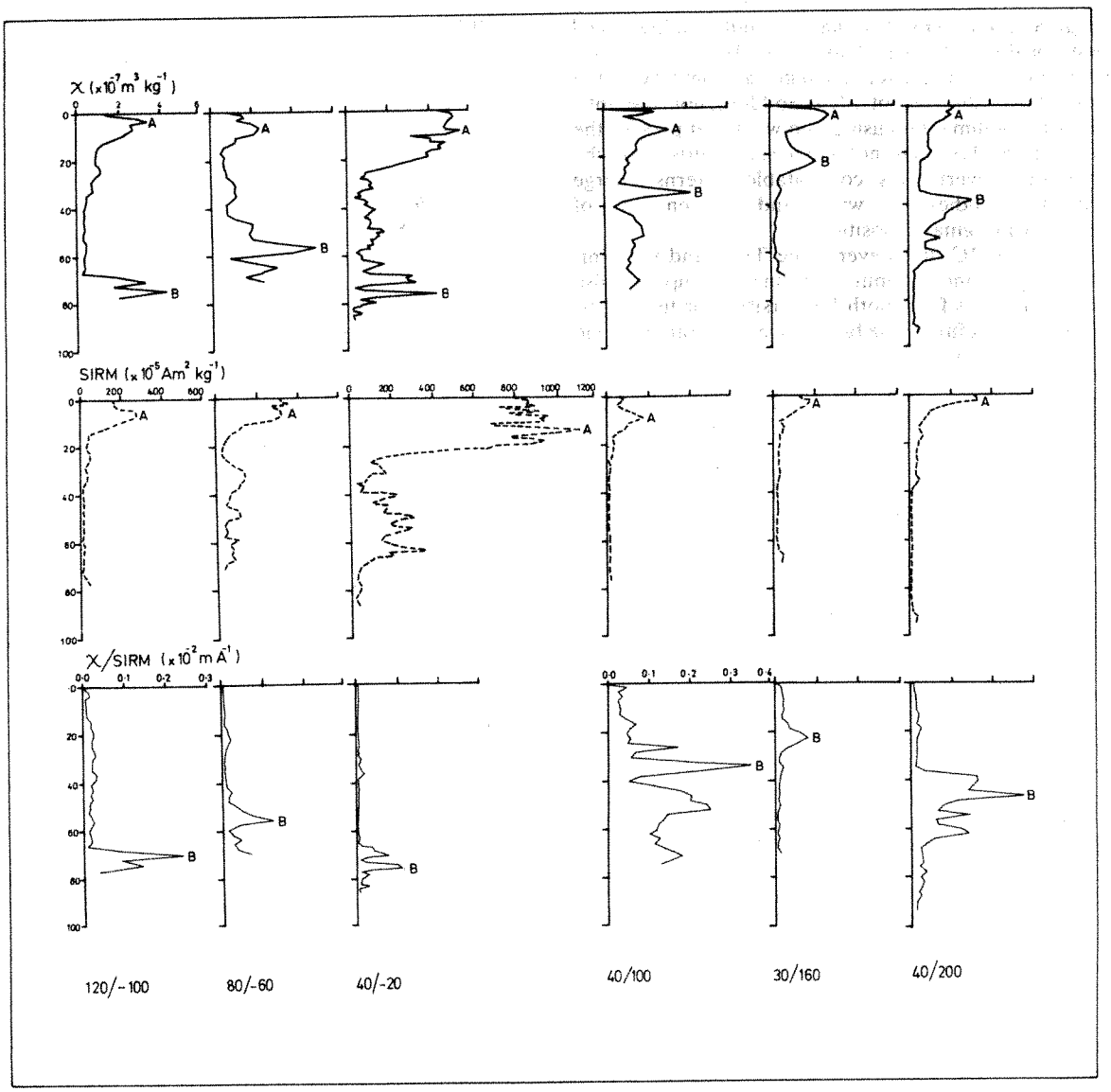


Fig. 2 Llyn Goddionduon. Single sample plots of χ, SIRM and χ/SIRM for six widely separated cores from the main grid. In all cases, the upper peak in χ and SIRM which gives low χ/SIRM values corresponds with the influx of magnetic minerals subsequent to a forest fire in 1951. The lower peak is clearly distinguishable from this in all cores by virtue of its high χ/SIRM ratios.

automatically immediately after sensing and it was thus possible to plan day-to-day field sampling priorities in the light of the results obtained within a few hours of taking each set of cores.

Subsequent studies based on extruded sediment have included measurements of specific susceptibility (χ), the intensity and direction of natural remanence (NRM), anhysteretic remanence (ARM), saturation isothermal remanence (SIRM) and coercivity of SIRM (B_{CR}). These magnetic measurements provide a wide range of rapidly calculated parameters and ratios which may be used to test core-correlation schemes. In addition chemical, particle size, loss-on ignition, pollen-analytical and radiometric studies are in progress.

Figure 1*b* shows whole core susceptibility traces for a selection of cores from the main grid of cores for the whole lake. In view of the paucity of Flandrian (Post-glacial) sediments in the tiny trench zone (<1 m in all 27 cores from only 5% of the lake), the complex deposition pattern of these cores has little effect on 'whole lake' investigations and the trench results can be satisfactorily assessed on the main 20-m grid. A 'tie-line' has been drawn down the length of the lake. The tie-line cores have been provisionally correlated using the scheme of whole core susceptibility peak matching indicated in Fig. 1*c* and substantiated in more detail by the plots of specific magnetic parameters from dried contiguous 1- or 2-cm subsamples presented in Fig. 2. The central zone in Fig. 1*b* reflects an area of more extended deposition within which the main susceptibility peak used in correlation lies below the depth of sediment penetrated by 1.3-m cores.

Figure 2 shows down core χ, SIRM and χ/SIRM ratios for dried sub-samples in, six widely separated cores located on Fig. 1. Peak χ/SIRM ratios associated with the main tie-line linking the sites, and low χ/SIRM ratios characteristic of the near surface peak (masked by the core tops in whole-core measurements) are common to all the cores and support the proposed correlation scheme. The uppermost sediment with low χ/SIRM ratio is attributable to the forest fire of 1951 (ref. 11) which occurred on the north-east edge of the drainage basin.

Studies in progress are designed to confirm the full pattern of correlation, to date each stage in accumulation, to characterise sedimentologically, chemically and magnetically the variations in deposition rate portrayed, and to relate them to the history of land use at the site decipherable in the pollen record. These studies will provide a basis for empirically based calculations of changing net sediment input versus age for almost all the lake basin for the time interval spanned by the majority of the cores.

Magnetic measurements now open up the prospect of core scanning and correlation with a speed, and on a scale, orders of

magnitude greater than conventional techniques allow. Well founded empirical calculations of total sediment input versus time thus becomes feasible even for lakes as complex as the present example. In the case of Llyn Goddionduon the anticipated pattern of sediment focusing into what is at present the deepest part of the lake has not been characteristic of the Flandrian period. Nevertheless, correlatable patterns emerge from the magnetic studies even within and between zones of relatively shallow marginal deposition.

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^{210}Pb dating of annually laminated lake sediments from Finland

USE of ^{210}Pb dating is increasing rapidly and applications include studies of accelerated eutrophication in major lakes[1], salt-marsh accretion[2], the recent history of heavy metal pollution[3] and accelerating soil erosion resulting from subsistence agriculture[4]. As dating models have increased in variety and complexity, it is important to compare models against precise and unambiguous independently derived time scales. In each area of application of ^{210}Pb dating, the inferences drawn from the calculated age–depth curves and the estimates of changing flux rates are often highly dependent on the ^{210}Pb dating model used. In this report ^{210}Pb-derived estimates of lake sediment age and dry-mass sedimentation rates are compared with ages and rates calculated directly by counting annual laminations. The results support a model of ^{210}Pb dating which assumes a constant net rate of supply (c.p.s.) of unsupported ^{210}Pb to the sediment despite fluctuations in dry mass sedimentation rates. Our findings underline the need for empirical evaluation of alternative ^{210}Pb dating models in the widest possible range of contexts. They also cast doubt on some published studies in which strongly 'kinked' profiles of unsupported ^{210}Pb concentration have been interpreted within the framework of conventional constant initial concentration (c.i.c.) assumptions.

Most workers using ^{210}Pb dating have assumed the initial unsupported ^{210}Pb concentration to be constant throughout a dated profile, or at least in large sections of the profile. This assumption can be satisfied in theory by a direct association between sedimentation and unsupported ^{210}Pb supply[5], or by a combination of constant unsupported ^{210}Pb flux and constant dry-mass sedimentation for all of[6], or segments[7] of, each profile. Authors using c.i.c. models have dealt with sharply inflected or non-monotonic ^{210}Pb-versus-depth profiles, or individual 'anomalous' values, by invoking alternative explanations such as physical mixing[8], bioturbation[9], diffusion[10] and co-precipitation of ^{210}Pb with manganese[11]. Where authors have referred to the alternative basic assumption of a constant net rate of supply (c.r.s.) of unsupported ^{210}Pb to the sediment independent of fluctuations in bulk sedimentation rate this alternative has usually been dismissed in favour of some variant of the c.i.c. model[12,13], or else it has been used but not empirically tested in a rigorous manner[2,14,15]. Recent applications of c.r.s. model calculations[16] to lake sediment sequences in Northern Ireland, the Highlands of Papua New Guinea[17] and Lake Michigan[18] provide strong, varied, but essentially circumstantial evidence for the validity of c.r.s.-derived age–depth relationships and dry-mass sedimentation rates. In each of these case studies, as in the example by Robbins[15], the choice between alternative models has a major impact on the results and their interpretation.

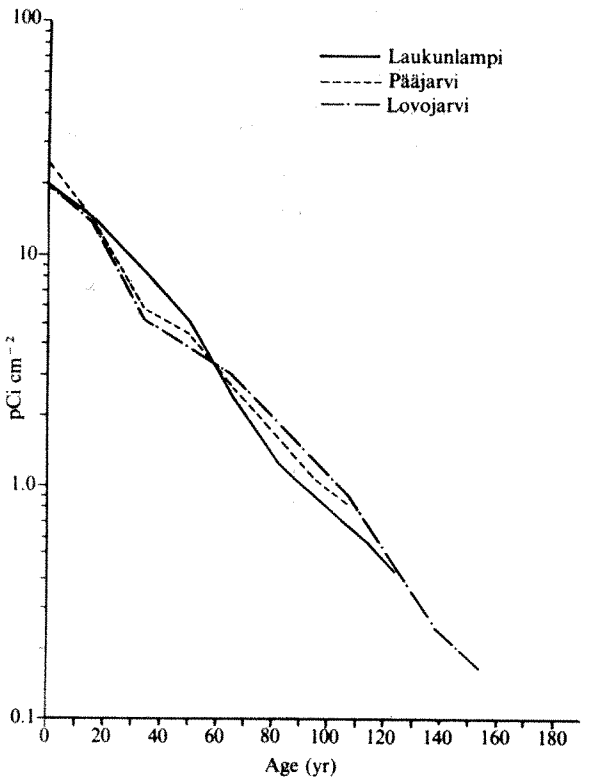


Fig. 1 Cumulative residual unsupported ^{210}Pb versus age in three annually laminated sediment profiles from lakes in east Finland. The approximation to a negative exponential function in each case confirms the validity of this as the appropriate dating parameter.

The sites from which the present evidence has been obtained provide a very precise and unambiguous basis for testing the alternative dating models. Not only are their recent sediments in the form of distinctive annual laminations, confirmed both biostratigraphically[19,20] and by ^{239,240}Pu and ^{137}Cs analyses (T. Jaakola, K. T., P. H. and S. Tiainen, unpublished), but they have also experienced disturbance in each catchment which has given rise to fluctuations in sedimentation rate strong enough to 'kink' the unsupported ^{210}Pb concentration-versus-depth curve.

In the c.r.s. model, the age t of sediments of depth x is calculated from[16]

$$A(x) = A(0)\,e^{-kt} \qquad (1)$$

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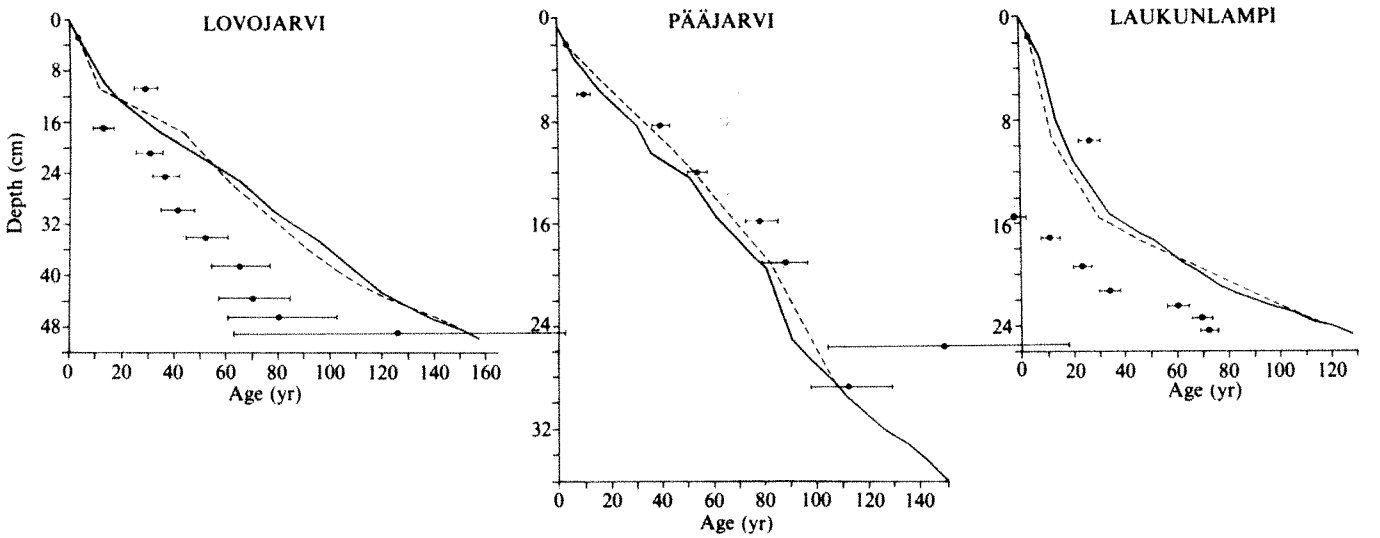


Fig. 2 Age–depth curves based on annual laminae- and on c.r.s.-model ^{210}Pb dating. ——, Laminae based curve; - - - -, c.r.s. model curve; ●, points on c.i.c. model.

where $A(x)$ is the residual unsupported ^{210}Pb beneath sediments of depth x, and k is the ^{210}Pb radioactive decay constant. The values of the dating parameter $A(x)$ for the Finnish lakes were calculated by numerical integration of the unsupported ^{210}Pb concentration with regard to the cumulative dry mass. The residual unsupported ^{210}Pb beneath the lowest datum point was estimated by assuming that the mean ^{210}Pb flux rate during the period covered by the observations was similar to the mean ^{210}Pb flux rate before this period. In Fig. 1, $A(x)$ is plotted against time for all three profiles, confirming the close approximation of this parameter to the theoretical relation expressed by equation (1). There is a close correspondence in the cumulative residual unsupported ^{210}Pb between sites, and hence in the ^{210}Pb flux rates, despite their physical separation, varied morphometry and contrasted sedimentation rates. The location and morphometric characteristics of each lake and drainage basin are summarised in Table 1, which also records mean ^{210}Pb flux rates, and total residual unsupported ^{210}Pb contents of each core.

Figure 2 compares for each site, the age–depth relationship derived from counting annual laminae, with the c.r.s. based ^{210}Pb age–depth profiles. In accordance with the assumption used in estimating the initial value of the dating parameter, the curves are synchronised at the lowest data point. The age–depth points derived using the c.i.c. model are also shown, although no attempt has been made to translate these into an age–depth

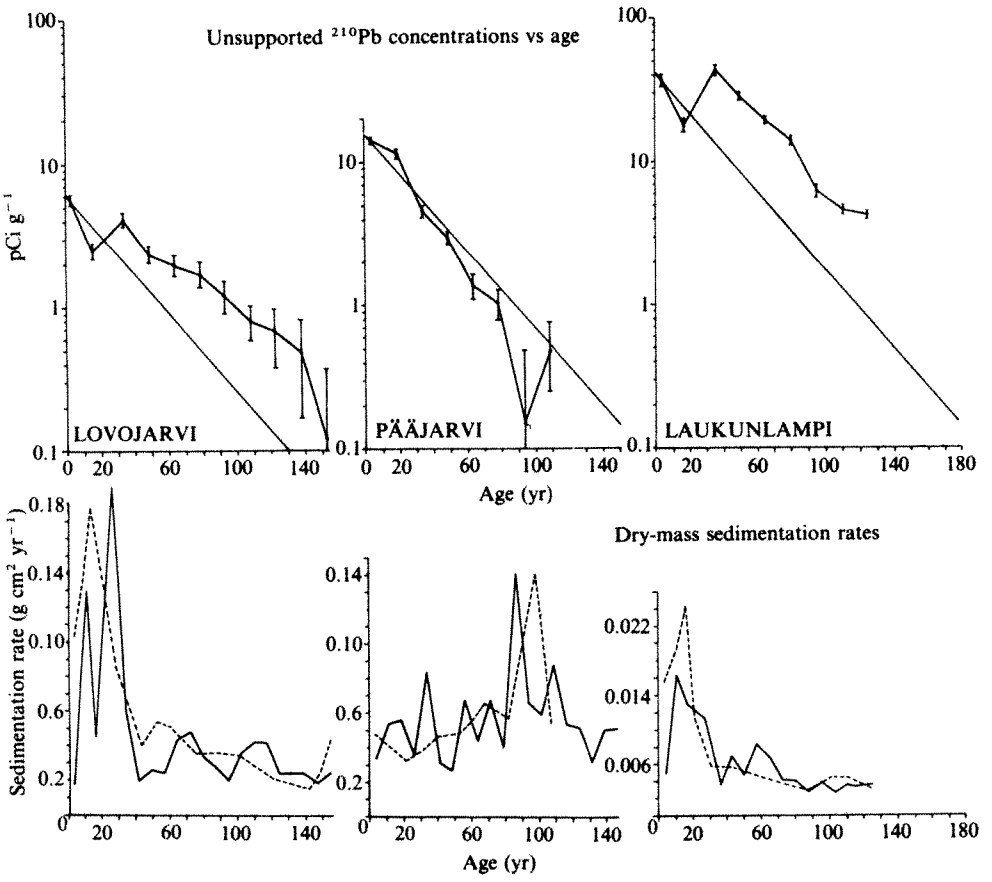


Fig. 3 Unsupported ^{210}Pb concentrations plotted against age (upper) and dry mass sedimentation rates based on annual laminae thickness and on c.r.s. model ^{210}Pb calculations (lower). In each sediment profile, the concentration-versus-age curve deviates significantly from the negative exponential function representing the expected radioative decay of ^{210}Pb and shown by the straight line. Each major inflection in the concentration-versus-age profile corresponds with peak dry-mass sedimentation rates determined from the annual laminae (——). Sedimentation rates based on c.r.s. model ^{210}Pb are shown as dashed line (- - -).

Table 1 Morphometric data, mean unsupported ^{210}Pb flux and total residual unsupported ^{210}Pb content

Lake	Area (A, hectare)	Drainage area (D)	D/A	Maximum depth (m)	Volume (m^3)	No. of direct inflows	Mean unsupported ^{210}Pb flux (pCi cm^{-2} yr^{-1})	Total residual unsupported ^{210}Pb content (pCi cm^{-2})
Lovojärvi 61°85′ N, 25°92′ E	4.8	570 hectare	119.1	17.5	36.9×10^4	6	0.65	20.09
Pääjärvi 61°04′ N, 25°05′ E	1,342.4	244 km^2	18.18	87	205.7×10^6	25	0.66	24.45
Laukunlampi 62°40′ N, 29°10′ E	8.4	21 hectare	2.5	26	—	0 (closed basin)	0.64	20.47

relation in view of their generally poor match with true age irrespective of which adaptation of the c.i.c. model is used.

Figure 3 shows changing unsupported ^{210}Pb concentrations plotted against time for each site compared with the dry-mass sedimentation rates derived from both the laminae counts and the c.r.s. calculations. In each case, depressed concentrations correspond with independently verified peak sedimentation rates, as required by the c.r.s. model.

At no point in any profile do the laminae- and c.r.s.-derived ages differ by more than 10 yr. At major points of inflection in each concentration-versus-depth profile, whether the inflections date from 5 to 10 yr or 100 yr before the year of sampling (1977–78), the c.r.s. calculations give an excellent approximation to true age. The close agreement is confirmed despite the need to interpolate between data points, an argument previously used to cast doubt on the validity of c.r.s.-based dating[13]. For dry-mass sedimentation rates, the agreement between laminae-based and c.r.s.-based estimates is less close partly because of the short time scale over which sedimentation rates appear to have varied in comparison with the ^{210}Pb sampling interval, and partly because the c.r.s. calculations of sedimentation rates are intrinsically more sensitive to the effect of experimental error than are the age–depth calculations. Nevertheless, the general characteristics of the changes in sedimentation rate are reasonably well portrayed by the c.r.s. calculations, within the limits of accuracy imposed by the sampling interval. Further studies incorporating mixing models[18], procedures for estimating patterns and rates of sediment focusing from ^{210}Pb measurements, comparison between ^{210}Pb derived ages and precisely dated influx events in non-laminated sediments, and systematic error analysis[21] are in progress. Treatment of over 40 ^{210}Pb profiles from 18 contrasted lacustrine and near-shore marine environments confirms that when independent tests of internal consistency and external validation can be applied to cases where dry mass sedimentation rates have varied through time, the c.r.s. model provides a better approximation to true age than do any of the alternatives. In no case encountered so far are any of the alternative dating models more accurate when used as a basis for estimating rates of sedimentation or mineral flux.

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Cinnamic acid inhibition of detritus feeding

AN important advance in ecology has been the general acceptance of Fraenkel's postulate that certain chemicals in plants deter herbivores[1–3]. Such chemicals are usually termed secondary plant substances because they are not involved in primary metabolic pathways. Very little of the annual production of biomass by higher plants is consumed by herbivores or phytopathogens[4]. Instead, most of the biomass becomes litter and eventually decays through the activity of decomposers. The secondary compounds that deter grazers while the plants are alive do not disappear immediately when plants senesce and die. We have therefore investigated whether these anti-herbivore substances continue to inhibit consumption by organisms feeding on litter or detritus. We report here that cinnamic acids, one type of secondary plant substances found in detritus, inhibit feeding by detritivores. This inhibition occurs at concentrations found in nature and may be a major factor controlling the rate of decay of organic matter.

In salt marshes most of the annual production by plants becomes litter. The most prominent saltmarsh plants in the eastern coast of the US are grasses of the genus *Spartina*. Unlike some grasses[5], tissues of *Spartina alterniflora* contain few or none of the compounds, such as condensed tannins, alkaloids or cyanogenic glucosides, which are the major deterrents to grazing in many terrestrial plants[2,6]. It seems likely that in *Spartina* the substances deterring herbivores are the soluble and insoluble esters of glucosides of hydroxyaromatic acids[7–9], also found in

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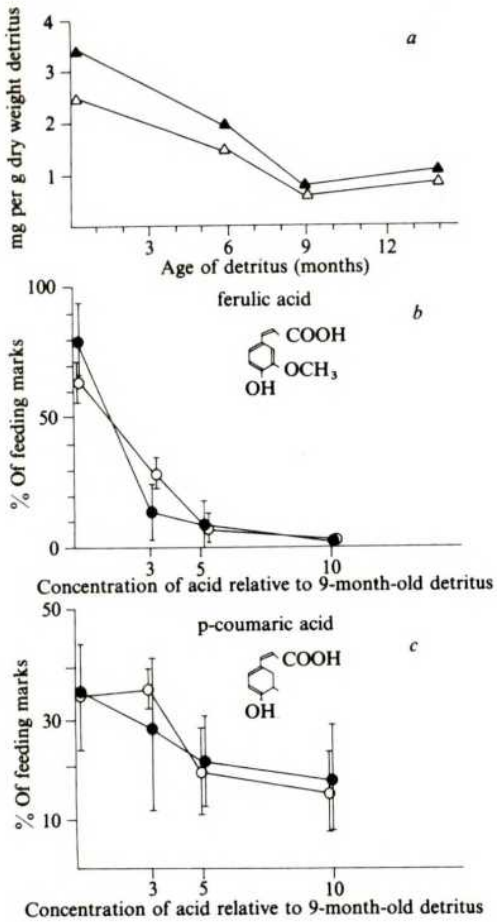


Fig. 1 *a*, Concentration of ferulic acid (▲) and coumaric acid (△) in litter of *Spartina alterniflora* of increasing age. *b*, *c*, Feeding response of *Orchestia grillus* (●) and *Melampus bidentatus* (○) to increased ferulic acid (*b*) and *p*-coumaric acid (*c*) in their food.

Sorgum bicolor[10,11]. These compounds are readily hydrolysed by hydrolases in the plant to form the actual deterrents, the corresponding free acids[10,11]. Our work focused on ferulic acid and *p*-coumaric acid (Fig. 1), cinnamic acids that are abundant in the cell walls of *S. alterniflora*.

We extracted free cinnamic acids from tissues of dead *S. alterniflora* in 80% methanol at 85 °C for 10 min. The extract was cooled for 20 min and filtered through a Millipore filter; the procedure was then repeated. The unhydrolysed extract of detritus of four different ages was analysed by TLC and spectrophotometry for ferulic acid (320 nm) (Fig. 1*a*). Both acids decreased in concentration with the age of the detritus so that after 9 months of natural ageing in the field only one-third of the initial concentration of cinnamic acids remained in the detritus.

We tested the effect of ferulic and *p*-coumaric acids on feeding by two saltmarsh detritus feeders, the amphipod *Orchestia grillus* and the snail *Melampus bidentatus*. We suspended ground, dead grass in agar in a Petri dish with four compartments. This agar suspension method of testing palatability was developed by Gieselman[12]. The amphipods and snails fed very well on the suspension and both species fed on the agar only when detritus was present.

We tested the effect of ferulic and *p*-coumaric acids by adding them to the agar suspensions of 9-month-old detritus of *S. alterniflora*, plating the suspensions, allowing the test animals to feed. The detritus was aged naturally in the field. We added 0, 3, 5 and 10 times the amounts of acids found in 9-month-old detritus, and each of these four doses was included in every Petri dish. To each of these dishes we introduced four amphipods or snails. The dishes were incubated in the dark for 3 h. Feeding was assessed by the number of feeding marks left on the surface of the agar suspension. Marks were easily counted and clearly reflected the difference in mouth parts and feeding mechanisms of the two species (Fig. 2).

An increase in the concentration of both ferulic and *p*-coumaric acids decreased feeding activity by both *O. grillus* and *M. bidentatus* (Fig. 1*b*, *c*). Ferulic acid was more effective than *p*-coumaric acid. When feeding on recently dead plant tissues, the detritus feeders are exposed to concentrations of these acids up to three times that found in 9-month-old detritus (Fig. 1*a*). We can expect, therefore, that detritus of 9 months or older could be significantly more palatable than new detritus due to the lower content of ferulic acid. Concentrations of more than five times the amount of *p*-coumaric acid in 9-month-old dead grass reduced feeding. Because we did not find such high concentrations in litter or detritus of *S. alterniflora*, *p*-coumaric acid may not be as important as ferulic acid in deterring detritus feeders.

The mechanism that makes ferulic acid distateful is unclear. Detritus feeders must taste the acidity as well as other flavours of the compound. In preliminary tests with ferulic acid buffered at neutral *p*H amphipods, but not snails, were inhibited, suggesting that some detritus feeders are deterred by acidity, whereas others are inhibited by sour or bitter tastes.

Other investigators have looked at microbial density and carbon and nitrogen contents as providing different ways of measuring the food quality of aquatic detritus[13,14]. Our evidence suggests that secondary plant substances such as ferulic acid are important in determining whether organic matter is consumed by detritus feeders. Further, because there are marked changes in the amounts of these compounds in litter as it ages and becomes detritus, the time at which food may become palatable

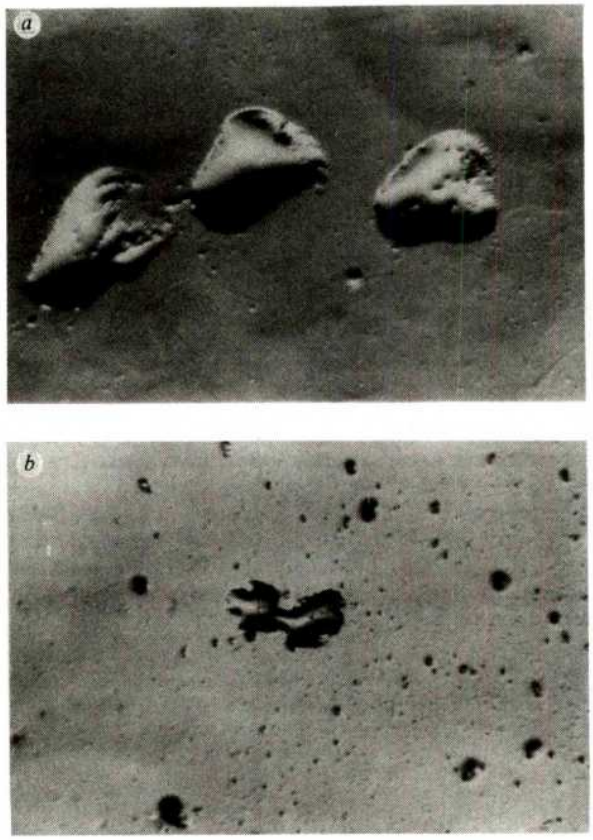


Fig. 2 Feeding marks of *Melampus bidentatus* (*a*) and *Orchestia grillus* (*b*) on the surface of an agar–detritus suspension. The marks are about 0.4 mm for *M. bidentatus* and 0.3 mm for *O. grillus* at their longest dimension. The radular marks left by the snail (*M. bidentatus*) are single whereas the amphipod (*O. grillus*) leaves double marks as a result of biting with paired mandibles.

to decomposers may also be determined by secondary plant substances.

The rate of decay of organic matter is accelerated by the comminuting of detritus by detritivores. The smaller particles provide new surfaces for microbial attack. Further, passage of detritus through the digestive system of detritivores also alters the nature of the particles, facilitating microbial reinvasion and also accelerating rates of decay. Secondary plant substances present in detritus, by inhibiting feeding by detritivores, thus delay the rate at which organic matter decays. It is also possible that microbial activity itself may be inhibited by such substances. This needs study, but it is clear that cinnamic acids, inherited from live plants, and perhaps other compounds, have a critical role in determining rates of decomposition of detritus. Thus, secondary plant substances may be a major control mechanism in ecosystems such as marshes where the detritus food web dominates the flow of carbon.

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Isolation and cultivation of diatom symbionts from larger Foraminifera (Protozoa)

THERE has been increasing interest in larger Foraminifera that have symbiotic algae which apparently adapt them to thrive in certain shallow, nutrient-poor tropical seas where they are responsible for a significant or even dominant fraction of the carbon fixed in the benthos[1,2]. Studies of fine structure of several species of these giant protozoa have suggested that their algal symbionts are diatoms[6–12] without frustules (shells). This suggestion is itself remarkable because almost all marine invertebrate–brown algal associations (for example, those of corals and tridacnid clams) involve dinoflagellates. Unfortunately, knowledge of the architecture of the frustule is needed for the identification of a diatom. However, because the formation of the outer envelopes of the dinoflagellate and chlorophyte endosymbionts of other large species of Foraminifera is repressed within the hosts[6,713] but not in culture[5,14], we hoped that if the diatom endosymbionts could be isolated and cultivated they would form characteristic frustules. We report here the successful isolation and cultivation of diatom symbionts from foraminifera.

With the aid of SCUBA, *Heterostegina depressa*, *Amphistegina lessonii*, *A. lobifera* and *A. papillosa* were collected at 20–35 m in the Gulf of Elat (Red Sea) near Geziret Faraoûn anchorage. Individual animals were placed immediately in sterile dishes and under a dissecting microscope they were meticulously and aseptically brushed, scraped and washed through successive baths of antibiotic-containing sterile seawater to remove all external algae[5,14]. After processing, each animal was examined with a water immersion lens of a compound microscope to assure removal of all external algae. Individual animals were transferred aseptically to test tubes of sterile seawater and crushed carefully with sterile glass rods to release the symbionts. The algae were then inoculated into isolation media[15].

Growth of algal colonies was observable under a dissecting microscope after 4–8 d. The growth of large numbers of colonies of the same types[15] from each host is strong evidence that the algae isolated were not contaminants. If the isolates were undigested food organisms, then their hosts were filled with them. Growth of transfers was poor and restricted to media containing vitamins B_{12}, thiamine, biotin or mixtures of vitamins (media 15, 17 and 18)[15]. The isolates are now being maintained in a natural seawater medium (~40‰) enriched with: 10 mg % (w/v) yeast extract, 1 ml % (v/v) soil extract, 0.1 μM vitamin B_{12}, 1 μM biotin,and 1 μM thiamine.

All isolates were small pennate diatoms (3–14 μm), and two were previously undescribed. Some Foraminifera contained two

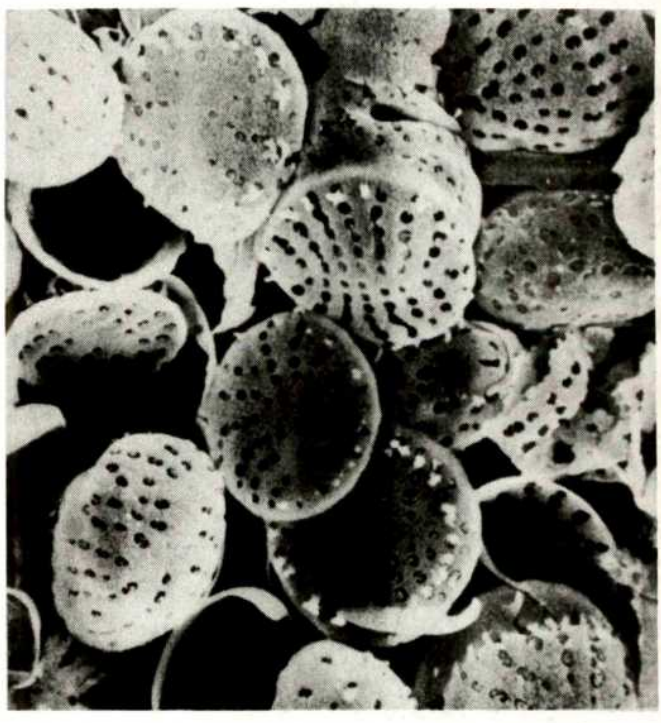


Fig. 1 A new species of zooxanthellae, *Fragilaria* sp., isolated from specimens of the large Foraminifera, *Amphistegina lessonii*, *A. lobifera* and *A. papillosa* collected in the Gulf of Elat (×8,100).

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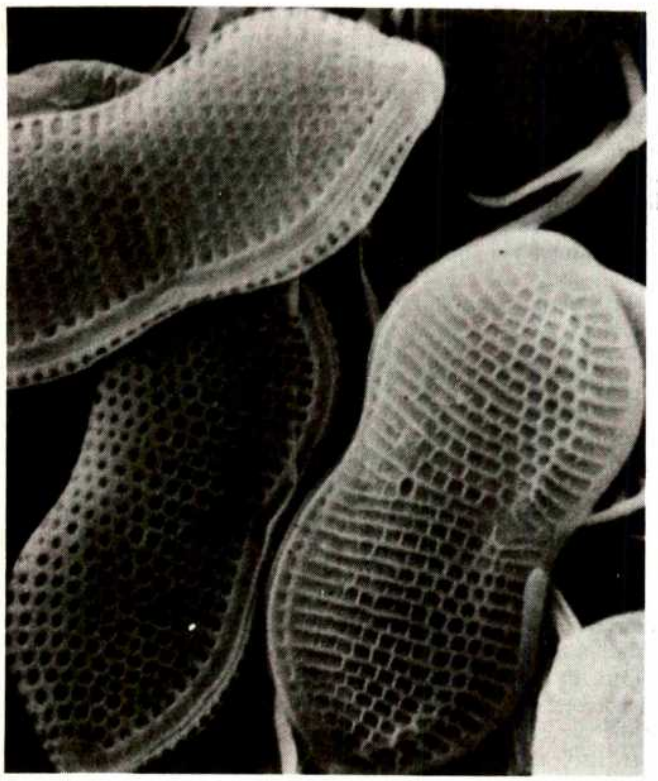


Fig. 2 *Nitzschia panduriformis* v. *continua* Grun. isolated from all specimens of the larger foraminiferan *Heterostegina depressa* and a few specimens of *Amphistegina lobifera* collected in the Gulf of Elat (×5,575).

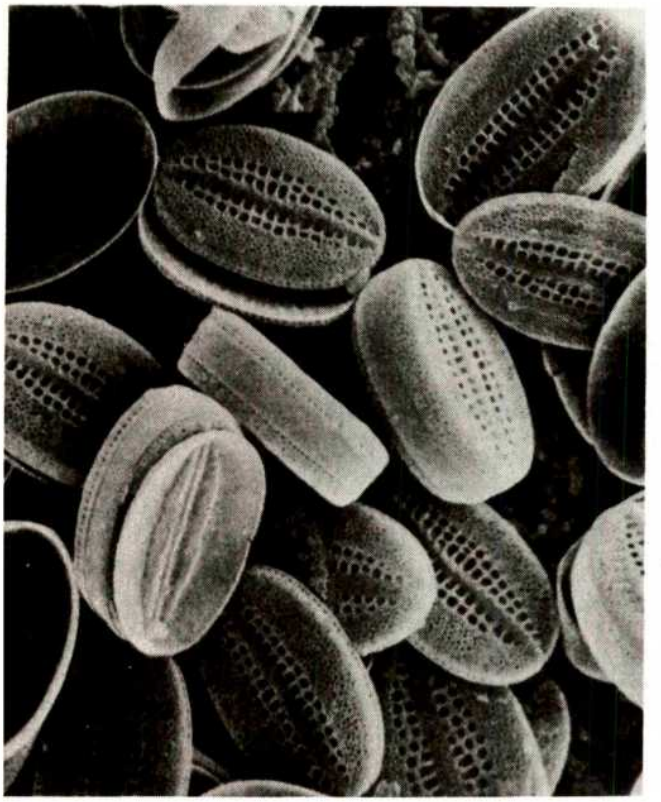


Fig. 3 A new naviculoid diatom isolated from some specimens of *Amphistegina papillosa* collected in the Gulf of Elat (×2,400).

or rarely three different diatom species. A very small new species of *Fragilaria* (Fig. 1) was found in specimens of all three species of *Amphistegina*. *Nitzschia panduriformis* v. *continua* was found in *Heterostegina depressa* and a few *A. lobifera* (Fig. 2). Several *A. papillosa* contained an unusual new naviculoid diatom (Fig. 3). *Amphora tenerrima*, perhaps an episymbiont, was isolated from the exterior surfaces of both *H. depressa* and *Amphisorus hemprichii*. A manuscript containing full descriptions of the new diatoms is in preparation. None of the symbiont-diatom species was abundant in the epiphytic community at the time of collection.

Traces of chlorophyll *b* in extracts of pigments from intact animals suggested that *Amphistegina* spp. and *Amphisorus hemprichii* also contained symbiotic zoochlorellae. We isolated and grew clones of green algae from them.

The isolation of these symbionts paves the way for physiological–ecological studies which will advance our understanding of the carbon budgets and depth distributions of their hosts. When it is possible to obtain aposymbiotic hosts without killing them, we should be able to satisfy Koch's postulates and explore questions of host specificity and host–symbiont interactions. Research along these lines will be particularly exciting because algal endosymbiosis may have been a driving force in the evolution of these giant protozoa and a key to their great success.

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Changes in the circuitry of the kitten visual cortex are gated by postsynaptic activity

MOST neurones in the visual cortex of normal adult cats are binocular and respond very selectively to elongated contours of a particular orientation[1]. If a kitten is deprived of vision in one eye during a critical period in early life the afferents from this eye lose the ability to excite cortical cells[2]. This functional deafferentation is, however, reversible if, still within the critical

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period, the previously open eye is closed and the other opened[3]. These changes in afferent connectivity are attributed to competitive interactions between the pathways from the two eyes[4,5], which have been shown to occur at the level of the striate cortex[6]. We previously demonstrated[7] that afferents from the deprived eye remain functional when activity from the open eye is enhanced but fails to drive the common cortical target cell. This had suggested that the competitive suppression of one subset of converging afferents requires, in addition to imbalance in presynaptic activity, that the postsynaptic neurones actually respond to the more active pathways. Such postsynaptic gating was first postulated by Hebb[8], and since then it has been assumed in most models on adaptive neuronal connections. In the study reported here more direct evidence for the participation of postsynaptic factors both in competitive suppression and in functional recovery of converging afferents was obtained. The results support Hebb's early hypotheses and demonstrate that both changes in connectivity are in fact guided by postsynaptic response properties.

Three kittens were dark reared until the age of 5 weeks and then, with one eye closed, exposed to a normal environment for 9 consecutive days. Control experiments and a number of previous studies have shown that by this time most cortical cells have become dominated by the open eye and display orientation selectivity comparable to adult standards. At this age the initial shift in ocular dominance can still be reversed when the previously open eye is closed and the deprived eye opened[3]. If this functional reorganisation of afferent connectivity required responses from postsynaptic cells the following prediction can be made: when the spectrum of orientations visible by the open eye is restricted to a narrow range, reversal in ocular dominance should occur only for those neurones whose response properties enable them to react to the range of visible orientations. Cells with other orientation preferences that have no predisposition to respond to the signals from the open eye should remain connected to the eye that was open first and had experienced the normal environment.

We used artificial astigmatism[9] to produce effective restriction of the range of visible orientations during this second exposure period. A cylindrical lens of −25 dioptres was mounted in a polyurethane mask and fitted in front of the initially deprived eye while the other, previously open, eye was occluded. Photographs taken through the lens of the environment in which the kittens were exposed confirmed that only orientations within ±10° of the zero axis of the lens remained visible. Apart from the minimal counter-rotation of the eyes (~10% of tilt angle[10]) this range remains constant with respect to retinal coordinates regardless of head and body movements. The kittens were therefore allowed to freely explore their environment, which contained high-contrast contours of all orientations. In two kittens vision was restricted to horizontal orientations and in one to vertical orientations. After 10 days of 10 h exposure daily (during the night the kittens were returned to the dark room) the kittens were again kept in the dark for a few days until the experiment. For convenience the eye that was open first is referred to here as the 'first eye', and the eye that had seen only the restricted range of orientations is referred to as the 'second eye'.

The preparation and recording techniques were conventional and are described elsewhere[11]. The orientation selectivity and the ocular dominance of single neurones in area 17 were determined from manual field plots and from additional averaged response histograms.

As expected, after sequential monocular occlusion, the large majority of neurones were monocular (Fig. 1*a*); but the reversal of ocular dominance was incomplete. The second eye had regained dominance only over those cells whose receptive field orientations matched the visible spectrum of orientations (Fig. 1*b*). In these cells the afferents from the first eye had become ineffective; they remained, however, efficient in neurones whose orientation preference did not correspond to the range of orientations seen by the second eye (Fig. 1*c*). This indicates that circuit changes had occurred only for those neurones whose functional predisposition had enabled them to react to the activity from the second eye.

This conclusion is further supported by the distribution of orientation preferences of cells in different ocular dominance classes. Comparison of Fig. 1*e* and *f* shows that these polar distributions are complementary to each other. As expected, the cells which are now dominated by the second eye (ocular

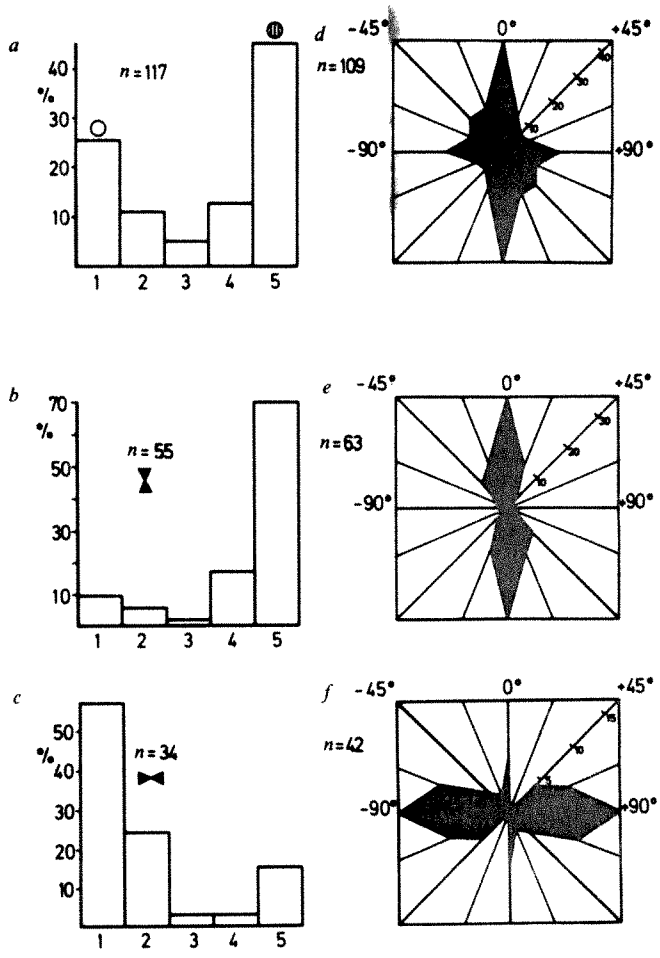


Fig. 1 Ocular dominance (OD) distributions (*a–c*) and orientation preferences (*d–f*) of neurones in the visual cortex of three kittens after reversal of monocular deprivation and selective exposure of the newly open eye to a narrow range of orientations. Number of neurones analysed, 176; those responsive to light, 127 (72%); those visually unresponsive, 49 (28%). Both ocular dominance and orientation selectivity fully determined, 117. Orientation selective or orientation biased, 109 (93%); non-orientated, 8 (7%). Ocular dominance distributions are plotted separately: *a*, for all neurones together irrespective of their orientation preference; *b*, for neurones with orientation preferences corresponding to the orientation (±22.5°) experienced by the second eye; and *c*, for neurones with orientation preferences orthogonal (±22.5°) to those experienced during the second stage of exposure. OD classes 1 and 5 refer to monocular cells excitable exclusively from the originally open eye (class 1) and the eye that was open last and viewed a narrow range of orientations (class 5). OD class 3 comprises binocular cells that are equally responsive to either eye and classes 2 and 4 contain binocular cells in which the first or second eye, respectively, is dominant. Polar plots of preferred orientations: *d*, independent of ocular dominance; *e*, for neurones driven monocularly or predominantly by the second eye; *f*, for neurones driven monocularly or predominantly by the first eye. The radius of the graph at each intersection gives the number of cells with that particular orientation preference. Angles from 0 to ±90° correspond to the difference between the orientation that was seen by the second eye and the neurones' preferred orientations.

dominance classes 4 and 5, Fig. 1*e*) have orientation preferences that match the narrow range of orientations visible during the second stage of exposure. In addition, cells with these properties are under-represented in the population of cells still dominated by the first eye (ocular dominance classes 1 and 2, Fig. 1*f*). This complementarity in orientation preference indicates that: (1) cells now dominated by the second eye were recruited from the pool of neurones initially dominated by the other eye; and (2) this recruitment must have been guided by the response properties of the cortical cells recruited.

These results all support the hypothesis that both suppression and enhancement of excitatory transmission in converging pathways are gated by a matching process between the pattern of presynaptic activity and the response properties of the common postsynaptic target cell. Thus, for the present paradigm, Hebb's[8] classical postulates on adaptive synaptic connections appear to be fully confirmed. Transmission is enhanced selectively in those pathways that are capable of exciting the postsynaptic target and are contingently active with the latter. On the other hand, those afferents which fail to excite the postsynaptic target become suppressed while the target is activated by other converging afferents. Such a mechanism implies then, that activity from the eyes can only select from among preexisting connections and only within the limits set by the response properties of the postsynaptic targets. Moreover, as functional recovery after reversal was guided by postsynaptic responses, as apparent from the complementary orientation distributions, one has to infer that the initial disconnection has not been completed by the time of reversal. In view of the short period of preceding monocular deprivation and the rapid recovery after reversal it seems possible that, at this early stage of monocular deprivation, synaptic transmission through the deprived pathway is only weakened while the connections themselves persist. This would allow at least sub-threshold activation or even occasional spiking of the cortical target cells in response to stimulation of the deprived eye. Such activation would then act as the postsynaptic signal for initiating the re-establishment of fully functioning synaptic connections.

While this manuscript was in preparation Cynader and Mitchell[6] reported experiments in which previously inexperienced kittens were exposed with one eye to a restricted range of orientations while the other was allowed to view normally. They found orientation-dependent changes in ocular dominance and therefore concluded that the competitive interactions between afferents from the two eyes occur at the cortical level. Our present data fully support this conclusion and advance the results of Cynader and Mitchell in that they provide the neuronal mechanism through which the response properties of postsynaptic target cells gate the consolidation and the disruption of converging afferents. The generality of the mechanism proposed in this report is emphasised by the fact that it not only predicts correctly the disruption of pathways observed by Cynader and Mitchell but also, as described above, the selective recovery of previously inactivated connections.

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Orientation of horizontal cell axon terminals in the streak of the turtle retina

NEURONES which respond preferentially to slits of light of a given orientation have been found at the ganglion cell level of the retina[1], as well as at higher centres of the visual system[2–4]. Present knowledge about the morphology of such cells and the organisation of the inputs to these cells does not account for the origins of their specialised receptive field properties. We report here that the horizontal cells impaled in the visual streak of the turtle retina (a specialised linear area centralis[5] analogous to the fovea of higher vertebrates) respond preferentially to slits of light oriented parallel to the streak and thus parallel to the horizontal meridian of the animal's visual field[5]. Anatomical studies demonstrate that these horizontal cells are all elongated and oriented in the direction of the streak. Thus, our findings show that a class of neurones exists in the retina whose morphologies may form the basis for their specialised receptive field properties.

The techniques used for intracellular recording from neurones in the eyecup of the turtle, *Pseudemys scripta elegans*, and the criteria for horizontal cell identification, have been described previously[6–8]. The light stimulus was a slit, 0.1 mm wide and 3.0 mm long. The centre of the impaled unit's receptive field was determined as the position yielding the largest response to flashed slits of light oriented along two mutually perpendicular axes.

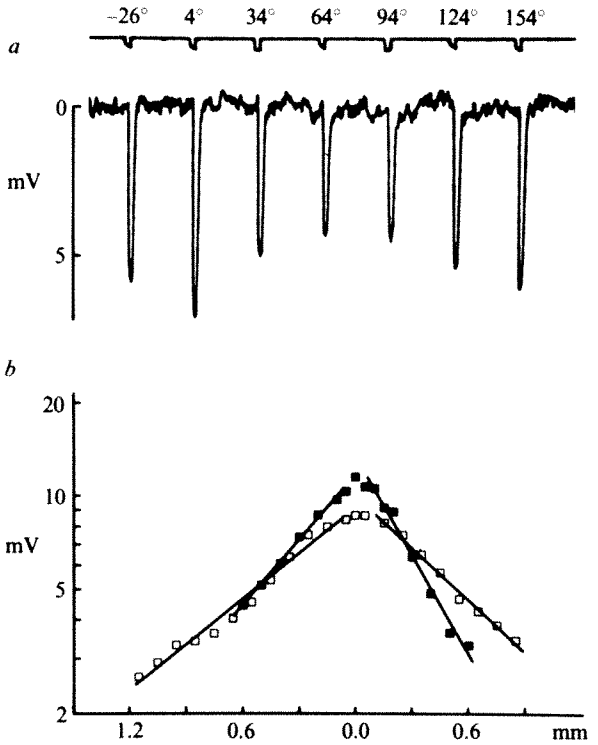


Fig. 1 Receptive field asymmetries in an L_1-type horizontal cell in the visual streak of turtle retina. *a*, Photoresponses to slits of dim light centred over the receptive field and flashed at 6 s intervals with the orientations given on top of the figure. Angles were measured with respect to the streak orientation (defined as 0°). *b*, Semilogarithmic plot of the peak amplitudes of photoresponses to slits of light as a function of displacement of the slit along directions parallel (□) and orthogonal (■) to the streak. Lines drawn through data points are the best fit to an exponential as determined by a least squares criterion. The slit intensity in B was 3 times that used in A. The maximum response elicited in this unit with bright, 3.2 mm diameter spot illumination was 40 mV.

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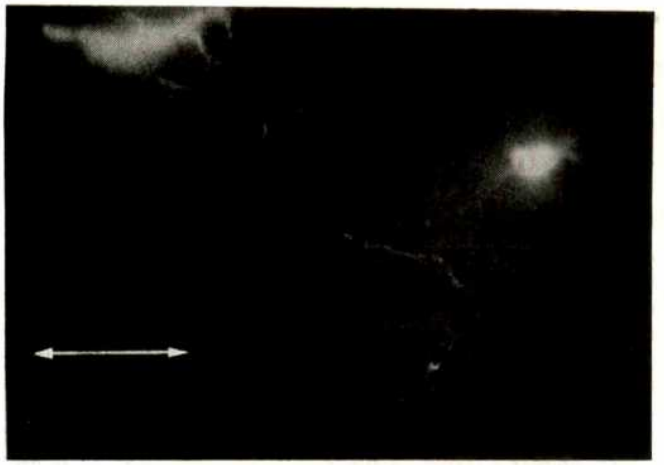


Fig. 2 Lucifer-stained horizontal cell from which intracellular responses of Fig. 1 were recorded. Lucifer has stained the axon terminal (upper left), axon and cell body. The recording site was the axon terminal as indicated by Fast Turquoise marking. Streak direction and scale are indicated by arrow. Scale bar, 30 μm.

The receptive field properties of seventeen luminosity type horizontal cells (L_1-type) lying within 75 μm of the visual streak of the turtle retina were studied. A slit of dim light intensity was centred over the unit's receptive field and flashed at various orientations. Typical responses are shown in Fig. 1*a*. In this unit, as in all others, the smallest and largest responses were elicited by slits of light oriented approximately perpendicular and parallel to the streak, respectively. The ratio of the smallest to the largest amplitudes recorded (the response eccentricity) was 0.59 for the unit of Fig. 1*a* and the average for all seventeen units was 0.66 ± 0.1 (s.d.).

This non-isotropy in the receptive fields was further investigated in eight units by measuring space constants[9] in directions parallel and orthogonal to the streak. Figure 1*b* shows a semi-logarithmic plot of peak voltage against slit displacement. The response evoked when the slit was centred over the unit (0.0 in Fig. 1*b*) and parallel to the streak (filled squares) was larger than that evoked by a slit orthogonal to the streak (open squares). Furthermore, the decay of potential with distance from the centre of the receptive field was approximately exponential along each axis and the fall off was greater for slit displacements in the direction perpendicular to the streak (filled squares). The space constants measured on each side of the unit's receptive field centre were different and were averaged to give a mean space constant for each direction. For the unit of Fig. 1*b*, the mean space constants for displacements orthogonal and parallel to the streak were 535 μm and 831 μm, respectively. The ratio between the two space constants provides an index of the asymmetry of the receptive field, and was 0.64 for this unit and 0.73 ± 0.09 on the average.

Lamb[9] has shown that for dim intensities, the response V, evoked by a slit of width a, centred over a horizontal cell receptive field is related to the space constant λ, by the function $V = E(1 - \exp(-a/2\lambda))$ where E is the response of the cell to a full field stimulus. In this model, λ is related to the sheet and membrane resistances, R_s and R_m, respectively, by $\lambda = (R_m/R_s)^{1/2}$. We have modelled the horizontal cell network in the streak as a two dimensional conductive sheet where the sheet resistance differs in directions parallel and perpendicular to the streak. For this non-isotropic model, the ratio of the voltages calculated for λ values parallel and perpendicular to the streak, $V_\perp/V_\parallel$, gives an expected response eccentricity which can then be compared to the measured response eccentricity. For the conditions of the experiments described here, $a/2\lambda \ll 1$, so this ratio can be approximated by $V_\perp/V_\parallel \simeq \lambda_\parallel/\lambda_\perp$. Because the measurements of response eccentricities and space constants were both functions of intensity, data obtained with the same intensity slit were used for a meaningful comparison. The mean difference between predicted and measured response eccentricities was 0.01 ± 0.08. These findings support the view that the receptive field asymmetries of L_1 horizontal cells as revealed by measurements of space constants and response eccentricities reflect summation within a linear, but anisotropic network where the attenuation of signals is greater in the direction perpendicular to the streak than parallel to it.

Intracellular injection of a mixture of Fast Turquoise[10] (1.2%) and the fluorescent dye, Lucifer[11] (3%), was attempted in all seventeen L_1 units and an example is shown in Fig. 2. All six successful stains revealed axon terminals which were elongated and oriented parallel to the streak. In some instances, a faintly stained axon and cell body were also visible (Fig. 2). Luminosity type horizontal cells in the turtle, as in other species, have an axon terminal and a cell body which are connected by a fine axon[11–13] but which are electrically independent[7,12]. The brighter stains of axon terminals, and the Fast Turquoise marking indicate that the L_1 units are axon terminals.

Anatomical studies of Golgi-Colonnier impregnated horizontal cells in the streak region of the turtle retina provided a morphological correlate for the physiological findings described above. All Golgi-impregnated axon terminals of horizontal cells in the streak region were observed to be elongated and oriented parallel to the streak as shown in Fig. 3. Each axon terminal consisted of a thick, tuberous process 8 μm in diameter and 50 μm in length with numerous small terminals projecting to the photoreceptors. Each axonal arborisation occupied an approximately rectangular space on the retina, with mean dimensions of 20×50 μm. Also shown in Fig. 3 is a cell body of a horizontal cell with its fine axon.

The actual dimensions of the axonal arborisations (Fig. 3) cannot account for the large receptive fields measured in L_1 units of the turtle streak. There is considerable physiological[7,9,14–18] and anatomical[19–23] evidence that horizontal cells in the vertebrate retina are extensively electrotonically coupled via gap junctions, thereby forming a conductive sheet across the retina. The elongated shape of the axon terminals in the turtle streak, and their common orientation parallel to the streak suggests that

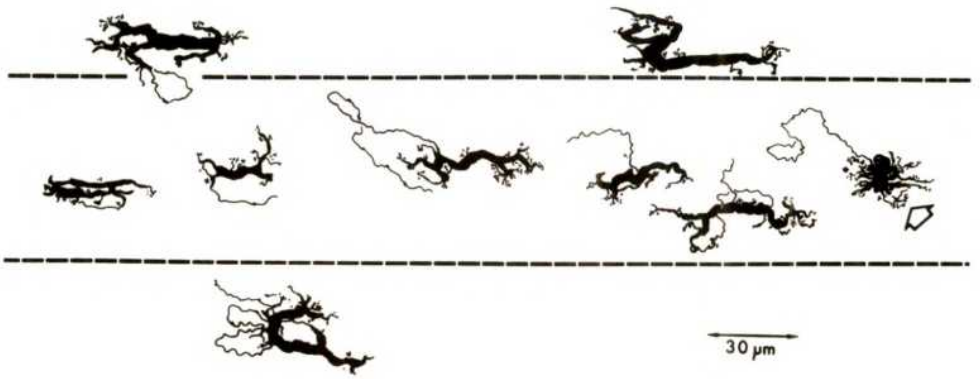


Fig. 3 Drawings of Golgi-Colonnier impregnated horizontal cell axon terminals in the visual streak region (area between dashed lines). The axon terminals are connected via the thin curly axon to horizontal cell bodies of the type illustrated (arrow). The axon terminals have arborisations elongated in the direction of the streak.

the mean distance between gap junctions linking neighbouring L_1 units should be greater in the direction parallel to the streak than perpendicular to it. Assuming that cytoplasmic resistance is much lower than junctional resistance, the signal attenuation will occur mainly at these junctions. We further assume a uniform distribution of gap junctions along the cell surface. The signal attenuation per unit length normal to the slit stimulus will then be greater in the direction perpendicular to the streak resulting in receptive fields elongated along the streak.

The mechanism described above is based upon a network of L_1 units each of which has a common orientation. A random distribution of orientations of elongated cells would result in more equal mean distances between junctions along any two orthogonal directions. This type of anatomical arrangement is in fact seen in Golgi-impregnated L_1-type horizontal cells in the peripheral retina. Here, our preliminary physiological findings indicate that L_1-type horizontal cells have response eccentricities closer to 1.0 with no consistently observed preferred orientation along any particular axis.

In this paper, we have shown that the streak region of the turtle retina contains at least one population of cells with specialised properties; the axonal arborisations and the receptive fields of L_1 horizontal cells in this region are elongated in the direction of the streak. The functional significance of the specialisations in the streak region may be better understood from ganglion cell recordings in this region. However, such measurements have only been made in the visual streak of another species, the rabbit[1]. Amongst rabbit ganglion cells in this region, orientation selective units were found which responded only to slits of light which were oriented either parallel or perpendicular to the streak. Although a full understanding of receptive fields at the ganglion cell level requires a greater knowledge of signal processing in the inner and outer plexiform layers, the oriented horizontal cell receptive fields described here may have significance for the development of orientation specificity in the visual streaks of species whose retinas contain this specialisation.

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Involvement of cyclic GMP phosphodiesterase activator in an hereditary retinal degeneration

CYCLIC NUCLEOTIDES mediate many aspects of normal cellular metabolism[1]; thus, degradation as well as synthesis of these intracellular mediators must be strictly regulated. Phosphodiesterase (PE), the enzyme of cyclic nucleotide catabolism, is present in mammalian tissues in multiple forms, which differ in substrate specificity, kinetic characteristics and subcellular localisation[2]. Moreover, a calcium-dependent protein activator (now called calmodulin) has been characterised that specifically activates at least one of the PDE types[3,4] although other types of PDE are known to be activator independent[5,6]. Thus, several mechanisms are present *in vivo* which allow strict control of PDE. A unique cyclic GMP-PDE is compartmentalised in the outer segments of retinal photoreceptor cells[7–10]; its activity is low in the dark-adapted state but increases dramatically on light adaptation[10–12]. The resulting drop in cyclic GMP content could serve as a chemical 'signal' in the normal visual process[13,14]. However, despite much investigation of various cyclic nucleotide systems, no definitive information has been obtained which clearly links a disorder of cyclic nucleotide metabolism with a disease process elsewhere than in retina[15,16]. We have recently presented preliminary evidence that an abnormality in cyclic GMP metabolism could be present in the

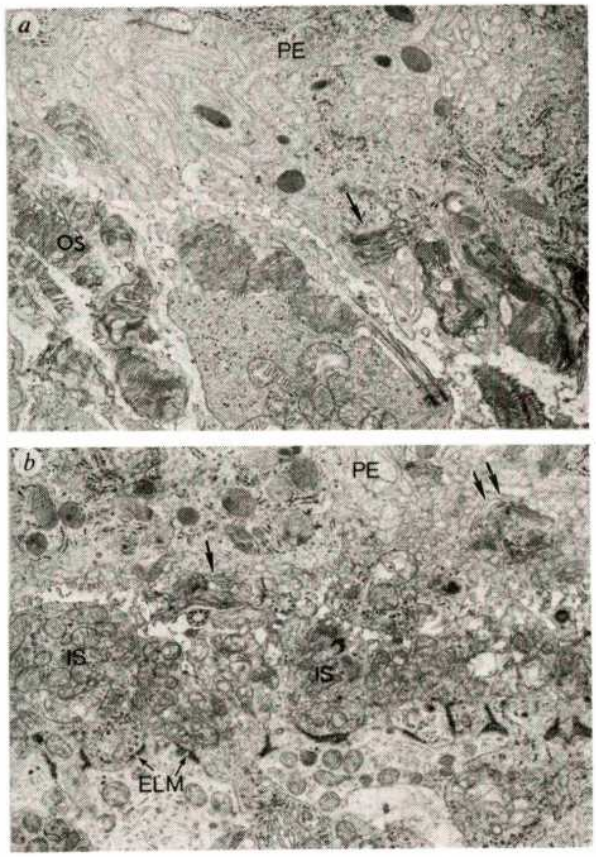


Fig. 1 *a*, Photoreceptor layer from a 13-d-old normal Irish setter. Photoreceptor outer segments have begun to elongate and contain well organised stacks of outer segment (OS) lamellar disks. Some of these seem to have been engulfed (arrow) by the pigment epithelium (PE). *b*, Photoreceptor layer from a 13-d old affected Irish setter. Small photoreceptor inner segments (IS) project through the external limiting membrane (ELM). There is minimal outer segment material; some is apposed to the PE apex (arrow) and some seems to be located within the PE cytoplasm (double arrow) ×10,400.

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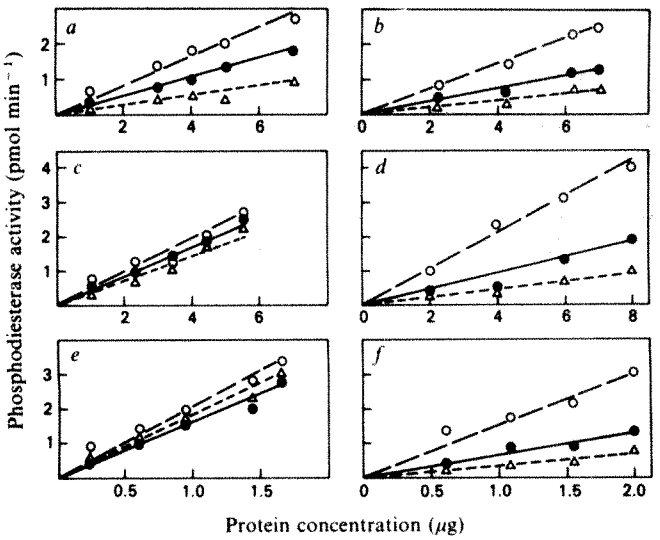


Fig. 2 Cyclic GMP phosphodiesterase activity in retinas of different ages. *a*, Control 9-d-old; *b*, affected 9-d-old; *c*, control 31-d-old; *d*, affected 34-d-old; *e*, control 48-d-old; *f*, affected 48-d-old. ●, No additions; ○, 10 μg purified brain activator added; △, 250 μM EGTA added. Experimental points are averages of triplicates which differed by no more than 15%.

retinas of Irish setter dogs with inherited rod–cone dysplasia[15] that could lead to greatly increased cyclic GMP content, as had been reported in mice with inherited retinal degeneration[16]. We now report that the basic defect in the disease seems to be a failure to switch PDE type and a concomitant decrease in protein activator concentration early in postnatal development, at the time of photoreceptor differentiation.

The Irish setters used were bred to develop rod–cone dysplasia, and controls were heterozygous Irish setters unaffected by the disease. Tissues were obtained as previously described[15]; briefly, animals were anaesthetised with pentobarbital, their eyes enucleated and the retinas quickly dissected on ice and frozen in liquid nitrogen. Protein was determined by the method of Lowry *et al.*[17] with bovine serum albumin as standard. Bovine brain activator and activator-deficient PDE were prepared as previously described[18]. The activator-deficient enzyme had a basal activity of 930 pmol cyclic AMP hydrolysed per mg protein per min; this was increased fourfold in the presence of optimal concentrations of Ca^{2+} and activator protein. PDE activity was measured using an assay mixture containing 40 mM Tris-HCl (*p*H 8.0), 3 mM $MgSO_4$, 50 μM $CaCl_2$, 1 μM ^{3}H-cyclic AMP (36.6 Ci mmol^{-1}) or ^{3}H-cyclic GMP (8.28 Ci mmol^{-1}) (NEN) and an appropriate amount of retinal homogenate in a total volume of 100 μl. When appropriate, 250 μM EGTA or 10 μg of purified brain activator was added before PDE assay. Samples were incubated for 12 or 15 min at 30°C, the reaction was terminated by boiling for 45 s, and 50 μg snake venom (*Crotalus atrox*, Sigma) was added. Samples were incubated for a second 10-min period at 30°C and 1.0 ml of IRP-58 resin (200–400 mesh, Rohm and Haas) added. After centrifugation, ^{3}H-adenosine or ^{3}H-guanosine in the supernatant was determined by liquid scintillation spectrometry. Recovery of ^{3}H-nucleoside was >90%. Retinal protein activator was assayed by testing its ability to stimulate the activity of activator-deficient, purified cyclic nucleotide PDE enzyme (0.68 μg per 0.1 ml assay mixture) using boiled retinal aliquots as the source of activator. One unit of activator is defined as the amount required to give half-maximal stimulation of the activator-deficient PDE enzyme.

The second week after birth is critical in retinal development in dogs, as the photoreceptors begin to differentiate during this period (Fig. 1). At day 9 (Table 1 and Fig. 2*a*, *b*), cyclic GMP-PDE activity was already lower in the dystrophic dog (244 pmol per mg protein per min) than in heterozygous controls (309 pmol per mg per min). However, cyclic AMP-PDE activity was the same in affected and control retinas at 9 days. Cyclic GMP-PDE activity in control retinas rose sevenfold (Table 1) by the time of photoreceptor maturation at 7 weeks[19]. Cyclic GMP-PDE activity observed in affected retinas during development was lower than that of control retinas, whereas no difference was observed in cyclic AMP-PDE activity (Table 1). Measurement of endogenous protein activator for cyclic GMP-PDE using activator-deficient brain PDE enzyme indicated lower amounts in affected than in control retinas in each age group studied. No differences were observed with the protein activator using ^{3}H-cyclic AMP as substrate (not shown).

Activity of cyclic GMP-PDE was then investigated in the presence of exogenous brain activator protein or EGTA (Fig. 2). The latter was used because Ca^{2+} is necessary for activator-induced increases in PDE activity. The addition of brain activator increased PDE activity in the 9-d control retina but added EGTA decreased activity (Fig. 2*a*). In the 9-d affected retina (Fig. 2*b*), the inhibitory effect of EGTA was also apparent, as was the stimulatory effect of brain activator. At about 5 and 7 weeks, neither brain activator nor EGTA had any marked effect on PDE activity in the control retina (Fig. 2*c*, *e*). In contrast, EGTA continued to exert an inhibitory effect in the dystrophic retina, and the addition of brain activator restored PDE activity to the levels seen in the control (Fig. 2*d*, *f*).

The expression of PDE activity in cells seems complex in that it can be regulated by several factors including activator protein and Ca^{2+} (refs 20, 21) in addition to the basic activity of the enzyme itself. It has been suggested[22] that the concentration of cyclic GMP in particular is controlled by a PDE protein activator. It was therefore not unexpected to find effects of activator and Ca^{2+} on cyclic GMP-PDE activity in 9-d control retinas. Morphologically, photoreceptor outer segments begin to develop at this time. However, it was surprising to find that after outer segment elongation and maturation added brain activator and Ca^{2+} had negligible effects on PDE activity in these retinas. This indicated that the activator-dependent PDE activity had switched to an activator-independent ('adult') type (Fig. 2). In contrast, in affected retinas, no such switch was found to occur as the outer segments began to develop. Exogenous activator markedly stimulated PDE activity throughout the 7-week experimental period, whereas endogenous activator levels fell to about half of that observed in control retinas. It is not known if endogenous activator levels are actually limiting in affected retinas. The finding that exogenous brain activator restores PDE activity to close to that seen in the control *in vitro*, however, could be important in future attemps to halt or at least slow the progress of the disease.

Table 1 Phosphodiesterase activity and protein activator activity in control and affected retinas

Animal age (day)	Animal type	Phosphodiesterase activity (pmol per mg protein per min)		Cyclic GMP-PDE activator concentration (units per mg protein)
		Cyclic AMP	Cyclic GMP	
9	Control	102	309	125
9	Affected	102	244	101
31	Control	104	887	53
34	Affected	104	326	23
48	Control	143	2,275	95
48	Affected	151	539	56

Phosphodiesterase activity was measured by determination of ^{3}H-nucleoside with an ion-exchange method (see text). Protein activator was determined in boiled retinal aliquots using purified bovine brain PDE which was deficient in activator. One unit of protein activator activity is that amount which is required to give half-maximal stimulation of the activator-deficient brain PDE enzyme. Values given are averages of triplicate determinations in two experiments which agreed to within 15%.

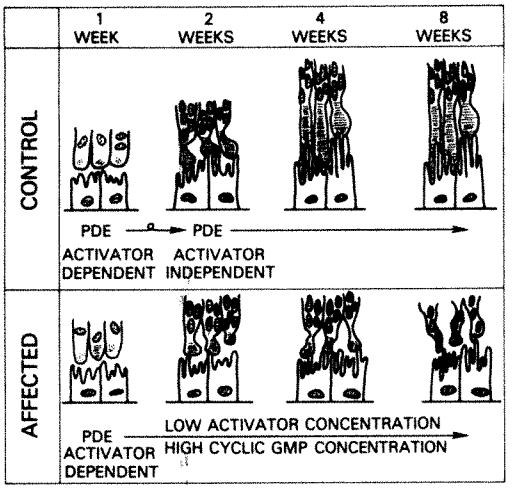


Fig. 3 Schematic outline of morphological and biochemical events in the development of outer segments (OS) in control and affected retinas. In both control and affected retinas, no OS are observed in the first postnatal week. OS appear in the second week, develop normally (4–5 weeks) in the control retinas but quickly degenerate in affected retinas. Biochemically, the cyclic GMP-PDE enzyme switches from an activator-dependent to an activator-independent type in control retinas but not in affected retinas. Protein activator concentration is substantially decreased in affected retinas.

It is well known that enzyme patterns change during development[23,24]. Recent evidence suggests that, at least in some tissues, PDE activity changes during differentiation with regard to its sensitivity to regulation by the Ca^{2+}-dependent protein activator[25,26]. Our data strongly indicate that a normal switch in PDE type fails to occur in affected retinas during photoreceptor development and that the low level of activator present in these retinas may not be sufficient to maintain adequate cyclic GMP-PDE activity (Fig. 3). It seems, therefore, that for the first time calmodulin can be directly linked to a disease process. It will be interesting to see if a similar situation exists in other degenerative diseases or if this biochemical defect is unique to inherited degeneration of the neural retina.

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Evidence that cyclic GMP regulates membrane potential in rod photoreceptors

CYCLIC NUCLEOTIDES have been proposed as mediators of the decreased Na^+ permeability caused by illumination of vertebrate photoreceptors[1,2]. We show here that cyclic GMP depolarises the rod outer segment (ROS) approximately to the Na^+ equilibrium potential within milliseconds after being injected intracellularly, that previous light adaptation antagonises this depolarisation, and that the injection of cyclic GMP without illumination initiates a repolarisation after a time lag which is longer than that following a light flash and proportional to the injection time. The results are interpreted in terms of a model in which cyclic GMP levels are controlled by the resultant of cyclase and phosphodiesterase (PDE) velocities. The PDE velocity of hydrolysis in the dark is increased by prior light adaptation in the presence of increased substrate (cyclic GMP). The data suggest that cyclic GMP is an important factor in the regulation of the membrane potential of the ROS.

Biochemical experiments on the cyclic nucleotide system of isolated homogenised ROS suggest that illumination decreases the concentration of cyclic GMP by activating a GTP-dependent PDE[3,4]. The rate of light-initiated hydrolysis of cyclic GMP by PDE is apparently sufficiently fast to mediate the rapid initial decrease in Na^+ permeabilty[5,6]. The cyclic GMP that is not hydrolysed presumably increases Na^+ permeability. The mechanism of increased Na^+ permeability of the ROS plasma membrane would be by phosphorylating a membrane protein[6,7] as proposed for other tissues. Superfusion with cyclic GMP or a PDE inhibitor has been shown to depolarise rods[8]. We have injected cyclic GMP intracellularly through the recording pipette into ROS of the isolated retina of the toad, *Bufo marinus*, to determine the effects of excess cyclic GMP on membrane potential, latency and amplitude of the light response. We reported[9] that this excess cyclic GMP increases both the latency and amplitude of the response. Here, using the same preparation and methods, we found that excess injected cyclic GMP depolarised the rod membrane. This depolarisation in dark-adapted preparations frequently exceeded the zero level (Figs 1, 2) and attained a maximum value of +11 mV (Fig. 1*a*, *d*). A reversal potential of 0 to +10 mV was obtained for the isolated *Necturus* rod[10]. A value of +11 mV implies an intracellular Na^+ concentration ($[Na^+]_i$) of 66 mM, based on our extracellular concentration of 103 mM. This compares with 50 mM $[Na^+]_i$ estimated using another technique[11]. Therefore, +11 mV seems to approximate to the equilibrium potential for Na^+. The maximum depolarisation we observe is consistent with the interpretation that the injected excess cyclic GMP renders the ROS plasma membrane permeable to Na^+.

Intracellular injection of cyclic GMP in the absence of illumination should initiate a recovery from depolarisation after a time proportional to the amount injected, provided the dark concentration is less than the K_m of PDE which is 70 μM cyclic

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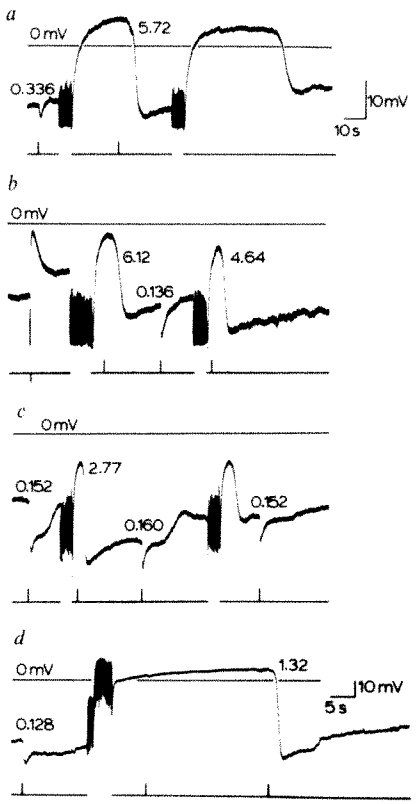


Fig. 1 Effects of intracellular injection of cyclic GMP in four different cells. Pen oscillations indicate injection of -2.5 nA; nominally 6×10^7 molecules cyclic GMP $nA^{-1}s^{-1}$ assuming transport number is 0.01. Numbers above membrane potential traces give latencies to light stimuli in seconds. All light flashes were 0.1 s, indicated by upward vertical lines on traces below each record. Light intensity is -3 log units on *A* and -2 log units for all other stimuli except the last on *D*, which is -1 log units. 0 log units = 5 $\mu W\,cm^{-2}$. Change in noise level during injection in *D* may be due to a pipette tip which broke in the cell, releasing a large amount of cyclic GMP such that the first stimulus after injection failed to elicit a response. However, note small deflection that may originate from outside the rod compartment (which is about 500 rods by electrical coupling[17,18]). Compartment size for cyclic GMP is not known.

GMP[4,5]. There are $5–10\times10^7$ molecules of cyclic GMP per ROS in the dark[12], or a concentration of 40–80 μM. The PDE should therefore be capable of higher velocities on injection of additional substrate. The V_{max} of PDE in the dark was estimated to be 10^4 times lower than in the light for frog ROS[5]. Nevertheless, a brief (40 ms) injection of cyclic GMP (Fig. 1*b*) causes an almost immediate depolarisation and a rapid repolarisation. Figure 2 shows that the time before repolarisation is proportional to the injection time. Each injection of additional substrate increases the PDE velocity, according to classical enzyme kinetics. The increased hydrolysis of cyclic GMP would tend to return the membrane potential to the baseline if cyclic GMP, acting through its kinase, determines membrane potential.

Following an intense flash (Fig. 3*a*) and after the baseline has returned to previous levels, injection of cyclic GMP fails to elicit



Fig. 2 Cell was dark adapted 10 min before injection; 2, 5 and 10 s injections of -2.5 nA, 25 mM cyclic GMP pipette.

a depolarisation. This suggests that light activation of PDE can potentiate the elevated velocity of PDE caused by additional substrate, thus preventing the depolarisation, or rapidly suppressing a brief depolarisation obscured by the pen oscillations. According to our model, the high V_{max} resulting from PDE light activation decays approximately with the time constant of dark adaptation (note no depolarisation after second injection, Fig. 3*a*, and small depolarisation 1 min later on, Fig. 3*b*). This persistently high V_{max} is unmasked by the injection of excess cyclic GMP in the dark.

Similar arguments may explain the length of latency, the increase in amplitude and the steeper repolarisation following a light flash delivered during the depolarisation after an injection of cyclic GMP. The light flash would briefly activate PDE, but the persistently high velocities of PDE caused by prior illumination and high substrate concentrations would be necessary for rapid hyperpolarisation of the membrane potential below the baseline. Illumination initiates hydrolysis by activation of PDE, but whereas the control hyperpolarisation in Fig 1*a* is complete in less than 0.5 s, the light-initiated PDE activity must have been augmented by increased substrate plus the light adaptation

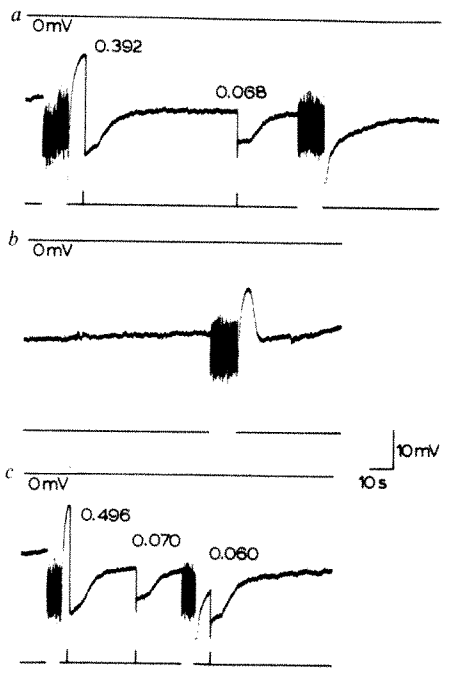


Fig. 3 *a*, *b* And *c* continuous recordings. Injections as in Figs 1 and 2, 25 mM cyclic GMP pipette. All light stimuli 0 log units.

effect mentioned above during the 5.72 s in which there is no visible response. The combination of increased substrate and light also seems to cause a persistent increase in PDE velocity, or undershoot, even after cyclic GMP levels are reduced, as judged by the increased plateau phase (Fig. 1*c*).

Figure 4 shows a control experiment in which the injection of the product of cyclic GMP hydrolysis, 5'-GMP, causes a small depolarisation. The response to illumination during this depolarisation is smaller than the control and of the same latency, in contrast to the opposite effects caused by cyclic GMP.

Excitation and adaptation of photoreceptors by light is a complex process that may involve molecules of the cyclic nucleotide systems, Ca^{2+} (refs 13–16) and other molecules. The experiments reported here are consistent with the interpretation that excess cyclic GMP injected into ROSs is capable of

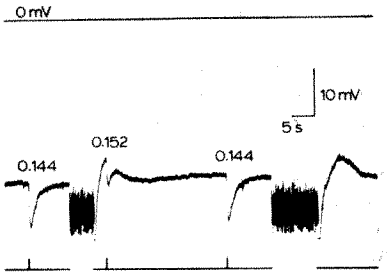


Fig. 4 Injections of −2.5 nA, 25 mM 5′-GMP pipette. Light stimuli −2 log units.

depolarising the rod plasma membrane to the Na^+ equilibrium potential, that both the injection and the previous adaptational history affect the intracellular concentration of cyclic GMP through the hydrolysing capacity of PDE, and that cyclic GMP must be hydrolysed by light-activated PDE so as to hyperpolarise the membrane to produce the normal response to illumination. These experiments suggest that normal intracellular levels of cyclic GMP may be an important determinant of the latency and other response characteristics of vertebrate rod photoreceptors.

We thank Ted Reid for helpful discussions. This work was supported in part by USPHS grant EY00089.

Note added in proof: Control experiments indicate that the zero level shown in the figures should be shifted upwards by 5 mV because of differences in tip potentials measured in Conway's and mock intracellular superfusates based on ref. 11.

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Opposite effects of intracellular Ca^{2+} and glucose on K^+ permeability of pancreatic islet cells

MANY cells possess a potassium permeability system that is activated by an increase in the concentration of Ca^{2+} in the cytosol. This Ca^{2+}-sensitive permeability was originally described[1] and is best characterised[2] in erythrocytes, where its function is still not known. However, a similar mechanism could represent a physiological control of the membrane conductance in excitable tissues such as nerve[3,4] or cardiac muscle[5]. Physiological stimulation of salivary glands results in a Ca^{2+}-mediated release of potassium[6] and hyperpolarisation[7]. In contrast, glucose depolarises pancreatic B cells[8,9] by decreasing their potassium permeability[10,11], but also stimulates Ca^{2+} uptake by islet cells[12–14]. I report here an investigation into the possible interplay between these two effects of glucose. Evidence that intracellular Ca^{2+} increases the K^+ permeability in pancreatic islet cells and that the glucose-stimulated Ca^{2+} inflow may represent a feedback control of the glucose-mediated decrease in K^+ permeability of B cells is presented.

The K^+ permeability of pancreatic islet cells was evaluated by continuously monitoring the efflux of $^{86}Rb^+$ from preloaded isolated rat islets in a perifusion system[10,14,15]. The simultaneous measurement of $^{86}Rb^+$ and $^{42}K^+$ loss from islets labelled with both tracers has shown that the efflux of the more convenient isotope $^{86}Rb^+$ is a valid index of the qualitative changes in K^+ efflux from islet cells (data not shown).

A sudden rise in the extracellular Ca^{2+} concentration (0.5 to 10 mM) led to a reversible increase in the rate of $^{86}Rb^+$ efflux from islet cells perifused with a medium containing 3 or 6 mM

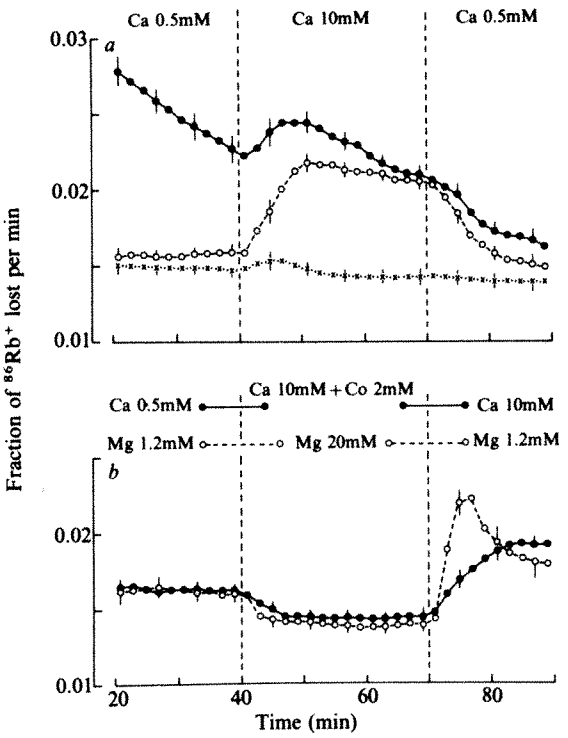


Fig. 1 Effect of a high concentration of extracellular calcium on rubidium efflux from perifused rat islets. *a*, Ca^{2+} concentration was increased from 0.5 to 10 mM between 40 and 70 min; glucose concentrations were kept constant at: ●, 3 mM; ○, 6 mM; ×, 10 mM. *b*, 2 mM Co^{2+} was added to the medium containing 10 mM Ca^{2+} (●), or the concentration of Ca^{2+} was kept constant (2.5 mM), while the concentration of Mg^{2+} was raised to 20 mM (○); the glucose concentration of the medium was constant at 6 mM. In (*a*) and (*b*), PO_4^{2-} was omitted to avoid precipitation of insoluble salts. Values are means ± s.d. of four experiments except in (*a*) where $n = 7$ at 6 mM glucose. Techniques have been detailed previously[10,14,15] and are only outlined below. Isolated rat islets were obtained by collagenase digestion of the pancreas of fed male Wistar rats. All experiments were carried out at 37 °C, with a Krebs–Ringer bicarbonate solution, *p*H 7.4, containing 6 mM K^+ and supplemented with 0.5% (w/v) bovine serum albumin. When Ca^{2+} was partially or completely omitted, it was replaced by Mg^{2+}; when its concentration was increased, Na^+ was isosmotically decreased. Groups of 60 to 90 islets were first incubated for 150 min in 0.5 ml medium containing 3 mM glucose and 0.2 mM $^{86}RbCl$ (350–480 mCi mmol^{-1}, Radiochemical Centre). After three washings with non-radioactive medium, the islets were placed in perifusion chambers[15]. $^{86}Rb^+$ was counted by measuring the Cerenkov radiation[10] in the effluent fractions, that were collected at 2 min intervals, and in the tissue at the end of the experiment. Results are expressed as fractional efflux of $^{86}Rb^+$.

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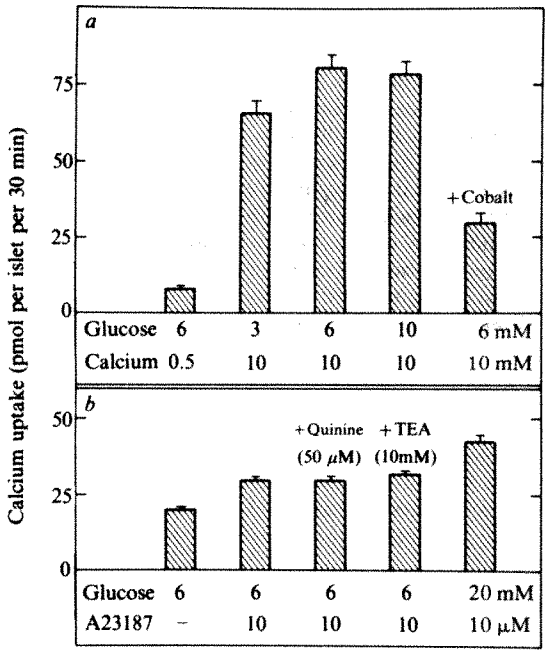


Fig. 2 Calcium uptake by islet cells. $^{45}Ca^{2+}$ uptake by rat islets was measured in relation to that of sucrose which is restricted to the extracellular space. The method has been described previously[14]. Islets were incubated in 100 μl medium layered on silicone oil and separated from the radioactive solution by centrifugation through the oil at the end of the 30-min uptake period. The radioactivity entrapped in the tissue pellet was counted by liquid scintillation. Blanks without islets did not differ from the background. *a*, After preliminary incubation for 40 min in the presence of 0.5 mM $CaCl_2$ and the same concentration of glucose as for the incubation period (3, 6 or 10 mM), batches of 10 islets were transferred into the prewarmed incubation medium. This phosphate-free medium contained the indicated concentration of glucose, 0.25 mM [6,6'-^{3}H]sucrose (0.1 Ci mmol^{-1}) and either 0.5 mM $^{45}CaCl_2$(60 mCi mmol^{-1}) or 10 mM $^{45}CaCl_2$ (3 mCi mmol^{-1}). In one series, 2 mM Co^{2+} was also present. Values are means ± s.e.m. of 14–17 batches of islets. *b*, After preliminary incubation for 40 min in the presence of 2.5 mM $CaCl_2$ and 6 mM glucose, batches of 10 islets were transferred into the incubation medium. The concentrations of A23187 and glucose were as indicated. The medium contained 2.5 mM $^{45}CaCl_2$ (12 mCi mmol^{-1}). Values are means ± s.e.m. of 12–16 batches of islets.

glucose, but not in the presence of 10 mM glucose (Fig. 1*a*). By contrast, increasing the Mg^{2+} concentration (1.2 to 20 mM) slightly depressed $^{86}Rb^+$ efflux (Fig. 1*b*), showing that the effect observed with Ca^{2+} is not due to damage of the cell membranes by a high level of divalent cations. The mere presence of 10 mM Ca^{2+} in the medium is not sufficient to increase the K^+ permeability of islet cells. Thus, $^{86}Rb^+$ efflux was not increased, but even somewhat decreased when the Ca^{2+}-antagonist[14], cobalt, was added to the medium simultaneously with the rise in extracellular Ca^{2+} (Fig. 1*b*). Figure 2*a* shows the increase in Ca^{2+} uptake that occurs in islet cells when they are transferred from a medium with a low (0.5 mM) to a medium with a high (10 mM) concentration of Ca^{2+}. Using this experimental protocol, it is difficult to know exactly what proportion of Ca^{2+} has been adsorbed or has entered the cells. Note, however, that only cobalt, and not 10 mM glucose, reduced Ca^{2+} uptake, although both conditions prevented the increase in $^{86}Rb^+$ efflux that is otherwise produced by the rise in extracellular Ca^{2+}.

To evaluate the role of intracellular Ca^{2+} in the activation of $^{86}Rb^+$ efflux further, the effects of the divalent cationophore A23187 (ref. 16) were studied. Addition of 10 μM A23187 to a medium containing Ca^{2+} steadily increased $^{86}Rb^+$ efflux in the presence of 6 mM glucose, but this effect was considerably reduced at 10 mM glucose (Fig. 3*a*). In the absence of extracellular Ca^{2+}, A23187 was almost ineffective, whereas the subsequent introduction of Ca^{2+} into the medium was followed by a prompt and marked increase in $^{86}Rb^+$ efflux, which was completely reversible when both A23187 and Ca^{2+} were withdrawn (Fig. 3*a*). It is thus clear that the ionophore, which has a weak affinity for monovalent cations[16], does not substantially transport Rb^+ ions from islet cells and that, again, Mg^{2+} cannot substitute for Ca^{2+} in activating K^+ permeability. The Ca^{2+}-activated K^+ permeability is specifically inhibited by quinine in the erythrocyte[2] and is sensitive to tetraethylammonium (TEA) in certain neurones[3]. Figure 3*b* shows that the increase in $^{86}Rb^+$ efflux brought about by A23187 + Ca^{2+} was reduced by TEA and stopped by quinine or 20 mM glucose. Only the effect of quinine was not reversible. In the presence of 6 mM glucose, A23187 produced a 50% increase ($P < 0.001$) in Ca^{2+} uptake by islet cells (Fig. 2*b*); this stimulation was unaffected by quinine or TEA and was increased by 20 mM glucose. Thus, the inhibition of Ca^{2+}-activated Rb^+ efflux by these agents is not due to a primary reduction of Ca^{2+} transport into the cells

Since islet cells[17,18], as most cells, possess an energy-dependent system to sequester Ca^{2+}, metabolic inhibitors should cause cellular organelles to release stored Ca^{2+} and increase the levels of the cation in the cytosol. Figure 4*b* shows that the rate of $^{45}Ca^{2+}$ efflux from perifused islets was augmented after addition of the mitochondrial uncoupler, dicumarol, and that this effect was unaffected by TEA or quinine. Dicumarol simultaneously increased the rate of $^{86}Rb^+$ efflux (Fig. 4*a*); TEA partially and reversibly prevented the increase, whereas quinine suppressed it almost completely. Omission of extracellular Ca^{2+} did not alter

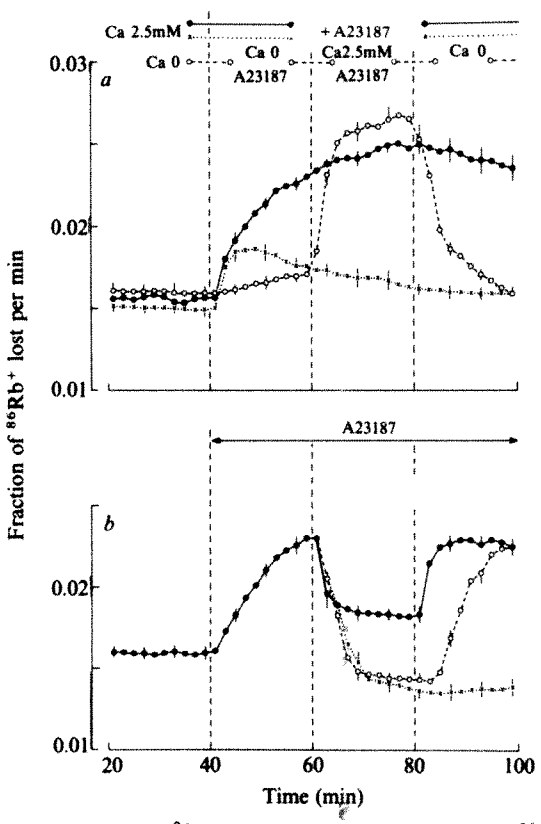


Fig. 3 Effect of Ca^{2+}-ionophore A23187 (10 μM) on $^{86}Rb^+$ efflux from perifused rat islets. Experimental procedure as for Fig. 1. Glucose concentrations: *a*, ● ○, 6 mM; ×, 10 mM; *b*, 6 mM. A23187 (Lilly) was disolved in dimethyl sulphoxide and the solvent was added alone (0.5%) in control solutions. In (*a*), A23187 was added from 40 to 100 min (● and ×) or 40 to 80 min (○); Ca^{2+} (2.5 mM) was present throughout or only between 60 and 80 min (○). Values are means ± s.d. of four experiments. In (*b*), Ca^{2+} was present throughout and A23187 from 40 to 100 min; between 60 and 80 min, TEA (●, 10 mM) or quinine (×, 50 μM) was added, or the glucose concentration was increased from 6 to 20 mM (○). Values are means ± s.d. of nine experiments until 60 min and of three experiments thereafter.

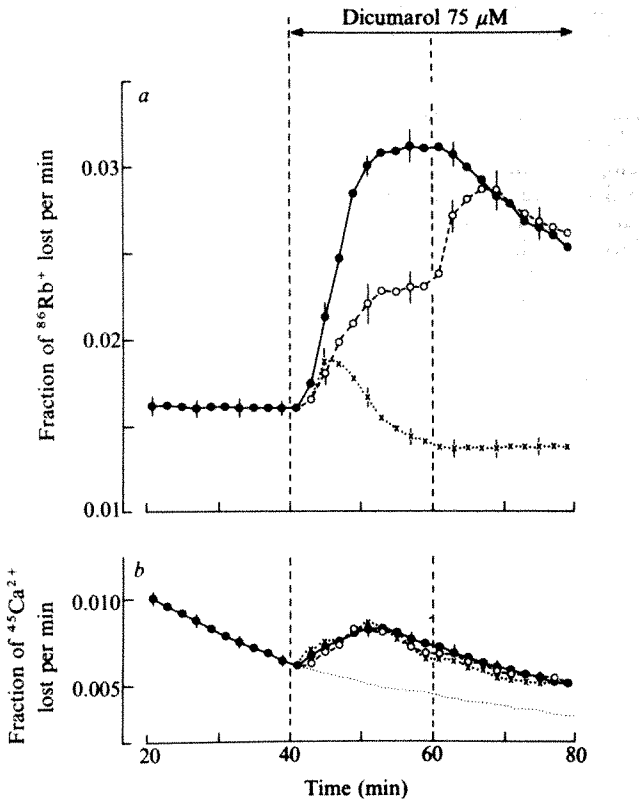


Fig. 4 Effect of dicumarol (75 μM) on $^{86}Rb^+$ (*a*) or $^{45}Ca^{2+}$ (*b*) efflux from perifused rat islets. Experimental procedure as for Fig. 1, except that for studying $^{45}Ca^{2+}$ efflux, islets were first incubated for 150 min in 0.5 ml medium with 1.25 mM $^{45}CaCl_2$ (200 μCi mmol^{-1}) and experiments were carried out in the absence of extracellular Ca^{2+}. Dicumarol (added from a 20 mM stock solution in 0.1 M NaOH) was present from 40 min onwards, while TEA (○, 10 mM) or quinine (×, 50 μM) was added between 40 and 60 min. The glucose concentration was constant at 6 mM. Values are means ± s.d. of nine experiments until 40 min and of three experiments thereafter.

the increase in $^{86}Rb^+$ efflux produced by dicumarol (not shown). Although a direct effect of dicumarol on the plasma membrane cannot be ruled out, these observations indicate that the activation of K^+ permeability by intracellular Ca^{2+} is not obligatorily linked to an influx of the divalent cation and are in keeping with the report[8] that uncouplers of oxidative phosphorylation hyperpolarise B cells.

The experiments described above strongly suggest that a rise in intracellular Ca^{2+} increases the membrane permeability to potassium in islet cells as in other tissues[1-6]. Although these experiments were made with whole islets comprising only two-thirds of B cells, the comparison of the changes in K^+ permeability with electrical recordings in B cells and with insulin release suggests physiological implications for our findings. When glucose is increased to a stimulatory concentration (>5 mM), the membrane potential of B cells first decreases from the resting potential to a constant threshold level and then starts to oscillate in slow waves[19]. These waves are the regular alternation of depolarisation phases with bursts of spikes and silent periods of repolarisation. The duration of these phases of activity increases with the concentration of glucose and is correlated with insulin release[19]. It is also known that the depolarisation from the silent to the active membrane potential[9,20,21] and perhaps also the spike activity[22] depend on the entry of Ca^{2+} into the cells. However, high concentrations of extracellular Ca^{2+} shorten the periods of depolarisation[20] and decrease insulin release[14,23]. On the other hand, TEA[24] and quinine[21] suppress the polarisation phases of the slow waves, thus producing continuous activity, and markedly increase insulin release[15,25]. In the light of these observations, this report supports the suggestion that an activation of K^+ permeability by Ca^{2+} may be involved in the hyperpolarisation which terminates the bursts of activity. Within this framework, glucose-stimulated Ca^{2+} uptake might seem irreconcilable with the prolongation of the phases of depolarisation. However, the key observation is that high glucose levels reduce or even suppress the Ca^{2+} sensitivity of that K^+ permeability system. Lew and Ferreira[26] have shown that the Ca^{2+} sensitivity of the potassium gating mechanism depends on the metabolic state of the erythrocyte. As glucose has to be metabolised by islet cells to control their K^+ permeability[10], the present system could represent a link between B cell metabolism and membrane permeability.

In conclusion, like the membrane of bursting neurones[3], the B cell membrane possesses a Ca^{2+}-activated K^+ permeability. Its glucose sensitivity makes the system a sensitive control mechanism of the periodical oscillations of the membrane potential and insulin release.

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Variability of cell cycle times measured *in vivo* in embryonic chick retina by continuous labelling with BUdR

IN this report, cell cycle times were measured in the embryonic chick retina by continuous labelling with 5-bromodeoxyuridine (BUdR) and the fluorescence plus Giemsa[1] (FPG) technique. The measurement of cell cycle times in embryonic chick tissues *in vivo* is preferably carried out by continuous rather than pulse labelling because a single injection of a nucleotide precursor into an egg can form a long-lasting pool which remains available for at least 24 h (ref. 2). Continuous labelling with BUdR provides information about the number of S phases a metaphase cell has encountered since the start of labelling whereas the previous continuous labelling procedure[3] using tritiated thymidine indicates that a mitotic cell has encountered an S phase but does

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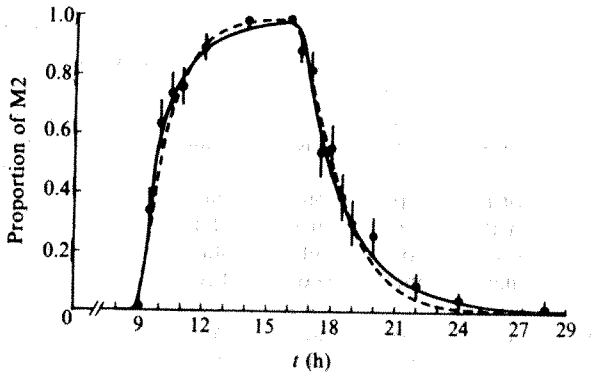


Fig. 1 The proportion of M2 at various times, t, after the start of labelling with BUdR. Each datum point (●) is the mean of five animals. Lines are drawn to 2 s.d. on either side of each datum point. 500 metaphase spreads were observed in each animal; the spreads which were not M2 at $t < 16$ h were either cells which had not incorporated BUdR or those which had incorporated it for one S phase; at $t \geq 16$ h, with very few exceptions they were cells which had incorporated BUdR for three or more S phases. To obtain these data, chick eggs incubated at 38 °C were injected at 72 h with 2×10^{-5} g BUdR and then sampled t hours later. Colchicine (1×10^{-5} g) was injected 1 h before sampling. Metaphase spreads were prepared from the neural retina as described previously[2]. For FPG, the spreads were stained in 1×10^{-4} g ml^{-1} 33258 Hoechst, mounted in 0.2 M sodium phosphate–citrate buffer, pH 7.2, and exposed to sunlight for 8–16 h, on successive days when necessary, and then stained in 3% Giemsa. The dose of BUdR was the lowest at which FPG differential staining could be achieved consistently. The continuous line is the curve of best fit obtained when the variances for phases SG_2EP and V were zero and when the phase G_1SAT (see text for definitions of these phases) was a constant plus a gamma distributed variable. The dashed line is the curve of best fit when G_1SAT was a constant plus an exponential variable. The curve for an inverse gaussian variable is not shown but it is close to that for the gamma.

not show how many. BUdR labelling thus gives more detailed data for estimating the means, variances and distributions of the durations of the cell cycle and its phases. Analysis of the data by statistical modelling has shown that the cell cycle is made up of a phase of constant duration and one of variable duration and we were able to test the appropriateness of different models for the variable phase. The variable phase was described well by a gamma distribution which was clearly not exponential in form and, somewhat less well, by an inverse gaussian distribution. A random transition in the cell cycle would be indicated by an exponentially distributed period; therefore, for the random transition model[4,5] of the cell cycle to hold, there would have to be an exponentially distributed component within the variable phase. Our results do not exclude this but they do show that such a component could only be a minor part of the variable phase.

The procedure was to inject BUdR into eggs, sample the retina after various times and prepare[2] metaphase spreads. Colchicine was injected 1 h before sampling. The number of metaphase spreads which showed incorporation of BUdR for: one or no S phases, two S phases and three or more S phases, were counted in each sample. These three types of spread could be identified[2] by the FPG technique because it stains unsubstituted and unifilarly substituted chromatids darkly and bifilarly substituted chromatids lightly. The data are presented in Fig. 1 as the proportion of metaphases which had incorporated BUdR for two S phases (M2 metaphases) at various times after the start of labelling.

To describe the statistical analysis of the data, it is necessary to divide the cell cycle in a non-conventional way. A line is drawn at a point in S, a second line is drawn in prophase and a third at the end of metaphase. The line in S marks the beginning of the minimum time a cell must spend in S so as to incorporate enough BUdR to be detectable by FPG when the cell has undergone another S phase. The line in prophase is at the beginning of the period during which the chromosomes could be rendered observable by the techniques for making metaphase spreads. This period is assumed to terminate at the end of metaphase. The new periods created by these boundaries were grouped as follows: G_1, the part of S following G_1 and the anaphase and telophase stages of mitosis were grouped together and called G_1SAT; the remainder of S, G_2 and a period approximating to early prophase were grouped and called SG_2EP; the period during which the chromosomes were observable was assumed to extend from about mid-prophase to the end of metaphase and was labelled V, for viewing period. For the analysis, the population of dividing retinal cells was modelled as an age-dependent branching process[6,7] up to the time at which cells were acted upon by colchicine. The population was assumed to be growing exponentially[7] from the time of BUdR injection and the parameters describing the population were assumed to remain constant from this time until the cells were collected. After colchicine began to act, it was assumed that all cells would be arrested in V. A parameter, c, known to be between 45 and 60 min (ref. 8), was introduced to denote the duration of colchicine action preceding t, the time of sampling. A theoretical curve for the proportion of V phase cells which were in M2 was derived as a function of t. This theoretical curve depended solely on c and the distribution of the durations of G_1SAT, SG_2EP and V and not on the individual distributions of G_1, S, G_2 and mitosis. This was why the phases of the cycle were grouped in the way described. Mathematical details of the analysis will be reported elsewhere[9].

The theoretical curve was fitted to the data using normal, gamma, displaced gamma or inverse gaussian (reciprocal normal) distributions for G_1SAT, SG_2EP and V. The parameters for the best-fitting curves and the value of c were found by minimising a weighted sum of squares by computer search methods. The best fit in the initial trials always occurred when the variances of SG_2EP and V were zero. In subsequent searches, therefore, SG_2EP and V were modelled as constants and different distributions were tried for G_1SAT. Neither the

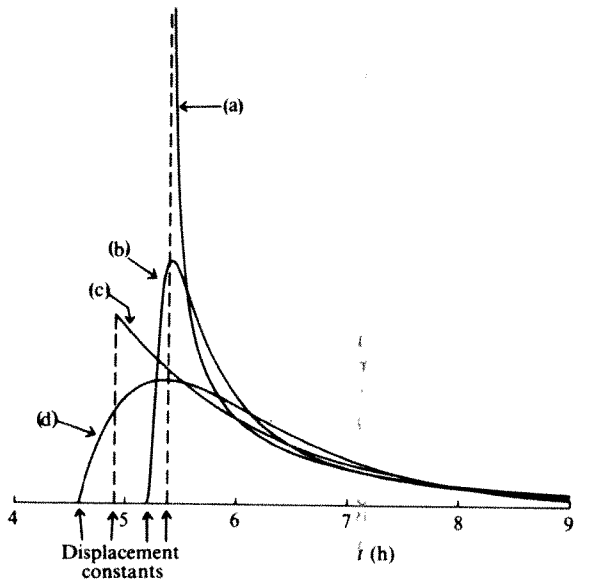


Fig. 2 Distributions for the variable phase in the cell cycle. Each curve is the probability density function obtained when G_1SAT was modelled by a displacement constant and a variable. The variables were: (a) a gamma with p unconstrained and estimated to be 0.44; (b) an inverse gaussian; (c) an exponential; (d) a gamma with p constrained to be 2; (a) gave the best-fitting theoretical curve and (b) was nearly as good; (c) was worse than either (a) or (b), and (d) gave a poorly fitting curve. The densities are markedly different for times less than 6 h both in shape and in the value of the displacement constant.

Table 1 Estimates of the cell cycle time

	Phase	Constant period (h)	Variable period (h) p	b	mean p/b	s.d. $p^{\frac{1}{2}}/b$	Total period (h) mean	median
Gamma	G_1SAT	5.4	0.44	0.34	1.3	2.0	6.7	5.9
	SG_2EP	1.7					1.7	1.7
	V	0.08					0.1	0.1
	Total cycle	7.2					8.5	7.7
Inverse gaussian	G_1SAT	5.2	0.48	0.30	1.6	2.3	6.8	6.0
	SG_2EP	1.7					1.7	1.7
	V	0.08					0.1	0.1
	Total cycle	7.0					8.6	7.8

The variable part of G_1SAT was modelled by a gamma density function

$$g(x) = b^p x^{p-1} \exp(-bx)/\Gamma(p) \qquad (x>0)$$

and an inverse gaussian density function

$$f(x) = \frac{p}{(2\pi b)^{1/2} x^{3/2}} \exp\{-\tfrac{1}{2}b(x-p/b)^2/x\} \qquad (x>0)$$

SG_2EP, V and the remainder of G_1SAT were constant. V is about 5 min (ref. 13); $c = 0.75$ h.

normal nor the gamma distributions alone gave good fits. The sum (convolution) of a normal distribution and an exponential distribution fitted best when the variance of the normal component was zero. This established that part of G_1SAT was also a constant. The fit was much improved when the variable part of G_1SAT was modelled by either the inverse gaussian or the gamma distribution instead of the exponential. The estimates for the cell cycle durations from the best-fitting curves are given in Table 1. The gamma distribution gave the best fit and for this the cell cycle time had a minimum of 7.2 h, a mean of 8.5 h and a standard deviation of 2.0 h.

These procedures show that SG_2EP, V and most of G_1SAT are constant in duration and constitute a constant phase in the cell cycle. The remainder of G_1SAT is variable and can be called the variable phase of the cell cycle.

The fit of the distributions assumed for the variable phase was assessed by the value of the minimised weighted sum of squares (WSS). The gamma distribution gave the lowest WSS and was thus the best fit. The inverse gaussian was nearly as good, with a WSS 1.24 times greater. The exponential distribution, on the other hand, gave a WSS 1.86 times that of the gamma. A more powerful test for the exponential distribution, because it is a special case of the gamma when the shape parameter p is equal to 1, was the value of p when a gamma distribution was assumed for the variable phase. We estimated $p = 0.44$ with an approximate standard error of 0.05 for the best-fitting theoretical curve. A value of $p = 1$ is thus approximately 11 standard deviations away from $p = 0.44$ and so it is very unlikely that our best-fitting curve had an exponentially distributed variable phase. The estimate of p shows that the total variability in cell cycle times cannot be attributed to a single random transition, nor can it be attributed to multiple transitions[10] because they lead to a gamma distribution in which p, equal to the number of transitions, is an integer ≥ 2 and this is further from the estimate of p.

The existence of a random transition in the cell cycle depends on whether the total variability can be partitioned into an exponential distribution and some other distribution. Computations showed that a gamma distribution with $p < 1$ cannot be partitioned in this way. Our inverse gaussian distribution can have an exponential component but the mean of the exponential cannot sensibly be more than 20% of the mean of the variable phase. Therefore, either the exponential component does not exist or it is small, and a decision as to which of these two is so rests on the power to discriminate between the gamma and the inverse gaussian; this depends in particular on data about the very shortest cell cycle times (Fig. 2).

The advantage of our method is that it gives more information about the distributions of subdivisions in the cell cycle than do methods[4,11] based on observations of total cell lifetimes. The latter methods have very little ability to distinguish between alternative distributions for periods in the cell cycle.

A gamma distribution with $p < 1$ does not have a simple mechanistic explanation. An inverse gaussian distribution can have a simple interpretation such as the time taken to reach the critical cell size at which division occurs[12].

The model used for the analysis of the data assumes that the cells were in a steady state of exponential growth from the time of BUdR injection. The assumption is justified because (1) there are several thousand cells in a chick retina at 3 days incubation and so the population would have been asynchronous, and (2) independent observations by us (unpublished) have shown that the parameters describing the population did not change between 3 and 5 days incubation. These observations were the frequencies of unlabelled DNA strands in metaphase chromatids of chick retinal cells after continuous exposure to BUdR between 3 and 5 days incubation. The frequencies agreed well with those predicted by the model and hence show that the assumption of a steady state from the time of BUdR injection is valid.

We consider our observations to have been made on a homogeneous collection of cells and not on a mixture of subpopulations of cells cycling at different rates. The possibility that cell cycle times change in succeeding generations has been excluded by the evidence referred to for steady-state conditions and so the mixture of generations which is present in the population could not be a source of subpopulations cycling at different rates. However, there could be subpopulations which remain steady with time and which each have an exponentially distributed variable phase. This would be a way of explaining our findings in accordance with the random transition model of the cell cycle, for, although it is easy to prove mathematically that a mixture of exponentials does not lead to a gamma distribution, it might be difficult with the present data to discriminate between a gamma and the distribution resulting from a mixture of exponentials. Even so, we know of no evidence to suggest that such subpopulations exist in an embryonic chick retina.

We have presented evidence that the variability in cell cycle times is derived from one part of the cell cycle only and it is therefore in this part of the cycle that the rates of cellular proliferation would be controlled.

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Decreased response to calcitonin in osteopetrotic microphthalmic mouse bone

OSTEOPETROSIS is an autosomal recessive disease characterised by an increase in skeletal mass, often associated with retarded bone growth and a deficiency of marrow cavity development, which is related to a failure of bone resorption to keep pace with bone formation[1,2]. The disease has been studied in man and in several genetic mouse and rat mutants[1]. These mutants generally have diminished osteoclast function in the skeleton based on a lack of ruffled borders[1,3], lowered acid phosphatase activity[4] and a lower level of parathormone-induced resorption *in vivo*[3,5] and *in vitro*[6]. It seems that osteopetrosis has a cellular basis as it can be cured by parabiosis with a normal littermate[7] or by administration of spleen or bone marrow cells[8]. However, the only report examining bone cell metabolism in osteopetrosis indicates that in the calvarium of the osteopetrotic incisor-absent rat, parathormone affects adenylate cyclase activity and lactic acid production to the same extent as in the bone from normal littermates[9]. Recently, specific hormone-sensitive biochemical activities in intact bone in culture[10,11] or in cultured bone cells[12,13] have been associated with the functions of osteoclasts and osteoblasts. Based on these biochemical parameters we show here that in osteopetrotic (micropthalmic) bone there is a defect in calcitonin response attributable to osteoclast function whereas osteoblast activity is apparently normal.

The animals used in all experiments were obtained by breeding our own C57BL/6J +/mi parental stock. Eye pigmentation pattern at birth was used to identify genotype. Calvaria were excised aseptically from 1–2-day-old homozygous dominant normals (+/+) and recessive (*mi/mi*) osteopetrotics and were cultured in minimum essential medium (GIBCO) supplemented with 10% fetal calf serum and the hormones indicated in Table 1. Air containing 5% CO_2 was the gas phase for all experiments except the resorption studies in which 50% oxygen, 5% CO_2 and 45% nitrogen[10] was used. Cyclic AMP was measured by radioimmunoassay[14] using the ^{125}I-tyrosine methylester of succinyl-cyclic AMP and rabbit anti-cyclic AMP antiserum (both from NEN). Parathormone had a biological activity >2,500 U mg^{-1}, and was isolated in our laboratory[15].

The biochemical parameters chosen to test osteoclast function were the stimulation of cyclic AMP formation by both parathormone and calcitonin, and stimulation of hyaluronic acid synthesis by parathormone and the inhibition of this action by calcitonin. For osteoblast function the activities were stimulation of cyclic AMP formation and inhibition of citrate decarboxylation by parathormone—both effects are unaltered by calcitonin. To measure overall response of the osteopetrotic bone to parathormone nett resorption of the tissue was measured.

Parathormone stimulated resorption in normal calvarium by more than 50% and calcitonin blocked this response (Table 1). In osteopetrotic calvarium basal resorption was similar to that in the normals but bone resorption was not stimulated by parathormone. The basal level of cyclic AMP in the normal and osteopetrotic calvarium was the same (Fig. 1). Parathormone increased cyclic AMP production to the same extent in both groups as did prostaglandin E_2, another bone resorptive agent that apparently acts through cyclic AMP[16]. In contrast, calcitonin increased cyclic AMP more than 10-fold in the normal bones but only threefold in the osteopetrotic calvarium. Basal and parathormone-stimulated levels of hyaluronate synthesis were the same in both normal and osteopetrotic bones (Fig. 2). In normal bone calcitonin reduced this parathormone-stimulated increase by more than 75%. In osteopetrotic bone calcitonin exerted no inhibitory effect. Parathormone, as well as 1,25-dihydroxycholecalciferol, inhibited citrate metabolism in the osteopetrotic calvarium as effectively as in normal calvarium (Table 2).

These results show that osteopetrotic bone is deficient in certain biochemical markers that have previously been characterised in osteoclasts[12,13]. With the demonstration that the tissue fails to resorb normally under the influence of parathormone the results are consistent with the morphological and cytological evidence[1] that the genetic lesion resides in osteoclasts. In contrast, osteoblasts of osteopetrotic bone responded normally to parathormone and other bone resorptive agents (prostaglandin E_2 and 1,25-dihydroxycholecalciferol). This observation,

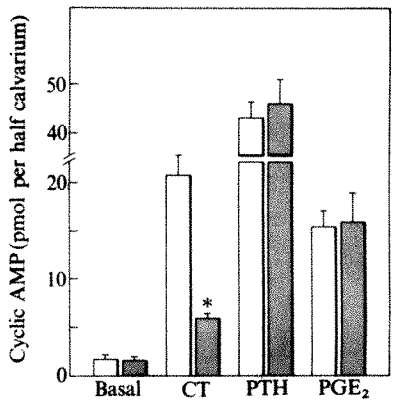


Fig. 1 The effect of calcitonin (CT, 12 nM), parathormone (PTH, 20 nM) and prostaglandin E_2 (PGE_2, 25 μM) on cyclic AMP formation in calvaria from normal and osteopetrotic mice. Half calvaria were placed in 1 ml of medium containing 0.5 mM theophylline and hormone additions as indicated. After 3 min cyclic AMP was extracted from the bones with 90% *n*-propanol[20] and was assayed by radioimmunoassay[14]. The dry weight and protein content of the normal and osteopetrotic were the same. Data are expressed as mean ± s.e.m. ($n = 4$). □, Normal (+/+); ▨, osteopetrotic (*mi/mi*). * Significantly different from normal, $P < 0.01$.

Table 1 Effect of parathormone and calcitonin on calcium release from normal and osteopetrotic calvaria

	Ca release (μmol per bone per 48 h)	
Treatment	Normal	Osteopetrotic
Control	0.271 ± 0.011	0.305 ± 0.008
Parathormone	0.414 ± 0.025*	0.335 ± 0.015
Parathormone + calcitonin	0.309 ± 0.008	0.330 ± 0.022

Whole calvaria (parietal and frontal bones) were individually cultured in 1 ml minimum essential medium containing 10% fetal calf serum. Parathormone (20 nM) and synthetic salmon calcitonin (12 nM, Armour) were added as indicated. Calvaria were maintained at 37 °C in a gas phase of 5% CO_2, 50% O_2, 45% N_2 for 48 h (ref. 10). Medium was removed and calcium concentration determined[19]. The release of calcium is defined as the difference between live bone and dead bone (frozen and thawed three times) for each group. Values are mean ± s.e.m.; $n = 7$ (for normal) and $n = 6$ (for osteopetrotic).

* Different from control, $P < 0.01$.

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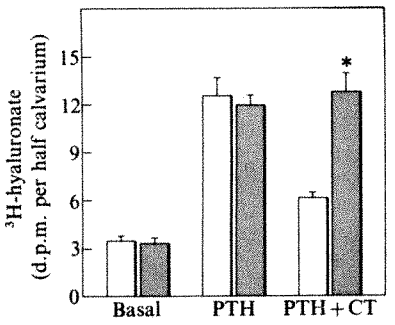


Fig. 2 Effect of parathormone (PTH, 20 nM) alone or in the presence of calcitonin (CT, 12 nM) on hyaluronic acid synthesis in half calvaria from normal and osteopetrotic mice. The bones were incubated for 24 h in medium (plus hormones as indicated) and then 3 μCi ^{3}H-glucosamine (NEN) was added to each bone. After 4 h the bones were rapidly frozen, digested with papain[11] and the ^{3}H-hyaluronic acid was isolated by differential cetylpyridinium chloride precipitation[21]. Data are expressed as mean ± s.e.m. (n = 6 for normal; n = 7 for osteopetrotic). Calcitonin alone had no effect on hyaluronic acid synthesis on either normal or osteopetrotic bone. □, Normal; ▨, osteopetrotic. * Significantly different from normal PTH + CT group, $P < 0.01$.

based on the degree of citrate decarboxylation[12,17], is consistent with that of Raisz *et al.*[6] who found that parathormone reduced collagen synthesis by osteopetrotic and normal bone by the same amount.

A paradoxical aspect of these findings is that whereas the osteopetrotic bone failed to respond to parathormone in terms of bone resorption, it exhibited normal response in terms of cyclic AMP formation and hyaluronate synthesis. It is possible that despite the seemingly normal activation of adenylate cyclase by parathormone, the specific response of the osteoclasts were nonetheless impaired. It has been shown that parathormone elicits substantially less cyclic AMP per osteoclast than per osteoblast[12]. This, together with the much larger number of osteoblasts than osteoblasts in calvarium[1], could have masked a decreased production of cyclic AMP by the osteoclasts. This is unlikely, however, as parathormone also increased the osteoclast marker hyaluronate synthesis to the same extent in osteopetrotic and normal bones.

The reduced response of osteopetrotic calvarium to calcitonin may be partly due to a decrease in the number of osteoclasts. Barnicot[18] reported that there was about a 30% reduction in osteoclast number in the grey lethal osteopetrotic mutant. However, the fact that the levels of basal and parathormone-stimulated hyaluronic acid synthesis in the microphthalmic bone were normal suggests that there was no difference in osteoclast number in our experimental conditions. It seems likely then that our results are better explained by a difference in the number or nature of calcitonin receptors, or an alteration in the mechanism for transmitting the calcitonin signal at the membrane level. The specific cause for such putative changes at the receptor level might be genetically based or secondary to alterations in circulating levels of calcitonin[22] or other agents that can affect bone function. Examination of receptor binding in the intact calvarium and in cells isolated from the osteopetrotic bone would help elucidate the nature of the genetic lesion.

Table 2 Effect of parathormone, 1,25-dihydroxycholecalciferol and prostaglandin E_2 on citrate decarboxylation in normal and osteopetrotic calvaria

Treatment	Citrate decarboxylation ($^{14}CO_2$ d.p.m. per half calvarium)	
	Normal	Osteopetrotic
Control	7,033 ± 1,383	7,305 ± 1,669
Parathormone	1,045 ± 114†	850 ± 107†
1,25-dihydroxycholecalciferol	1,675 ± 335†	2,091 ± 498*
Prostaglandin E_2	1,091 ± 173†	1,168 ± 351†

Calvaria were halved along the saggital suture and groups of 6 normal and 7 osteopetrotic half calvaria were individually cultured for each treatment group. Parathormone (20 nM), 1,25-dihydroxycholecalciferol (1 nM Roche) and prostaglandin E_2 (25 μM, Sigma) were added as indicated. After 48 h of culture the capacity of the bones to decarboxylate [6-^{14}C]citrate (NEN) was measured as previously described[10]. Data are shown as mean ± s.e.m.

* Different from control, $P < 0.05$.

† Different from control, $P < 0.01$.

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Melittin shares certain cellular effects with phorbol ester tumour promoters

MELITTIN, a 26-amino acid polypeptide of known sequence, is the major constituent of bee venom[1]. Its primary amino acid sequence reveals that the molecule has both basic and hydrophobic termini, which give the molecule an amphipathic character. Consequently melittin can insert into phospholipid bilayers and exhibits surfactant activity. There is evidence that the association of melittin with cellular membranes results in: (1) disturbance of the acyl groups of phospholipids such that they

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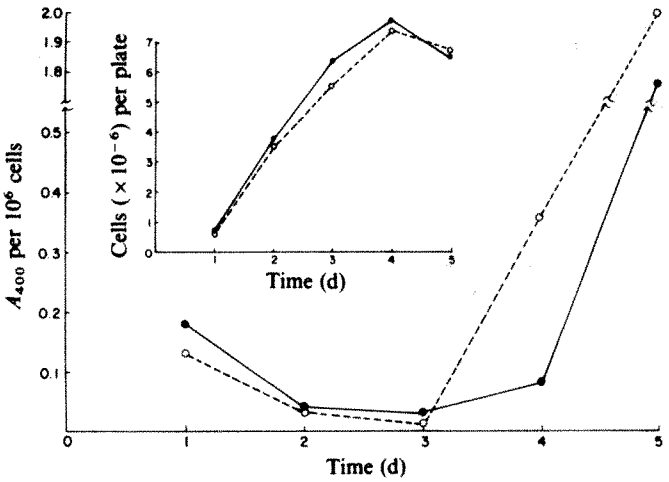


Fig. 1 The effect of melittin on the course of differentiation of C_3B16 melanoma cells. The cells were plated in medium containing 2 $\mu g\ ml^{-1}$ melittin in phosphate-buffered saline (PBS) or a control volume of PBS. After 24 h, fresh media containing either melittin or PBS were added. Cell counts and melanin determinations were carried out on replicate cultures every 24 h. The melanin content of cells plus medium was determined spectrophotometrically and is expressed in A_{400} units[17]. Cell number was determined with an electronic Coulter counter; inset shows total cell number per plate. All values are the means from three replicate culture plates; the s.e.m. was $\leq 10\%$ of the mean.

assume a broader range of angles with respect to each other[2,3]; (2) increased phospholipid susceptibility to hydrolysis by phospholipase[4,5]; and (3) increased synthesis of prostaglandins from the arachidonic acid released from the phospholipids[6]. Another amphipathic but structurally unrelated group of compounds consists of esters of the diterpene alcohol phorbol. These compounds are well known tumour promoters and have also recently been shown to have the ability to enhance phospholipid deacylation[7,19]. It is therefore of interest to determine whether melittin can produce some of the other phenotypic effects that can be induced in cells in culture by phorbol ester tumour promoters[8,9]. We report here that both melittin and the phorbol ester, 12-O-tetradecanoyl phorbol-13-acetate (TPA), inhibit differentiation of mouse melanoma cells, enhance anchorage-independent growth of adenovirus-transformed rat embryo cells, and induce arachidonic acid release and prostaglandin synthesis from C3H/10T½ mouse embryo fibroblasts.

Changes in arachidonic acid metabolism were examined in normal C3H/10T½ mouse embryo fibroblasts as it is known that *in vitro* transformation by chemical carcinogens or radiation is enhanced by TPA and related phorbol esters[10,11]. We found that TPA (2×10^{-8} M) and melittin (8×10^{-7} M) produced a two- to threefold enhancement of arachidonic acid and prostaglandin E_2 (PGE_2) release 3 h after addition to these cultures (Table 1). A low level of release of both arachidonic acid and PGE_2 in the control cultures was due to the presence of serum in the medium, an effect which has been previously reported[7,19]. Both TPA and melittin, however, enhanced arachidonic acid and PGE_2 release even in serum-free conditions. Time-course studies indicated that the release of both arachidonic acid and PGE_2 was maximal 3 h after adding either TPA or melittin. After 3 h the levels declined and had returned to control values by 24 h.

The phorbol esters are very potent inhibitors of differentiation in several cell culture systems (see ref. 9, for review),

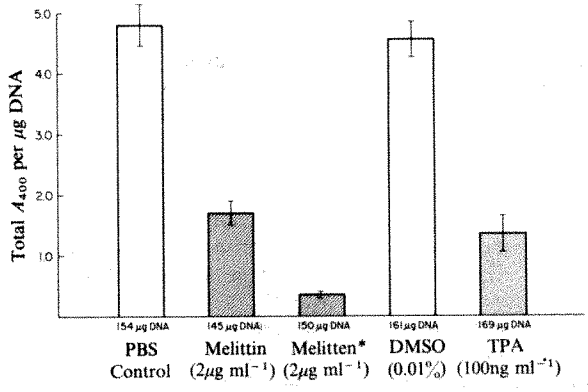


Fig. 2 The effect of melittin and TPA on the differentiation of C_3B16 cells. The cells were plated in medium containing melittin, TPA or the appropriate vehicle controls. After 24 h the cells were changed to fresh medium containing the appropriate compounds, except in one group (*) in which the melittin concentration was increased to 5 $\mu g\ ml^{-1}$. The cells and media were collected 5 d after plating. Melanin content of cells plus medium was determined spectrophotometrically[17], and the DNA content of the cells measured by the diphenylamine method[18]. Melanin levels are presented as the mean ±s.e.m. of determinations from four replicate culture dishes. DNA values are total μg per plate on day 5.

Table 1 Changes in arachidonic acid and prostaglandin release from C3H/10T½ cells

Addition	Arachidonic acid (c.p.m. $\times 10^{-3}$ per 200 μl medium)	Prostaglandin E_2 (c.p.m. $\times 10^{-1}$ per 200μl medium)
Phosphate-buffered saline	16 ± 2.0	28 ± 5.3
Melittin (8×10^{-7} M)	32 ± 3.7	68 ± 7.1
DMSO 0.1%	17 ± 1.5	25 ± 6.0
TPA (2×10^{-8} M)	40 ± 5.1	98 ± 11.0

C3H/10T½ cells (2×10^5) were grown for one day in 50-mm plastic tissue culture dishes in 4 ml of Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Then fresh medium containing 2.5 μCi of ^{3}H-arachidonic acid (60–100 Ci mmol^{-1}; NEN) was added. After labelling for 24 h this medium was removed and the cells were rinsed three times with 5 ml of serum-free medium. The labelled cultures were then incubated with 2 ml of medium containing 10% fetal calf serum and melittin, TPA or the corresponding solvents. Aliquots of the media were collected at 3 h and analysed for arachidonic acid and PGE_2 content by thin layer chromatography, as previously described[19]. The values given are means ± s.e.m. from three replicate culture plates. DMSO, dimethyl sulphoxide. Additional studies indicated that during the labelling period 90% of the incorporated ^{3}H-arachidonic acid was in cellular phospholipids and that the trace amounts of unincorporated radioactivity associated with the cells could not account for the radioactivity subsequently released into medium.

including melanogenesis in mouse melanoma cultures[12,20]. Cultures of the C_3 clone of B16 mouse melanoma cells (C_3B16) produce only small amounts of the pigment melanin until they reach confluence. There is then a marked and rather abrupt synthesis of melanin which accumulates in both the cells and culture medium.

Figure 1 indicates that when added to C_3B16 cultures shortly after plating, melittin (8×10^{-7} M) caused a delay of about 24 h in the onset of melanogenesis. Nevertheless, the melittin-treated cultures grew at the same rate and reached confluence at the same time as the control culture (inset Fig. 1). Thus, the delay in differentiation caused by melittin was not due to growth inhibition or cytotoxicity. These effects are similar to those produced by TPA and other phorbol-related tumour promoters[12]. Figure 2 compares in parallel the effects of melittin and TPA on pigment formation when either compound was added at the time the cells were plated. The amount of melanin was measured on day 5.

At 8×10^{-7} M (2μg ml^{-1}) melittin caused a 66% inhibition of pigment formation. An approximately equivalent inhibition was

Table 2 Enhancement of growth in agar of E11 cells by TPA or melittin

Compound added	No. of colonies	Agar cloning efficiency (%)
Control	58± 7.8	2.9±0.36
TPA (2×10^{-8} M)	192±22.8	9.6±1.14
Phorbol (3×10^{-7} M)	62± 8.4	3.1±0.42
Melittin (8×10^{-7} M)	174±16.8	8.7±0.84

Agar cloning efficiency of E11 cells was determined as previously described[15]. Cells 2×10^3 were suspended in 4 ml of Dulbecco's medium (0.1 mM Ca^{2+}, and 0.4% Noble Agar) supplemented with 7.5% fetal bovine serum. This suspension was overlaid on prehardened 4-ml base layers of 0.8% Noble Agar in the same medium in 50-mm culture plates. Parallel cultures contained TPA, phorbol or melittin incorporated in both agar overlay and base layers. Cultures were refed after 4 and 10 d by overlaying with medium containing 0.4% Noble Agar and the appropriate compounds. Colony number was determined by light microscopy after 14 d (colony size >0.1 mm). Results are reported as the average number of colonies in 4 replicate plates, ±s.e.m. and the agar cloning efficiency (%), ±s.e.m. Neither the solvents PBS or 0.1% DMSO affected cloning efficiency.

obtained with 1×10^{-7} M TPA (100 ng ml^{-1}). Increasing the melittin concentration to 2×10^{-6} M (5 μg ml^{-1}) one day after seeding produced a further inhibition of melanogenesis (Fig. 2). In these studies the DNA content of cultures was measured and the data obtained (Fig. 2) also indicate the absence of any growth inhibition by either melittin or TPA at the concentration used.

Phorbol esters have also been shown to enhance the expression of transformation-related properties in various cell cultures[8,9]. Growth of cells in soft agar (anchorage-independent growth) closely correlates with the tumorigenicity of cells *in vivo*[13]. Recent studies indicate that phorbol esters enhance the anchorage-independent growth of adenovirus-transformed rat embryo cells[9] and certain serially passaged mouse epidermal cells[14]. We examined, therefore, the effect of melittin on anchorage-independent growth of E11 cells, a clone of adenovirus-transformed rat embryo cells[15,16]. Table 2 indicates that both melittin (8×10^{-7} M) and TPA (2×10^{-8} M) caused approximately a threefold increase in the agar cloning efficiency of E11 cells, while phorbol, which is inactive as a mouse skin tumour promoter, did not enhance colony formation. Separate studies indicated that the concentrations of melittin and TPA used in these studies are approximately the optimal ones for these effects. Melittin concentrations greater than 2×10^{-6} M were cytotoxic.

The melittin preparation used in the above studies may contain trace amounts (~0.05 units per μg) of phospholipase A (Sigma) activity. This activity cannot, however, account for the results observed, as control studies using purified bee venom phospholipase A_2 (Sigma) in amounts several times higher than this level had no effect on melanogenesis by the B16 melanoma cultures, growth in agar of E11 cells, or PGE_2 synthesis.

Thus, it is rather striking that two structurally diverse compounds, melittin (a polypeptide) and TPA (a macrocyclic diterpene), have similar effects on three different properties of cells in culture: enhancement of the release of arachidonic acid and PGE_2; inhibition of melanogenesis; and enhancement of growth in agar. In view of the fact that melittin forms a very specific complex with the lipid phase of cell membranes[1-4], and the fact that a number of the early effects of TPA on cells are related to properties of cell membranes (see ref. 9 for review), it is likely that both compounds act by disturbing the structure and function of cell membranes. It is not clear, however, whether all the pleiotropic effects of the phorbol ester tumour promoters are secondary to such membrane effects or whether certain effects are mediated by non-membrane-related events. Melittin should prove highly useful for examining this question. In particular, it will be of interest to determine whether melittin itself has tumour-promoting activity. These studies are in progress.

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Quantitation of the adaptive response to alkylating agents

ESCHERICHIA COLI can acquire resistance to the lethal and mutagenic effects of alkylating agents such as *N*-methyl-*N'*-nitro-nitrosoguanidine (MNNG)[1]. We have called this the adaptive response and have shown that it is distinct from previously described forms of DNA repair, including the inducible error-prone pathway[2,3]. The induction of a similar response has recently been demonstrated independently in the liver cells of animals fed dimethylnitrosamine (DMN)[4]. The phenomenon is therefore apparently widespread and may conceivably protect us from mutagenesis and carcinogenesis induced by low levels of nitrosamines. The main mutagenic lesion produced by agents like MNNG and DMN is probably O^6-methylguanine (O^6-MeG)[5], which can be read as adenine during DNA replication[6] and transcription[7]; certainly, most of the mutations produced by MNNG are GC to AT transitions[8]. Therefore, it was not surprising to find that adapted bacteria contain less O^6-MeG after a challenge dose of ^{3}H-MNNG than do non-adapted bacteria[9]. However, the response seemed to have two distinct components, which we can call the fast and the slow reactions. Immediately after a 2-min exposure to ^{3}H-MNNG the adapted bacteria contain less O^6-MeG, as if some fast reaction were protecting them from accumulating alkylation at the O^6 positions; there is, however, a limit to the capacity of this part of the response, and at higher concentrations of ^{3}H-MNNG (or after longer periods of exposure) the adapted bacteria start to accumulate O^6-MeG (and mutations) at the same rate as the non-adapted bacteria. This suggests that the molecules which carry out the fast reaction are used up during their interaction with O^6-MeG (that is, each can function only once). During subsequent growth in the absence of MNNG, both adapted and

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Table 1 The effect of temperature and chloramphenicol on the adaptive response

Challenge	Outgrowth	Non-adapted bacteria O^6 c.p.m.	N^7 (c.p.m.)	O^6/N^7	Adapted bacteria O^6 (c.p.m.)	N^7(c.p.m.)	O^6/N^7
10 μg ml^{-1} ^{3}H-MNNG	—	594	4,874	0.12	64	7,888	0.008
10 μg ml^{-1} ^{3}H-MNNG	60 min, 37 °C	14	3,550	0.004	—	—	—
10 μg ml^{-1} ^{3}H-MNNG	60 min, 37 °C, 100 μg ml^{-1} CM	428	3,679	0.12	—	—	—
10 μg ml^{-1} ^{3}H-MNNG 100 μg ml^{-1} CM	—	—	—	—	42	8,071	0.005
10 μg ml^{-1} ^{3}H-MNNG	—	884	9,399	0.09	—	—	—
10 μg ml^{-1} ^{3}H-MNNG	60 min, 37 °C	0	7,907	0	—	—	—
10 μg ml^{-1} ^{3}H-MNNG	60 min, 0 °C	747	8,283	0.09	—	—	—
50 μg ml^{-1} ^{3}H-MNNG	—	—	—	—	718	40,542	0.02
50 mg ml^{-1} ^{3}H-MNNG	60 min, 37 °C	—	—	—	0	21,010	0
50 μg ml^{-1} ^{3}H-MNNG	60 min, 0 °C	—	—	—	346	20,054	0.02

Cultures of *E. coli* AB1157 were challenged for 2 min at 37 °C with ^{3}H-MNNG (56 mCi mMol^{-1}) and then filtered, resuspended and either processed immediately or allowed to undergo outgrowth and then processed as described previously[9]. In each case, the c.p.m. in DNA O^6-methylguanine and N^7-methylguanine are for samples of $\sim 3 \times 10^{10}$ bacteria. The adapted bacteria had been exposed, before challenge, to 0.5 μg ml^{-1} MNNG for 90 min. For challenge of adapted bacteria in the presence of chloramphenicol (CM) the CM was added 10 min before challenge.

non-adapted bacteria lose their O^6-MeG, but the process is completed much sooner in the adapted bacteria; thus, there is apparently a slow component to the adaptive response. This separation of the response into two components[9] was supported by the observation that the fast reaction occurs at 0 °C whereas the slow reaction does not (P. F. Schendel and P.R., unpublished results). However, further experiments reported here indicate that these two reactions are different manifestations of a single, rather unusual form of repair.

Although adapted bacteria lose their O^6-MeG during outgrowth sooner than non-adapted, this proved to be mainly because the fast reaction had ensured that they contained less O^6-MeG at the start of the period of outgrowth. When the challenge doses of ^{3}H-MNNG were adjusted so that the adapted and non-adapted cultures were left with the same absolute amount of O^6-MeG, the two cultures lost it at the same rate (Fig. 1). Thus, adapted bacteria seemed to differ from non-adapted solely in the presence of the fast reaction, and the slow reaction seemed to be some constitutive form of repair that could not be enhanced by adaptation.

Having reached that conclusion, we were therefore surprised to find that mutants, selected for their inability to adapt to MNNG, were unable to carry out either the fast or the slow reaction[10]. The explanation became obvious, however, when we remembered that the fast reaction is carried out by gene products that have accumulated during the period of adaptation and are apparently used up during the challenge. The slow reaction therefore could simply be the induced synthesis and immediate action of the gene products that carry out the fast reaction; following challenge, such synthesis would be occurring at the same rate in adapted and non-adapted bacteria. If this were correct, the slow reaction should be inhibited by chloramphenicol and should not occur at 0 °C (or in adaptationless mutants); in contrast, the fast reaction should not occur if chloramphenicol has been present during the period of adaptation[1], but it could conceivably occur at 0 °C and should not be inhibited by chloramphenicol present during the challenge. These predictions were borne out by the experiments shown in Table 1.

Far from being complicated, the adaptive response to O^6-MeG seems therefore to be rather straightforward. If we accept that it is carried out by the product of the *ada* gene (in which we have isolated mutants)[10] and assume that each of these molecules can dispose of only one O^6-MeG, the numerology is approximately as follows. During growth in adapting concentrations of MNNG or during outgrowth following a challenge, each cell can make roughly 100 adaptation molecules per min, and so bacteria that divide every 30 min will contain about 3,000 molecules per cell when fully adapted. Any challenge that creates less than 3,000 O^6-MeG per cell can therefore be handled immediately by adapted bacteria, and no O^6-MeG will be detectable at the end of the challenge. During subsequent outgrowth, all O^6-MeG produced in non-adapted bacteria and any residual O^6-MeG in adapted bacteria (that is, in excess of 3,000) will be handled at the rate of 100 per min. These numbers are derived from the published experiments using *E. coli* AB1157 (ref. 9). Other strains we have studied have a more effective response and seem to make about five times more of the gene product.

The most unusual feature of adaptation is that the molecules which mediate the response seem to be used up during the reaction. This could explain why large doses of alkylating agents will inhibit the capacity of cells to handle subsequent small doses[9,11]. Furthermore, this feature may account for the discrepancy between the high carcinogenicity of certain nitrosamines and their low mutagenicity in the Ames test[12]; for reasons of economy, carcinogenicity is usually measured at fairly high dose rates at which the animal's adaptive response is

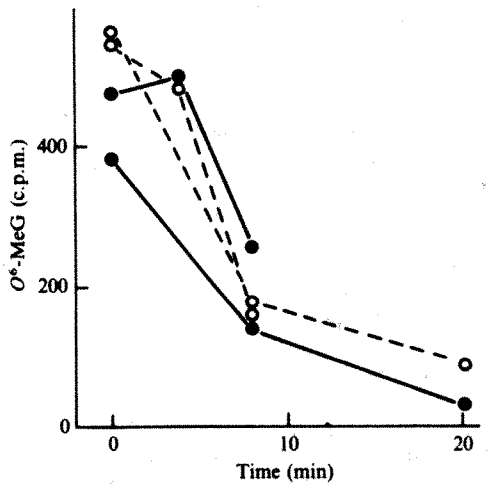


Fig. 1 The loss of O^6-methylguanine from the DNA of adapted and non-adapted *E. coli* AB1157. A culture was adapted by growth for 90 min in the presence of 0.5 μg ml^{-1} MNNG and then challenged with 50 μg ml^{-1} ^{3}H-MNNG for 2 min; to produce approximately the same starting level of O^6MeG, the control non-adapted culture was challenged with 10 μg ml^{-1} ^{3}H-MNNG. Both cultures were then filtered, resuspended and allowed to grow in the absence of MNNG. Samples containing 3×10^{10} bacteria were taken at various times and processed as described previously[9]. The results of two such experiments are shown. ●, Adapted bacteria; ○, non-adapted bacteria.

probably saturated, whereas bacterial mutagenicity is measured in growing cultures at low dose rates which are presumably within the capabilities of the bacterium's adaptive response.

Why the molecules that mediate adaptation can be used only once is not clear, but in this respect they resemble certain restriction enzymes which also can apparently act only once[13,14]. Fortunately, it is now possible to study the exact chemistry of the interaction between the mediator of bacterial adaptation and its substrate, O^6-MeG, because the process has been shown to occur *in vitro* (see accompanying paper[15]).

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Adaptive response to alkylating agents involves alteration *in situ* of O^6-methylguanine residues in DNA

THE mutagenic lesion O^6-methylguanine (O^6-MeG) is slowly but actively removed from DNA in *Escherichia coli* cells treated with alkylating agents[1]. In bacteria exposed to sublethal concentrations of chemical mutagens such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) or *N*-methyl-*N*-nitrosourea (MNUA), an apparently error-free repair system is induced that confers increased resistance to the mutagenic and lethal effects of challenge doses of the same or related compounds[2–4]. This adaptive response is associated with an increased ability of the induced *E. coli* to remove O^6-MeG residues from their DNA (see ref. 5 and preceding paper[6]). We have investigated the action of cell-free extracts from adapted *E. coli* on DNA containing O^6-MeG residues. Here we report that O^6-MeG disappears from alkylated DNA after incubation with a crude enzyme fraction from adapted cells, although no concomitant release of the methyl group or the alkylated base or nucleotide occurs. Instead, this process is due to a previously unrecognised DNA repair mechanism involving enzyme-catalysed structural alteration of the alkylated residue.

The standard substrate used to study the removal of O^6-MeG from DNA *in vitro* is *Micrococcus luteus* DNA (chosen because of its high guanine content) alkylated with [*Me*-^{3}H]MNUA. Because the enzymatic release of free 3-methyladenine from the alkylated DNA by a DNA glycosylase[7] complicates the analysis of the fate of O^6-MeG residues, the MNUA-treated DNA was preincubated for 16 h at 80 °C in neutral solution. In this way, purines methylated in the 7 and 3 positions were released while the double-stranded DNA structure was maintained. After dialysis, about 75% of the radioactivity present in the DNA

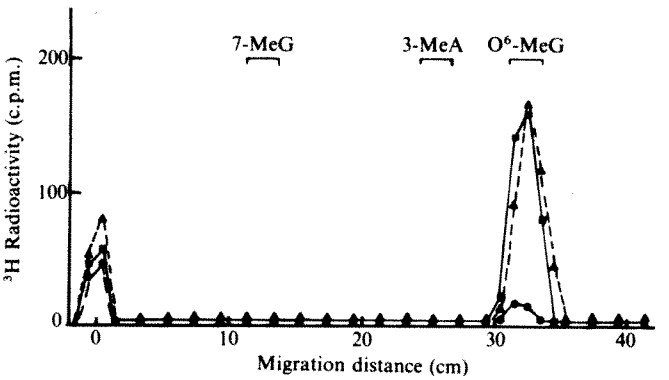


Fig. 1 Disappearance of O^6-methylguanine from DNA catalysed by extracts of adapted *E. coli*. *M. luteus* DNA (25 mg) in 5 ml of 0.2 M sodium cacodylate and 1 mM EDTA (*p*H 7.2) was incubated for 2 h at 37 °C with 1 mCi ^{3}H-MNUA (NEN, 1.03 Ci mmol^{-1}). The alkylated DNA was precipitated with 2 vols cold ethanol, dissolved in 5 ml of 0.1 M NaCl, 10 mM sodium citrate and 10 mM potassium phosphate (*p*H 7.4), and dialysed at 4 °C for 24 h against the same buffer. The DNA solution was then incubated at 80 °C for 16 h in a sealed glass ampoule, followed by dialysis at 4 °C for 24 h against 1 M NaCl, 10 mM Tris-HCl and 1 mM EDTA (*p*H 8.0), and for 4 h against the same buffer without NaCl. The DNA was stored frozen and had a specific radioactivity of 50,000 c.p.m. per mg. *E. coli* B/r was grown and adapted by exposure to MNNG (1 μg ml^{-1} for 90 min) as described by Jeggo *et al.*[3]. Adapted or non-adapted cells (3 g) were disrupted by grinding with three parts of sea sand and extracted with 12 ml of 70 mM HEPES-KOH, 1 mM dithiothreitol and 5% glycerol (*p*H 7.8). After removal of debris by centrifugation, extracts contained about 25 mg protein per ml. They were stored in small aliquots at −70 °C and were never refrozen. The reaction mixture (30 μl) contained 70 mM HEPES-KOH (*p*H 7.8), 1 mM dithiothreitol, 1 mM EDTA, 50 μM spermidine, 5% glycerol, 12 μg (600 c.p.m.) heated ^{3}H-MNUA-treated DNA, and cell extract (2 μl). After 10 min at 37 °C, the reaction was stopped by chilling to 0 °C and addition of 30 μl cold 0.8 M trichloroacetic acid. After 10 min at 0 °C, the mixtures were centrifuged at 10,000*g* for 15 min. The supernatant solutions, which contained less than 5% of the total radioactivity, were discarded. The precipitates were suspended in 30 μl 0.1 M HCl, supplemented with carrier O^6-methylguanine (30 μg in 3 μl H_2O), and the DNA was hydrolysed at 70 °C for 30 min. Each hydrolysate was applied to Whatman 3MM paper and chromatographed for 20 h in isopropanol/concentrated NH_3/H_2O (7:1:2). Reference methylated purines[7] were run in separate lanes and localised as UV-absorbing material. Strips containing individual samples were cut out and then cut transversely into 1-cm pieces. Each such piece was cut into small fragments, which were transferred to a scintillation vial and eluted with 2 ml H_2O. Radioactivity was determined after addition of 15 ml of Triton X-based scintillation fluid. Reaction mixtures contained no extract (▲), extract from non-adapted cells (■) or extract from adapted cells (●).

was in the form of O^6-MeG residues (Fig. 1). The residual radioactive material may be ascribed to the presence of alkylated pyrimidine nucleotides and phosphotriesters[8].

Incubation of this DNA substrate with crude cell extracts of *E. coli* B/r, adapted by growth for 90 min in the presence of 1 μg ml^{-1} MNNG (ref. 2), results in the disappearance of O^6-MeG residues from DNA. In the same conditions, extracts from non-adapted bacteria show no detectable activity. The method of assay involves incubation of the alkylated DNA with cell extract in an EDTA-containing reaction mixture, followed by precipitation of the DNA with cold ethanol or dilute trichloroacetic acid. After mild acidic hydrolysis of the precipitate to release free purines, paper chromatography of the hydrolysate is used to determine the amount of radioactivity co-migrating with authentic O^6-MeG (Fig. 1). With increasing amounts of adapted cell extract in the reaction mixtures, decreasing amounts of O^6-MeG are recovered (Fig. 2).

The method of assay clearly demonstrates the disappearance of O^6-MeG from DNA, but an additional striking feature of the reaction is that no radioactive material is released in an ethanol- or acid-soluble form at the end of the incubation period. In conditions where 90% of the radioactive O^6-MeG has been altered or removed, less than 5% of the total radioactivity is released in soluble form. On mild acidic hydrolysis of the precipitated DNA, using conditions allowing essentially quantitative release and recovery of O^6-MeG (ref. 1), the radioactivity associated with the enzymatically altered residues is converted into volatile form. Thus, the radioactive material is

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retained in the acid hydrolysate but is lost on evaporation or after application of the sample to a chromatography paper. Similar results have been obtained with DNA alkylated with either [*Me*-^{3}H]MNUA or [*Me*-^{14}C]MNUA, and a volatile secondary product (presumably methanol) is generated by acid treatment in either case. These data indicate either that the methyl group bound at the O^6 position of guanine is transferred to another site by the activity in adapted *E. coli* cells, or that O^6-MeG residues are destroyed (for example, by ring cleavage) without being released from DNA. Further, the enzymatically altered base residue is more acid labile than intact O^6-MeG.

The radioactive material present in reaction mixtures treated with adapted cell extract (90% disappearance of O^6-MeG from DNA) was not liberated in ethanol-soluble form (<10% release) by treatment with either Pronase (2 mg ml^{-1}) or pancreatic RNase (100 μg ml^{-1}) for 4 h at 30 °C. On the other hand, treatment with pancreatic DNase I (100 μg ml^{-1}, 0.005 M each of $MgCl_2$ and $CaCl_2$ added) for 4 h at 30 °C resulted in the conversion of more than 80% of the radioactivity into ethanol-soluble form. Similar results were obtained in control experiments with DNA incubated with non-adapted cell extract. Thus, the radioactive methyl groups in the ethanol-precipitable material generated from O^6-MeG residues by treatment with an extract from adapted *E. coli* remain in DNA and do not seem to be transferred to a protein or RNA molecule.

These data differ from previous reports on the fate of O^6-MeG residues in DNA after treatment with crude enzyme fractions. Kirtikar and Goldthwait[9] claimed that a DNA glycosylase activity that would release free O^6-MeG from alkylated DNA was present in a partly purified preparation of *E. coli* endonuclease II. However, an extensive search with different enzyme fractions from either non-adapted or adapted *E. coli* has failed to confirm the existence of such a DNA glycosylase (ref. 7 and our unpublished data). Pegg[10] showed that rat liver extracts can remove O^6-MeG from alkylated DNA, albeit about 1,000 times less effectively than the adapted *E. coli* extracts investigated here. The removal was not due to a DNA glycosylase, and the active enzyme was tentatively identified as a demethylase because part of the radioactivity from the substrate could be recovered as free methanol after prolonged incubation with the cell extract.

E. coli K-12 mutants defective in the adaptive response (*ada*) have recently been isolated[11]. We have analysed extracts from two such mutants, *ada*-5 and *ada*-6, and their *ada*$^+$ parent strain AB 1157, for their ability to alter O^6-MeG residues in DNA. Extracts (10–40 μg protein added to the standard reaction mixture) from both mutants were unable to cause the disappearance of detectable amounts (<10%) of O^6-MeG from alkylated DNA even though the bacteria had been pretreated with MNNG, whereas the parent strain extract was clearly active. However, as *E. coli* K-12 yields a somewhat weaker adaptive response than *E. coli* B (refs 4, 6) the extract (40 μg protein) from the *ada*$^+$ strain only caused the loss of 60% of the O^6-MeG residues. Growth rates in the conditions of adaptation were similar for both the parent and mutant strains. These data strongly indicate that the enzyme acting on O^6-MeG residues investigated here is related to the previously characterised repair pathway of adaptation *in vivo*. The simplest interpretation of the available results is that this inducible enzyme is the product of the *ada*$^+$ gene in *E. coli*.

There has been only preliminary characterisation of the properties of the *E. coli* enzyme activity associated with the adaptive response. The activity remains in the supernatant of crude cell extracts after precipitation of nucleic acids (and some proteins) with 0.8% streptomycin sulphate, and it can be recovered by precipitation between 1.6 M and 2.6 M ammonium sulphate followed by dialysis. It is heat labile (50% inactivation occurring after 1 min at 45 °C in the standard reaction mixture without DNA) and is partly inactivated by freezing and thawing. There is no apparent cofactor requirement of the ammonium sulphate-fractionated enzyme, and no detectable stimulation of activity by Mg^{2+} or ATP. Inclusion of spermidine (5×10^{-5} to 2×10^{-4} M) in the assay mixture results in slight (about twofold) stimulation of activity.

Although the present results show that O^6-MeG removal in adapted *E. coli* does not occur by nuclease, glycosylase or demethylase action, the precise nature of the enzymatic alteration of this alkylated residue remains unclear. The methyl group might be transferred to another, biologically less hazardous, site in DNA. However, the present data exclude methyl group transfer to the N^7 position of guanine, because no 7-methylguanine or any other acid-stable product is generated (Fig. 1). Alternatively, the miscoding O^6-MeG might be converted to a non-coding residue, such as by cleavage of the pyrimidine ring to generate a substituted imidazole residue remaining bound to DNA. Methyl-group transfer or ring-opening of the alkylated residue might also result in a lesion more readily recognised by other DNA repair enzymes. It is now of interest to define the structural alteration of O^6-MeG that occurs in adapted *E. coli*, and this will depend on the availability of suitable methods to release and purify the labile altered residues from enzyme-treated alkylated DNA.

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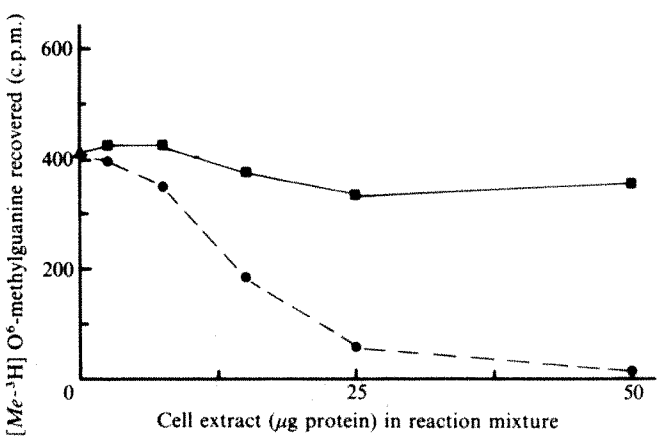


Fig. 2 Concentration dependence of the activity of an adapted *E. coli* cell extract on DNA containing O^6-methylguanine residues. Experimental conditions and symbols are as in Fig. 1. The extracts were adjusted to 25 mg protein per ml and diluted in cold reaction mixture (without DNA) immediately before use; 2 μl of each dilution was added to the standard reaction mixture.

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Hormonally specific phosphorylation of cardiac troponin I and activation of glycogen phosphorylase

WE recently reported that PGE_1 and isoprenaline have very different effects on the substrates of cyclic AMP-dependent protein kinase that regulate cardiac glycogen metabolism. Both agents elevated the cyclic AMP content and the protein kinase activity ratio in isolated perfused rat hearts, but only isoprenaline caused changes in the activity of the substrates of protein kinase (presumably phosphorylation, causing activation of phosphorylase kinase and inactivation of glycogen synthase)[1,2]. Thus, the activation of soluble protein kinase appears insufficient to cause alteration of the regulatory enzymes of glycogen metabolism. This suggests that the glycogenolytic responses to isoprenaline involve actions other than the activation of soluble protein kinase. We now report the effects of PGE_1 and isoprenaline on another substrate of protein kinase, the inhibitory subunit of cardiac troponin (TNI). Since the phosphorylation of TNI may mediate the positive inotropic effects associated with β-adrenergic stimulation and cyclic AMP accumulation[3], we felt that the hormonally specific expression of protein kinase activity might be used as a tool to determine whether similar specificity applies to the phosphorylation of TNI and to establish the functional relevance of that phosphorylation.

Although no biochemical consequence of TNI phosphorylation has been found to explain the positive inotropic action of drugs, some correlative data suggest a role for phosphorylation of TNI in the regulation of myocardial contractility. Cardiac TNI is phosphorylated in perfused hearts following exposure to β-adrenergic agonists and the phosphorylation correlates well with the time course and concentration-dependence of enhanced contractility[3]. When purified cardiac troponin is incubated with cyclic AMP-dependent protein kinase and [γ-^{32}P]ATP, the rate of phosphorylation is stimulated by cyclic AMP and inhibited by the heat stable inhibitor of protein kinase. The ^{32}P is incorporated specifically into the TNI subunit at rates two- to threefold greater than into mixed histones[4]. These results suggest that the rapid and specific phosphorylation of the I subunit of troponin may be a consequence of cyclic AMP accumulation in cardiac muscle. However, it is not clear whether phosphorylation of TNI always follows from elevation of intracellular cyclic AMP or results only from β-adrenergic stimulation[3,5]. Accordingly, we have compared the effects of isoprenaline and PGE_1 on cyclic AMP content, protein kinase activity, the phosphorylation of TNI, and contractility in the isolated perfused rat heart.

Measurement of the phosphate content of TNI depends for its accuracy on the purity of the material isolated from the perfused hearts. A densitometer tracing of a gel electrophorogram of the material isolated by affinity chromatography over troponin C columns (Fig. 1) shows that the major band of protein (>90%) moved with an apparent molecular weight of 26,100, in good agreement with the published value for TNI from other laboratories[3,4]. The purification by affinity chromatography and homogeneity with appropriate mobility on SDS-polyacrylamide gel electrophoresis are good criteria supporting the identity of the isolated protein as TNI. Small amounts of impurities (<10%) occasionally noticed on electrophorograms showed mobilities corresponding to those of TNC and TNT troponin subunits.

Both isoprenaline and PGE_1 increased the cyclic AMP content and the activity ratio of protein kinase in perfused rat hearts (Table 1). The activation of protein kinase occurred without changes in total activity (that measured in the presence of 2 μM cyclic AMP). Only isoprenaline treatment increased the phosphate content of TNI, and caused activation of phosphorylase and an enhanced rate of ventricular pressure development. PGE_1 did not show these effects despite its capacity to stimulate accumulation of cyclic AMP. The effects of 10 nM isoprenaline and 30 μM PGE_1 on cyclic AMP accumulation and protein kinase activation were additive, whereas the activity ratio of phosphorylase and the degree of phosphorylation of TNI were the same following treatment with isoprenaline alone and with the combination of isoprenaline and PGE_1.

We have found, like England[3], that treatment of perfused rat hearts with isoprenaline increases the phosphate content of TNI. Our values were up to 0.8 mol phosphate per mol TNI, in reasonable accord with the values reported by England for perfused rat heart[3] and comparable to values for perfused mouse heart[6]. These measurements are lower than the phosphate content of TNI prepared from rabbit heart (1–1.9 mol phosphate per mol TNI) as reported by Perry and co-workers[7,8] but are in good agreement with the *in vitro* experiments of Stull and Buss[4] who, using purified components, found that cyclic AMP-dependent protein kinase catalysed the maximal incorporation of 1 mol phosphate per mol TNI, suggesting a single site available for phosphorylation per TNI molecule. However, we emphasise the relative changes in TNI phosphate and the failure of PGE_1 to cause TNI phosphorylation.

Previous reports have demonstrated that not all changes in cardiac contractility correspond to an altered phosphorylation state of TNI. For instance, England[3] found that the inotropic effect of glucagon was maximal before significant TNI phosphorylation could be detected. Ezrailson *et al.*[9] reported that in perfused cat heart ouabain, increased frequency of stimulation, and elevated external calcium induced positive inotropic responses with no changes in TNI-phosphate. Frearson *et al.*[10] found no change in TNI phosphorylation when the inotropic state of perfused rabbit heart was altered by low-sodium and

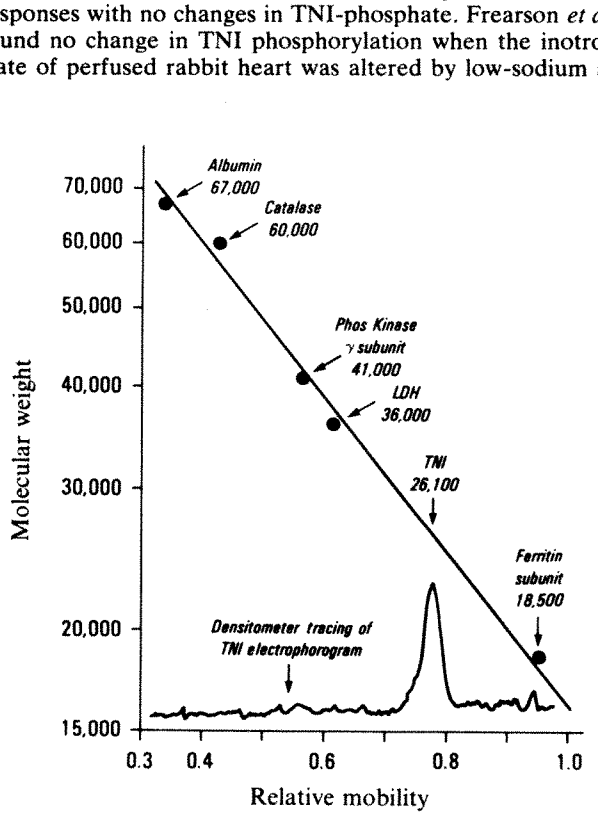


Fig. 1 SDS-polyacrylamide gel electrophoresis of TNI and calibrating proteins. Samples were solubilised as described by Stull and Buss[4]. Samples and standards (Pharmacia) were applied to 0.75 × 100 mm gel consisting of a 3.3% stacking gel and a 7.0% separating gel containing 0.5% SDS with Tris-glycine-SDS reservoir buffer. Following electrophoresis, gels were stained with Coomassie blue R (0.15%) in aqueous methanol (50%) acetic acid (7%), then destained with methanol acetic acid and equilibrated with acetic acid (7%). Stained bands were located and quantified by integration of areas under peaks of densitometer scans. The main figure shows a calibration curve (log of molecular weight versus mobility) indicating an apparent molecular weight of 26,100 for rat cardiac TNI. The inset shows actual densitometer tracing of gel with 2 μg of rat TNI applied. Purity of >95% was observed when up to 100 μg of TNI were applied.

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Table 1 Effects of isoprenaline and PGE_1 singly and in combination

Treatment (n)	Cyclic AMP (pmol per mg protein)	Protein kinase (−cAMP: +cAMP)	Phosphorylase (−AMP: +AMP)	Phosphate content of TNI (mol per mol)	dP/dt
Control (7)	5.0 ± 0.2	0.17 ± 0.01	0.05 ± 0.02	0.34 ± 0.03	1.00
INE (10 nM) (7)	8.6 ± 0.5**	0.26 ± 0.01**	0.36 ± 0.02**	0.49 ± 0.04**	1.83**
INE (100 nM) (5)	—	0.39 ± 0.01**	0.58 ± 0.03**	—	2.20**
PGE_1 (30 μM) (6)	7.3 ± 0.3**	0.24 ± 0.02**	0.08 ± 0.03	0.38 ± 0.06	0.94
INE (10 nM) + PGE_1 (30 μM) (6)	11.2 ± 0.7**	0.33 ± 0.01**	0.34 ± 0.03**	0.53 ± 0.07*	1.77**
INE (100 nM) + PGE_1 (30 μM) (4)	13.1 ± 1.1**	0.41 ± 0.02**	0.58 ± 0.03**	0.51 ± 0.05**	2.10**

Values are mean ± s.e.m. Asterisks indicate values differing significantly from control values by unpaired t-test analysis (*, $P < 0.02$; **, $P < 0.005$). dP/dt is expressed as a fraction of mean control value (1,680 ± 60 mm Hg s^{-1}). Hearts were excised from heparinised (1,500 U per kg), anaesthetised (pentobarbital, 100 mg per kg) male Sprague–Dawley rats (200–300 g) and immersed in ice cold saline until beating ceased. Hearts were perfused at 32 °C via the aorta without recirculation at an aortic pressure of 60 mm Hg with Krebs–Henseleit buffer as described by Neeley[17]. Mean coronary flow was 8 ml min^{-1}. Hearts were paced at 240 beats per min and equilibrated for 30 min before treatment. Left ventricular pressure and its rate of development (dP/dt) were monitored using an intraventricular needle connected to a pressure transducer. Following a 2-min perfusion with buffer containing drug or vehicle, hearts were frozen with Wollenberger clamps cooled in liquid N_2. The atria and great vessels were trimmed away and the remainder pulverised in a percussion mortar cooled in liquid N_2. The powder was stored at −70 °C until assayed. Cyclic AMP purified from acidic extracts of frozen tissue powder was quantified by the Gilman protein binding assay[18]. Protein kinase activity was assayed in the 300,000 g supernatant of a 1 : 10 homogenate of frozen tissue powder by the method of Keely *et al.*[19]. The activation state of protein kinase is expressed as the ratio of activities measured in the absence and presence of 2 μM cyclic AMP. Glycogen phosphorylase activity was assayed fluorometrically[20]; activities are expressed as the ratio of activities assayed in the absence and presence of 2 mM AMP. We have previously described these assays in more detail[2]. TNI was purified by troponin C affinity chromatography as described by Syska *et al.*[21] on TNC columns. For this procedure, 500 mg of frozen heart powder were homogenised in the presence of 8M urea which was removed only after the TNI was purified, so that enzymatic modification during purification was minimised[3]. The yield was ~120 μg TNI per g heart and was independent of the extent of phosphorylation. Purity of TNI was ≥90% as established for each sample by SDS polyacrylamide electrophoresis as demonstrated in Fig. 1. Protein-bound phosphate was measured by a combination of the general assay for phosphoester bonds in proteins[22] with the assay of inorganic phosphate of Itaya and Ui[23] and the ashing procedure of Ames[24], as detailed by Stull and Buss[4]. Protein was quantified by the method of Bradford[25] using bovine serum as a standard. We used a value of 23,550, as determined by amino acid analysis[26], as the molecular weight of TNI for our calculations.

high-calcium media. Nonetheless, the hypothesis that cardiac troponin may be phosphorylated *in vivo* in response to stimuli which increase cyclic AMP[5] and that this phosphorylation may be associated with increased contractile force[3] remains attractive. Our data suggest that this hypothesis must be modified since PGE_1 increases cyclic AMP but causes no net phosphorylation of TNI.

We have previously[2] discussed several possible explanations for this anomalous effect of PGE_1. A direct inhibitory effect of PGE_1 at a stage distal to protein kinase activation is unlikely since a high concentration of PGE_1 (30 μM) caused no inhibition of phosphorylase *a* formation, TNI phosphorylation, or the inotropic effects of 10 nM isoprenaline. We have not been able to detect an increase in phosphoprotein phosphatase activity due to PGE_1 (ref. 2). Maximally effective concentrations of the two agents produced the same activation of protein kinase as did isoprenaline alone, suggesting that we are not dealing with two populations of responsive cells. We must also consider the possibility that PGE_1 caused a transient change in protein phosphorylation such that our measurements, made on hearts freeze-clamped after 2 min of drug treatment, would not reflect the transient effect. Constant monitoring showed no transient change in dP/dt during the 2 min of treatment. The data of Keely (ref. 11 and personal communication) demonstrate that continuous perfusion with catecholamine or PGE_1 causes quick increases in cyclic AMP content and protein kinase activity ratios that are maintained for the duration of drug treatment (up to 10 min). During a 10-min treatment, catecholamine causes a sustained activation of phosphorylase whereas PGE_1 elicits no activation[11]. Thus, it seems that PGE_1 causes no transient increase in protein phosphorylation. England has demonstrated the persistence of TNI-bound phosphate following a pulse of isoprenaline[3]. His result suggests that a transient activation of TNI phosphorylation might still be noticeable as increased TNI-bound phosphate at a later time. Consideration of these points argues against a transient change in protein phosphorylation in response to PGE_1.

Two alternative explanations are attractive: that β-adrenergic stimulation supplies a 'factor' that PGE_1 does not, or that PGE_1 and isoprenaline activate protein kinases in different cellular compartments. We have previously discussed these possibilities with respect to the regulation of cardiac glycogen metabolism. If β-agonists supply a specific factor necessary for the expression of protein kinase activity, it would probably not be Ca^{2+}, since protein kinase is not known to require this cation[2]. In the present case, however, the discussion must be more tentative since the regulation of TNI phosphorylation and contractility by cyclic AMP-dependent protein kinase is by no means as clearly established as the involvement of protein kinase in the stimulation of glycogenolysis and because Ca^{2+} is intimately involved in the contractile process. The compartition of protein kinase within the cell and the activation of an ineffective pool of the kinase in response to PGE_1 are testable hypotheses. Several 'pools' of protein kinase are known—particulate and soluble, isozymes I and II (refs 12, 13); substrates and cyclic AMP, too, can be localised[14,15].

To test the possibility that the two isozymes of protein kinase might be selectively involved in the differing responses to isoprenaline and PGE_1, we have looked for hormonal specificity in hearts differing in their content of the isozymes—rat (>80% type I) and guinea pig (>90% type II)[12,16]. In both of these preparations, isoprenaline and PGE_1 increase intracellular cyclic AMP and cause comparable elevations of the protein kinase activity ratio, but only isoprenaline causes the activation of glycogen phosphorylase and an enhanced dP/dt. Thus, activation of protein kinase and the hormonally specific expression of protein kinase activity occur in hearts in which either isozyme of protein kinase predominates (data not shown).

The present results confirm that TNI-bound phosphate increases in perfused rat heart treated with isoprenaline. The phosphorylation of TNI is functionally relevant to the extent that enhanced dP/dt and increased TNI-phosphate occur concurrently; and when, following exposure to PGE_1, we record no change in dP/dt, we find no change in the phosphate content of TNI.

Our study indicates that TNI phosphorylation and other sequelae are not the consequence merely of increased intracellular cyclic AMP. β-Adrenergic stimulation must provide a signal more complex than a generalised stimulation of cyclic AMP formation, since neither enhanced dP/dt nor increased phosphate content of TNI nor phosphorylase activation results from treatment of perfused rat heart with PGE_1, even though

PGE_1 increases both cardiac cyclic AMP content and the activity ratio of soluble protein kinase (Table 1). England's results[3] suggest that glucagon may also provide the appropriate stimuli for enhanced protein phosphorylation and positive inotropism. This hormonally specific expression of protein kinase activity seems unrelated to the predominance of either of the isozymes of protein kinase. We are now extending these observations and considering the biochemical data in light of known functional and morphological compartments of the heart and in light of previous reports on functional pools of cyclic AMP and cyclic AMP-dependent protein kinases[12-15]. We are also aware that the compartition of cyclic AMP and protein kinases could have intriguing implications for the relationship of hormonal receptors, adenylate cyclase, and intracellular compartments.

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High-frequency generalised transduction by bacteriophage T4

GENERALISED transduction—the transfer of bacterial genes from one cell to another via bacteriophages—is one of a small number of processes by which bacteria can acquire exogenous genetic information. Transduction was first demonstrated in *Salmonella typhimurium* with the phage P22 by Zinder and Lederberg[1,2] and subsequently in *Escherichia coli* with the phages P1[3] and T1[4]. Bacteriophage T4 is the largest, and one of the most thoroughly studied of the virulent coliphages but has never been observed to mediate generalised transduction. In this report we describe a multiple mutant of T4 that displays the property of generalised transduction, and transfers *E. coli* genes with frequencies that, in general, are higher than those observed for other transducing phages.

The DNA of wild-type T4 contains glucosylated hydroxymethylcytosine (glu-HMC) in place of cytosine which enables the phage to escape host-controlled DNA restriction. Multiply mutated derivatives of T4 have been isolated which are defective in several genes involved in the synthesis of HMC and the degradation of cytosine-containing DNA[5]. We examined one such mutant, constructed originally for molecular cloning purposes, to see if it had the ability to transduce bacterial DNA. Mutant T4GT7 (T4 generalised transducer no. 7) was isolated as a pseudorevertant of the triple mutant *am*E51 (g*56*, dCTPase), *am*C87 (g*42*; dHMase), NB5060 (deletion spanning rIIB, *denB*, *ac*) on a restriction deficient, non-amber suppressing (Sup°) *E. coli* K host. The revertant carries an additional spontaneous *alc*$^-$ mutation (E. Kutter, personal communication) and yields progeny with cytosine-containing DNA. The progeny DNA is sensitive to *in vivo* restriction by the plasmid-specified *Eco*RI system, and *in vitro* the DNA is cleaved by many restriction endonucleases[6]. When the mutant is grown on an amber-suppressing host (Sup$^+$) permitting g*56* and g*42* to function, it produces progeny containing glu-HMC DNA which is resistant to restriction both *in vivo* and *in vitro*.

When auxotrophic strains of *E. coli* are infected with T4GT7 previously grown on bacteria carrying wild-type alleles, transductants arise at high frequency for most markers tested. Among the host markers for which we have observed transduction are the amino acids *leu* (2 min), *proA* (6 min), *his* (44 min), *arg G* (68 min) and *metB* (87 min), and the sugars *ara* (2 min), *lac* (8 min) and *xyl* (79 min) (ref. 7). In general, transductants arise at frequencies around 1 per 10^4 singly infected cells, but marker-dependent variation is observed (Table 1). For some markers, notably *thr* (0 min), *malA* (74 min) and *mtl* (80 min) no transductants at all have been observed. Wild-type T4 is unable to transduce any of the markers we have tested; P1 does so, but at a much lower frequency than T4GT7, around 1×10^{-6}. To date we have only observed co-transduction for the closely linked markers *ara* and *leu* which are co-transduced at the single marker frequency. The transducing capacity of T4GT7 lysates is not diminished by treatment with either DNase or RNase, eliminating the possibility that we are observing transformation rather than transduction. Finally, the number of transductants increases with increasing multiplicity of infection (MOI) through the range 0.001 to 0.5, then falls off sharply at higher MOIs as multiple infections reduce the number of singly infected, potentially transduceable cells (Fig. 1). Together these data firmly establish that the mutant T4 phage are capable of generalised transduction.

In addition to transducing chromosomal markers, T4GT7 transduces extrachromosomal, plasmid-borne markers. Lysates

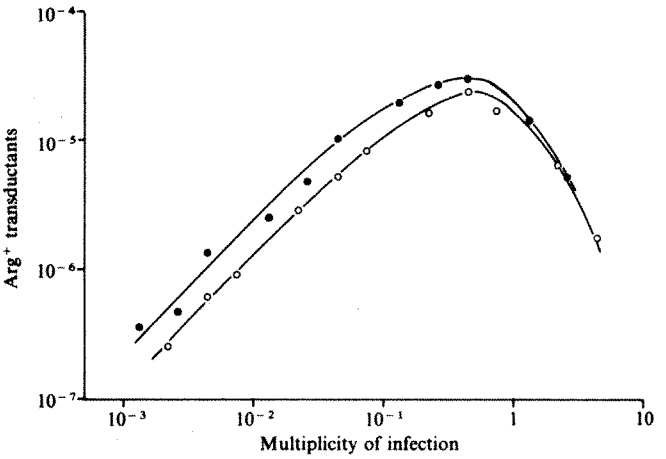


Fig. 1 Influence of multiplicity of infection on the formation of transductants. A culture of log-phase bacteria (JC411) was infected with T4GT7 at increasing multiplicities of infection and plated on minimal plates containing all the necessary supplements except arginine. The numbers of Arg$^+$ transductants were counted and are plotted here as the ratio of transductants to total cells plated. Data from two different experiments using independently prepared phage lysates are shown.

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Table 1 The frequencies with which T4GT7, T4 wild type and phage P1 transduce auxotrophic recipient strains JC 411 and AB 1157

	JC 411			AB 1157			
MetB	*ArgG*	*His*	*Leu*	*Leu*	*Ara*	*ProA*	*Thr*
T4GT7 3.6×10^{-4}	7.1×10^{-5}	7.8×10^{-5}	1.1×10^{-4}	8.3×10^{-5}	5.9×10^{-5}	3.5×10^{-6}	$<10^{-7}$
P1 cm 1×10^{-6}	3×10^{-6}	3×10^{-6}	1×10^{-5}	7×10^{-6}	3×10^{-6}	10^{-7}	$<10^{-7}$
T4 wild type $<10^{-8}$	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$

The phage were grown on *E. coli* K ED8689 (hsdR$^-$, Sup^0) either as liquid or plate lysates. Recipient cells were grown to log phase in supplemented M9 minimal medium, resuspended in fresh medium in the presence of 20 μg ml^{-1} L-tryptophan to a titre of approximately 4×10^8 cells per ml and infected with sufficient phage to achieve a multiplicity of infection in the range 0.05–0.25. After 15 min adsorption at room temperature, aliquots of the infection mixtures were spread onto minimal selection plates and incubated for 2 days at 37 °C to allow growth of transductant colonies. Immediately before infection, the recipient cells were titred to determine the actual MOI. The data are shown as ratios of the number of transductants observed to the number of singly infected cells plated, the latter calculated from the Poisson formula knowing the cell titre and MOI. The absolute frequency with which markers are transduced varies two- to threefold from lysate to lysate. This fluctuation seems to be due to differences in the intracellular growth times of different phage preparations (Fig. 2). Lysates prepared on Sup$^+$ donor bacteria have two- to threefold lower transducing capacities than lysates prepared on Sup^0 hosts.

of the phage prepared on donors containing the antibiotic resistance-determining plasmids pBR322 or RP4 were found to transduce the recipient strain JC411 to ampicillin resistance. To standardise for donor-dependent differences in transducing capacity in these experiments, transduction for the chromosomal *arg* marker was simultaneously measured. Routinely, ampicillin resistance was found to be transduced twice as frequently as Arg$^+$ from the pBR322-containing host, but only 1/20th as frequently from the RP4-containing host. As plasmid pBR322 exists in approximately 50 copies per cell[8] whereas plasmid RP4 exists in only a few copies[9], a diference in relative transduction frequency would be expected and is observed. The majority (170/171) of ampicillin-resistant transductants tested from the pBR322-grown lysate also were found to be tetracycline resistant, suggesting that the entire plasmid may be transduced although this has not been proved conclusively.

To determine at which stage during the development of T4GT7 transducing particles form, samples of a phage-infected bacterial culture were artificially lysed at various times after infection. The Arg$^+$ transducing capacity reached a maximum 30 min after infection and declined thereafter (Fig. 2). The maximum for Arg$^+$ transductants is about 10 times higher than the frequency observed in lysates that have been incubated for several hours. Most, but not all, other markers also showed an early maximum. The transducing capacity curve presumably reflects variation in the rates at which chromosomal fragments

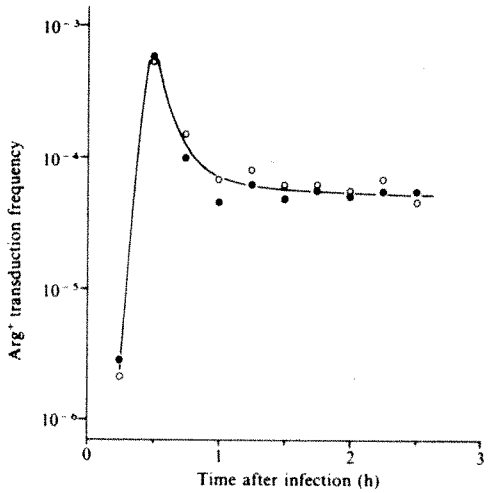


Fig. 2 Kinetics of transducing particle formation. A log-phase culture of ED8689 was infected at time zero with T4GT7 at a MOI of 5. Ten minutes later the culture was superinfected with the same multiplicity of phage to induce lysis inhibition. Starting at 15 min, and at 15-min intervals thereafter, samples of the infected cells were taken, lysed with chloroform, and the plaque-forming progeny titred. The infected cells were incubated at 37 °C with aeration throughout the experiment. Once the titre had been established, the Arg$^+$ transducing capacity of each sample was determined with JC 411 at MOI 0.5. Two independent estimates were made and both sets of data are shown.

and phage DNA molecules are packaged and accumulate and accounts for variation in transduction from lysate to lysate. Although the maximum transduction frequency is observed for the 30-min lysate, the formation of transducing particles does not cease at that time. Multiplying the transduction frequency by the viable phage titre at each time point during the lytic cycle indicates that transducing particles accumulate for at least an hour after infection and thereafter their number remains unchanged.

Possession of a circularly permuted phage chromosome and the ability to carry out generalised transduction often go hand in hand. Bacteriophage T4 is the classical circularly permuted phage[10], and although the wild-type phage is unable to transduce, the multiply mutated derivative T4GT7, described here, can. We are not yet certain whether all the mutations present in the mutant are critical for transduction. Since suppression of the *amber* mutations in genes *42* and *56* reduces the transducing capacity only slightly, it is probable that these two mutations are irrelevant. The remaining mutations, *denB*$^-$ and *alc*$^-$, are almost certainly required for transduction since both affect host and phage DNA after infection. The *denB*$^-$ mutation is a large deletion; in addition to abolishing the T4 cytosine-DNA-specific endonuclease IV, it also eliminates the *rIIB* and *ndd* functions[11], *ndd* being involved in the disruption of the host nucleoid[12]. The *alc*$^-$ mutation affects transcription from cytosine containing templates[5] and could also affect the *unf* function[13], responsible for unfolding the bacterial chromosome, as other *alc*$^-$ mutations are known to do[14]. Since T4GT7 was isolated as a plaque-forming derivative of the triple mutant, the possibility exists that it carries additional mutations beside *alc*$^-$. Further analysis is under way to determine the precise genetics of T4 transduction.

At present, we do not know the form in which host DNA is transduced by T4GT7. Like P1[15] and P22[16], T4GT7 might transduce continuous sections of the host chromosome in linear, headful-sized pieces. Alternatively it might scramble the chromosome and package otherwise unlinked fragments in the same head; or, like phage Mu[17], it may couple fragments of host and viral DNA and encapsulate hybrid molecules.

The observations presented here indicate that T4 is capable of efficient generalised transduction. Both the phage and its host are well understood from the genetic and physiological points of view[7,18]. T4 is twice the size of P1 and might be expected to co-transduce correspondingly larger sections of the host chromosome, perhaps up to 5-min as compared with 2-min for P1. The increased capacity, together with its higher transducing frequency, recommend T4GT7 as an alternative to P1 for practical laboratory use and other applications.

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Inability of circular mRNA to attach to eukaryotic ribosomes

IN contrast to prokaryotic systems, initiation by eukaryotic ribosomes seems to be restricted to a single site near the 5′-terminus of the message. However, nucleotide sequence analyses have not revealed a common 'recognition sequence' within the 5′-proximal portion of eukaryotic messages. We have previously suggested that the position of an AUG codon within a eukaryotic message—rather than the surrounding nucleotide sequence—might be the primary feature defining an initiation site. We proposed[1,2] that a 40S ribosomal subunit binds initially at or near the 5′-end of a message and migrates along the RNA chain until the first AUG codon is encountered, whereupon it stops, a 60S subunit joins, and polypeptide synthesis begins. Although this mechanism accounts for many of the peculiarities of eukaryotic translational initiation, it leaves unanswered the question of how ribosomes recognise the 5′-terminus of mRNA. The m^7G cap which blocks the end of most eukaryotic messages[3] is not responsible for directing ribosomes to the 5′-terminus since ribosomes initiate at the 5′-proximal AUG even in uncapped or unmethylated RNAs[4,5]. The ability of ribosomes to select the 5′-end of a message irrespective of its chemical structure (that is, whether the terminus is $m^7GpppN\ldots$, $GpppN\ldots$ or $pppN\ldots$) is difficult to explain. An intriguing possibility is that eukaryotic ribosomes attach to mRNA by a threading mechanism. A simple prediction of the threading mechanism is that circularisation of a message should abolish its ability to interact with ribosomes. The experiments described below fulfill this prediction. The possibility of threading by prokaryotic ribosomes was ruled out, several years ago, by the demonstration that *Escherichia coli* ribosomes were able to translate the circular DNA of phage fd[6,7]. Indeed, the simplest version of a threading mechanism (entry at the 5′-terminus of a message with translation beginning at the first AUG codon encountered by the ribosome) is contradicted by the ability of *E. coli* ribosomes to initiate at multiple internal sites in polycistronic messages, such as bacteriophage R17 RNA. Conversely, the threading mechanism is consistent with the monocistronic character of eukaryotic messages.

These experiments were designed to determine whether mRNA molecules retain the capacity to attach to eukaryotic ribosomes after circularisation of the message, catalysed by T4 RNA ligase[8]. Templates were sought which were able to bind with reasonable efficiency to both prokaryotic and eukaryotic ribosomes, and were small enough to undergo circularisation. Two kinds of synthetic polymers met these requirements. One series of experiments was carried out with linear and circular molecules containing a single AUG codon flanked by polyadenylic acid. A random copolymer of adenylic, uridylic and guanylic acid was used for another set of experiments.

Circular ^{32}P-labelled molecules containing a single AUG codon were synthesised as follows. (1) The dinucleotide UpG was converted to $UpG(pA)_n$ in a reaction catalysed by primer-dependent polynucleotide phosphorylase[9] (PNPase), with ^3H-ADP as precursor. In the presence of 0.5 M NaCl, a homo-

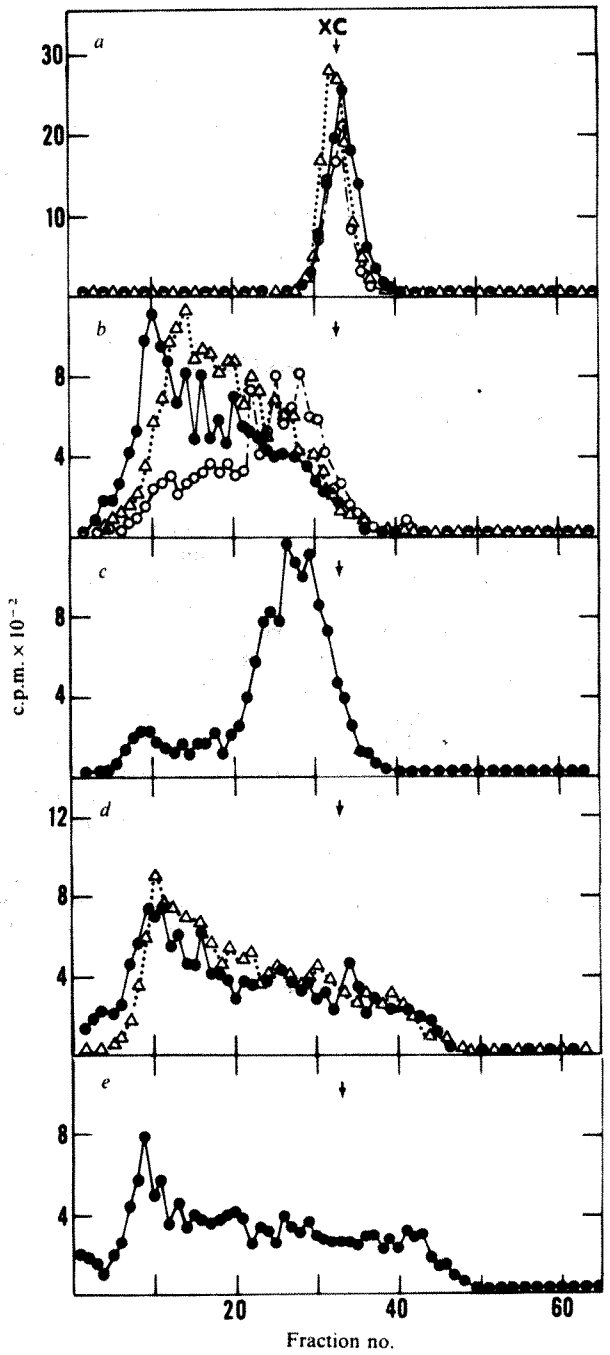


Fig. 1 Polyacrylamide gel electrophoresis of ^{32}P-labelled ribopolymers. The origin is at the left. Three forms of the polymer $UpG(pA)_{25}$ were analysed in separate gels, and the three profiles are superimposed in *a*: linear [5′-^{32}P]pUpG$(pA)_{25}$ before circularisation (△ . . . △); after reaction with RNA ligase (●——●); and after cleavage of the circular molecules with T_1 RNase (○-·-○). Incubation with T_1 RNase (40 U enzyme and 20 μg of RNA per 60 μl reaction) was for 20 min at 37 °C, followed by phenol–SDS extraction and ethanol precipitation. The same symbols in *b* represent three forms of the polymer $UpG(pA)_{110}$. After reaction with RNA ligase, an aliquot of $UpG(pA)_{110}$ was further treated with PNPase to degrade residual linear molecules (*c*). Electrophoresis of [5′-^{32}P]poly(A, U, G) linear (△ . . . △) and circular (●——●) molecules is shown in *d*. An aliquot of purified poly(A, U, G) circles, from which linear molecules had been removed with PNPase, was analysed in *e*. Composition of the gels and electrophoresis conditions were as described previously[10]. Arrows show the position of xylene cyanol FF blue (XC). Other markers were bromophenol blue (fraction 61) and transfer RNA (fraction 21).

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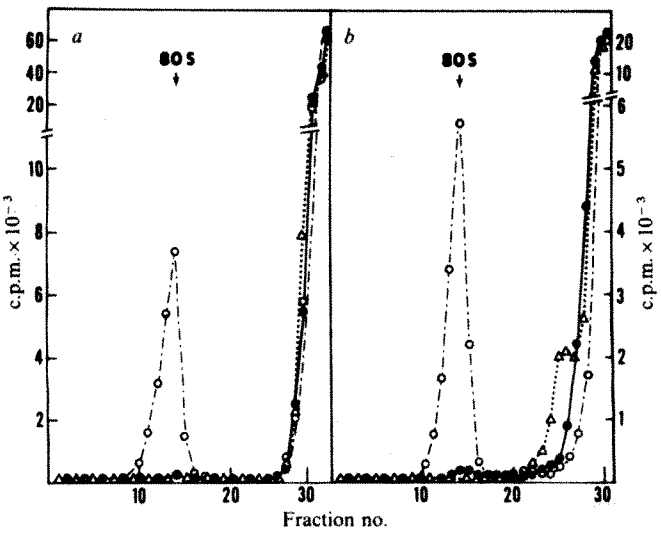


Fig. 2 Binding of ^{32}P-labelled pUpG(pA)$_n$ polymers to wheat-germ ribosomes. *a*, Binding of three forms of the polymer pUpG(pA)$_{25}$ (150,000 c.p.m. per 50 μl reaction); *b*, binding of three forms of the polymer pUpG(pA)$_{110}$ (80,000 c.p.m. per 50 μl reaction). Polymers were tested for ribosome binding before reaction with RNA ligase (△ . . . △), after circularisation with RNA ligase (●——●), and after cleavage of the circles with T_1 RNase (○ - · - ○). Reaction conditions with wheat-germ S23 extract were as described previously[2,10]. Following 6 min incubation at 19 °C, samples were chilled and layered on to 10–30% glycerol gradients. Centrifugation was for 3 h in a SW41 rotor. The direction of sedimentation was from right to left in all gradients. Gradient fractions (0.37 ml) were mixed with Beckman GP scintillation cocktail and counted for radioactivity. In each panel, profiles obtained from three parallel gradients have been superimposed.

geneous polymer containing ~25 adenylic acid residues was obtained (Fig. 1*a*, triangles). Higher molecular weight polymers (which were also more heterogeneous in size, Fig. 1*b*, triangles) were obtained by lowering the NaCl concentration[11]. (See Table 1 for reaction conditions.) Average chain lengths of 25 and 110 nucleotides for UpG(pA)$_n$ preparations 1 and 2, respectively, were determined from the ratio of ^{3}H-labelled adenosine/3′-AMP, following alkaline hydrolysis (not shown). (2) The linear molecules of UpG(pA)$_n$ were labelled at the 5′-terminus by phosphate transfer from [γ-^{32}P]ATP, catalysed by polynucleotide kinase. (3) Subsequent incubation with RNA ligase converted a substantial fraction of the 5′-terminal label to a form resistant to alkaline phosphatase (Table 1, 1*b* and 2*b*). As expected, the efficiency of ligation was greater with the shorter polymers (preparation 1) than with the longer molecules in preparation 2. Incorporation of 5′-^{32}P into an internal phosphodiester bond, suggested by resistance to alkaline phosphatase, was confirmed by analysing the products of alkaline hydrolysis. Whereas the linear polymer [5′-^{32}P]pUpG(pA)$_n$ yielded pUp as the only ^{32}P-labelled product (Table 1), after incubation with RNA ligase ^{32}P-radioactivity from preparation 1 was quantitatively recovered as 3′-AMP (Table 1). Thus, preparation 1*b* seemed to consist entirely of circular molecules. Preparation 2*b*, on the other hand, clearly contained residual linear monomers (and perhaps linear dimers) as only 43% of the ^{32}P-radioactivity was recovered as 3′-AMP after alkaline hydrolysis. The residual linear molecules were eliminated by treatment with PNPase, yielding the purified circle preparation 2*c* in Table 1. (Comparison of the polyacrylamide gel profiles of preparations 2*a* (Fig. 1*b*, triangles) and 2*c* (Fig. 1*c*) reveals that the smaller molecules in the linear preparation were preferentially circularised. Although the average chain length of the linear molecules was 110 nucleotides, the majority of the circular molecules were in the range 40–80 nucleotides. Thus, preparations 2*b* and *c* are represented as pUpG(pA)$_{40-80}$ in the following paragraphs.) (4) Finally, an aliquot of each pUpG(pA)$_n$ circle preparation was treated with ribonuclease T_1, which cleaves on the 3′-side of guanosine residues, thereby converting each circle into a linear molecule terminating in AUG. This provided a useful control for the ribosome binding experiments described below and the treatment with T_1 RNase also confirmed that putative pUpG(pA)$_n$ circles had not been randomly cleaved by contaminating nuclease(s) at any stage (as the size of the linear (Ap)$_{25}$UpGp molecules obtained after T_1

Table 1 Characterisation of linear and circular ribopolymers

^{32}P-labelled polymer preparation	Resistance to alkaline phosphatase	Resistance to polynucleotide phosphorylase	Products of alkaline hydrolysis	
			pUp	3′-AMP
1*a* Linear [5′-^{32}P]pUpG(pA)$_{25}$	0.2%	not done	100%	0%
1*b* [5′-^{32}P]pUpG(pA)$_{25}$ after reaction with RNA ligase	88%	90%	1%	99%
2*a* Linear [5′-^{32}P]pUpG(pA)$_{110}$	0.3%	5%	100%	0%
2*b* [5′-^{32}P]pUpG(pA)$_{110}$ after reaction with RNA ligase	29%	25%	57%	43%
2*c* Circular ^{32}P-labelled pUpG(pA)$_{110}$ after hydrolysis of residual linear molecules with PNPase	80%	(100%)	11%	89%
3*a* Linear [5′-^{32}P]poly(A, U, G)	0.4%	10%	not done	
3*b* [5′-^{32}P]-poly(A, U, G) after reaction with RNA ligase	76%	73%	not done	

Polymer preparation 1*a* was synthesised in a 200-μl reaction mixture containing 0.2 M Tris·Cl (*p*H 8.3), 0.5 M NaCl, 10 mM magnesium acetate, 0.7 mM UpG, 25 mM ^{3}H-ADP (1.8 mCi mmol^{-1}, NEN) and 1.25 U primer-dependent PNPase (PL Biochemicals). After incubation for 6 h at 34 °C the product was extracted with phenol, and unincorporated precursors were removed by filtration through Sephadex G-25. Similar conditions were used for preparation 2*a*, except that the concentration of UpG primer was 0.2 mM, and 0.2 M NaCl was used. Linear polymers (1*a*, 2*a*, 3*a*) were labelled at the 5′-terminus in reaction mixtures (125 μl) containing 36 mM Tris·Cl (*p*H 8), 120 mM NaCl, 10 mM $MgCl_2$, 10 mM dithiothreitol (DTT), 3.5 μg bovine serum albumin (BSA), 50 μg of polymer, 40 μC of [γ-^{32}P]ATP (33 Ci mmol^{-1}, NEN) and 3 U polynucleotide kinase (PL Biochemicals). Following incubation for 60 min at 37 °C the labelled polymers were extracted with phenol and filtrated through Sephadex G-25. Circular polymers (1*b*, 2*b*, 3*b*) were obtained by reaction with T4 RNA ligase, using the low temperature conditions suggested by Bruce and Uhlenbeck[12]. Reaction mixtures (450 μl) contained 1.6×10^7 c.p.m. of 5′-end labelled polymer, 11 U of RNA ligase (PL Biochemicals), 14 μg BSA, 10% (v/v) dimethyl sulphoxide, 50 mM HEPES (*p*H 7.6), 10 mM $MgCl_2$, 4 mM DTT and 0.2 mM ATP. Incubation was for 19–24 h at 4 °C. Hydrolysis of linear molecules with PNPase (preparation 2*c* and column 3) was accomplished by incubation for 40 min at 35 °C with 15 U ml^{-1} of primer-independent PNPase (type 15, PL Biochemicals) in 50 mM Tris·Cl (*p*H 8.8), 175 mM NaCl, 10 mM K_2HPO_4, 10 mM $MgCl_2$, 1 mM EDTA and 17 μg BSA per ml. Carrier RNA was present at 100 μg ml^{-1}. For preparation 2*c*, the reaction was terminated by extraction with phenol-SDS, and low molecular weight products were removed by filtration through Sephadex G25. For the analytical PNPase analyses in column 3, radioactive polymers were precipitated with 5% trichloroacetic acid (TCA) at 4 °C and then counted on Millipore filters. Resistance to alkaline phosphatase (column 2) is based on TCA-precipitable radioactivity following incubation at 37 °C for 40 min with bacterial alkaline phosphatase (2 U ml^{-1}, Worthington), in 20 mM Tris·Cl (*p*H 8). The products of alkaline hydrolysis were identified by high voltage paper electrophoresis following incubation in 0.5 M KOH at 37 °C for 18 h.

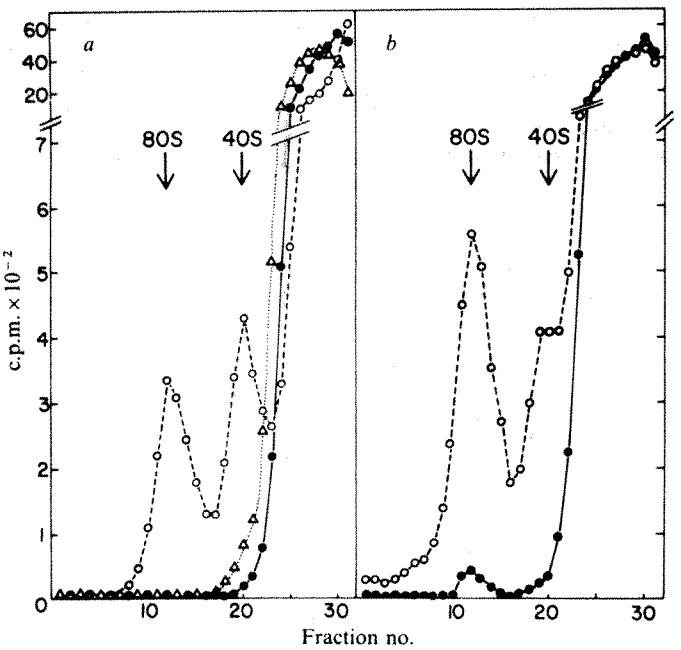


Fig. 3 Binding of linear and circular ^{32}P-labelled polymers to reticulocyte ribosomes. *a*, Three forms of the polymer pUpG(pA)$_{110}$ were incubated with rabbit reticulocyte ribosomes: △...△, linear molecules before ligation; ●——●, circles; ○---○, circles which had been cleaved with T_1 RNase. Reaction conditions were as described previously[13]. Incubation was for 8 min at 32 °C. Profiles from three parallel gradients have been superimposed. In *b*, linear (○---○) and circular (●——●) forms of poly (A, U, G) were tested. Each 200-μl reaction mixture contained 30,000 c.p.m.

RNase digestion of the circle preparation in Fig. 1*a* (open circles) was identical to that of the starting pUpG(pA)$_{25}$ linear molecules (triangles)). With the second preparation, the average size of the T_1 RNase-treated material (Fig. 1*b*, open circles) was smaller than the original linear pUpG(pA)$_{110}$ molecules (Fig. 1*b*, triangles), consistent with the interpretation that only the smaller chains had been circularised. The third polymer preparation used in these studies was a random copolymer of adenylic, uridylic and guanylic acid. Poly(A, U, G) (Miles) was treated with alkaline phosphatase to generate a 3′-hydroxyl terminus and was subsequently labelled at the 5′-end with [γ-^{32}P]ATP (Table 1, 3*a*). An aliquot of [5′-^{32}P]poly(A, U, G) was incubated with RNA ligase, resulting in ~75% circularisation (Table 1, 3*b*). For some of the ribosome binding studies described below, residual linear molecules in the poly(A, U, G) circle preparation were hydrolysed with PNPase. The resulting purified poly(A, U, G) circle preparation was 90% resistant to alkaline phosphatase. Figure 1*d* and *e* show that, although there was considerable heterogeneity in the size of the poly(A, U, G) circles, most of the molecules were larger than 80 nucleotides.

Linear and circular forms of polymers containing a single AUG codon, flanked by poly(A), were tested for ability to interact with wheat-germ (Fig. 2), reticulocyte (Fig. 3*a*) and *E. coli* ribosomes (Fig. 4*a*). One reason for the selection of [5′-^{32}P]pUpG(pA)$_{25}$ as substrate for the RNA ligase reaction is that, before circularisation, the polymer does not contain an AUG codon. Thus, the linear precursor showed no detectable stable binding to wheat-germ ribosomes (Fig. 2*a*, triangles). Although circularisation of pUpG(pA)$_{25}$ creates an AUG initiator codon, circular pUpG(pA)$_{25}$ was also unable to bind to wheat-germ ribosomes (Fig. 2*a*, closed circles). When the circles were hydrolysed with RNase T_1, however, the resulting linear molecules were able to form stable 80S complexes (Fig. 2*a*, open circles). Thus, inability of wheat-germ ribosomes to bind to circular pUpG(pA)$_{25}$ cannot be attributed to absence of a proper initiation sequence. The sequence (Ap)$_n$UpGp (in a linear molecule) is adequate for stable attachment of wheat-germ ribosomes, although the level of binding (~15%) was lower than would be observed with most natural messages. Failure of pUpG(pA)$_{25}$ circles to interact with wheat-germ ribosomes might, however, be due to the small size of the circles. Indeed, the preparation of circles tested with wheat-germ ribosomes in Fig. 2*a* also failed to bind stably to *E. coli* ribosomes (not shown). Thus, the experiment in Fig. 2*a* was repeated using bigger polymers. The gradient profile in Fig. 2*b* (closed circles) shows only a very low level of binding (<1%) when circular molecules of the form pUpG(pA)$_{40-80}$ were incubated with wheat-germ ribosomes. In contrast, 16% of the linear molecules resulting from T_1 RNase digestion (Fig. 2*b*, open circles) bound to wheat-germ 80S ribosomes. Figure 3*a* shows that reticulocyte ribosomes, like the wheat-germ system, discriminated against circular forms of the polymer pUpG(pA)$_{40-80}$.

In control experiments, linear and circular molecules of pUpG(pA)$_{40-80}$ were tested for the ability to bind to *E. coli* ribosomes. Even without an AUG codon linear [5′-^{32}P]pUpG(pA)$_{110}$ showed a fairly high level of binding to *E. coli* ribosomes, varying from 3 to 10% of the input polymer. Therefore, to show that circular molecules can attach to *E. coli* ribosomes, binding had to be carried out using a purified preparation of pUpG(pA)$_{40-80}$ circles (Table 1, line 2*c*) which was free of residual linear molecules. Figure 4*a* (closed circles) shows that 12% of the purified circles formed 70S initiation complexes with *E. coli* ribosomes which were fixed with glutaraldehyde[14] before centrifugation. Binding to *E. coli* ribosomes was also detectable although at a reduced level without glutaraldehyde fixation (Fig. 4*a*, open circles). In contrast, the

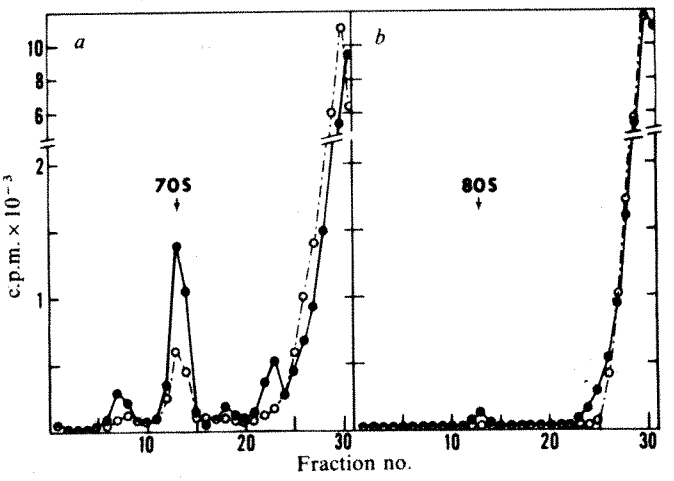


Fig. 4 Binding of purified ^{32}P-labelled pUpG(pA)$_{40-80}$ circles to: *a*, *E. coli*; and *b*, wheat-germ ribosomes. Each 50-μl reaction mixture contained 60,000 c.p.m. of the polymer described in 2*c* of Table 1. For binding to *E. coli* ribosomes (*a*), the 50-μl reaction mixture contained 50 mM HEPES(NH$_4$), *p*H 7.5, 100 mM NH$_4$Cl, 6.6 mM magnesium acetate, 0.1 mM EDTA, 0.6 mM DTT, 0.5 mM GTP, 55 μg of *E. coli* MRE600 tRNA charged with methionine in formylating conditions, and 2.3 A_{260} units of low salt washed *E. coli* Q13 ribosomes. The ribosomes (retaining initiation factors, but free of elongation factors) were obtained by pelleting from an S30 extract and washing twice with 20 mM HEPES (*p*H 7.5), 60 mM NH$_4$Cl, 10 mM magnesium acetate and 6 mM β-mercaptoethanol. Incubation of the ^{32}P-labelled polymer with *E. coli* ribosomes was for 8 min at 35 °C followed by sedimentation through a 10–30% sucrose gradient containing 20 mM HEPES (*p*H 7.6), 50 mM NH$_4$Cl, 7 mM magnesium acetate, 0.1 mM EDTA and 6 mM β-mercaptoethanol. Centrifugation was for 3.25 h at 4 °C in the SW41 rotor. Reaction conditions used with wheat-germ ribosomes (*b*) were as described in Fig. 2. After incubation with either wheat-germ or *E. coli* ribosomes, half of each sample was directly applied to a gradient (○-·-○); the remainder was fixed by addition of 7 μg BSA and 2 vol 2.5% glutaraldehyde in 50 mM HEPES (*p*H 7.5), 50 mM KCl and 5 mM magnesium acetate. After 5 min at 4 °C the glutaraldehyde-treated samples (●——●) were layered on to gradients and centrifuged.

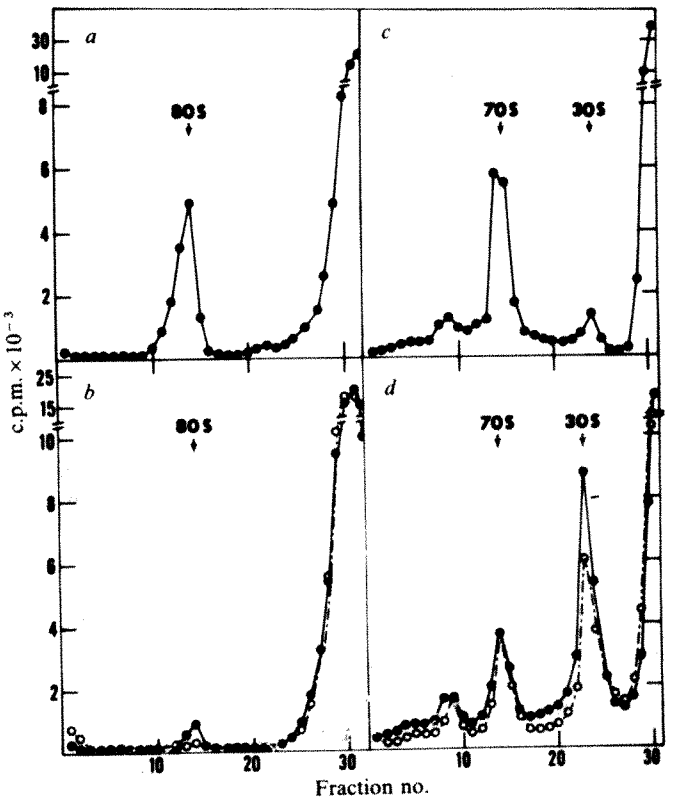


Fig. 5 Binding of ^{32}P-labelled linear and circular poly(A, U, G) to wheat-germ and *E. coli* ribosomes. Reaction conditions were as described in Figs 2 and 4, and initiation complex formation was assayed by centrifugation through gradients. Binding of linear [5′-^{32}P]poly(A, U, G) is shown with wheat-germ ribosomes (*a*) and with *E. coli* ribosomes (*c*). Circular poly(A, U, G) was incubated with wheat-germ ribosomes in *b* and with *E. coli* ribosomes in *d*. In *b* and *d*, binding is shown for the poly(A, U, G) circle preparation described in line 3*b* of Table 1 (●——●) and also for a more highly purified preparation (○- - -○) from which residual linear molecules had been removed by hydrolysis with PNPase. Each reaction mixture contained 75,000 c.p.m. Samples were not fixed with glutaraldehyde.

same preparation of purified pUpG(pA)$_{40-80}$ circles showed no stable interaction with wheat-germ ribosomes, with or without glutaraldehyde fixation (Fig. 4*b*).

Another series of ribosome binding experiments was carried out with linear and circular forms of poly(A, U, G). Linear [5′-^{32}P]poly(A, U, G) bound with approximately the same efficiency to wheat-germ (Fig. 5*a*) as to *E. coli* ribosomes (Fig. 5*c*). Circularisation of the template strikingly reduced its capacity to bind to wheat-germ ribosomes (Fig. 5*b*, closed circles). The very low (3%) level of binding to wheat-germ ribosomes was reduced even further when residual linear molecules were eliminated from the circle preparation (Fig. 5*b*, open circles). Even after fixation with glutaraldehyde, binding of circular poly(A, U, G) to wheat-germ ribosomes could not be detected (data not shown). Poly(A, U, G) circles also failed to form stable complexes with reticulocyte ribosomes (Fig. 3*b*, closed circles). In contrast, circular poly(A, U, G) attached to *E. coli* ribosomes (Fig. 5*d*) even more efficiently than the corresponding linear template. The reasons for the enhanced binding of circular templates, and for accumulation of some of the circular poly(A, U, G) molecules in 30S complexes, are not known.

The validity of the control experiments with *E. coli* ribosomes requires that the circular polymers are not nicked by endonucleases which might contaminate the *E. coli* translational system. Three observations suggest that circular RNA templates were not converted to a linear form before binding. (1) As mentioned previously, circular pUpG(pA)$_{25}$ failed to bind to *E. coli* (as well as to wheat-germ) ribosomes, presumably due to the small size of the circles. The same polymer preparation was able to attach to *E. coli* ribosomes after cleavage of the circles with T_1 RNase. Thus, the level of endonuclease activity associated with *E. coli* ribosomes was apparently insufficient to convert circular pUpG(pA)$_{25}$ to a linear form. (2) The pattern of binding of poly(A, U, G) circles to *E. coli* ribosomes was qualitatively different from the binding of linear poly(A, U, G). That is, a large proportion of the circular molecules accumulated in 30S complexes. Although the reason for this is not known, the different binding patterns obtained with linear and circular poly(A, U, G) suggests that the circular molecules were not cleaved during the incubation. (3) Attachment of both linear and circular poly(A, U, G) templates to *E. coli* ribosomes was complete within 2 min of incubation. The rapid binding suggests that conversion of circular molecules to a linear form is not a prerequisite for attachment to *E. coli* ribosomes.

The present experiments suggest that eukaryotic ribosomes might thread on to the 5′-end of mRNA. Even in the absence of the m^{7}G cap, the 5′-terminus of mRNA apparently has an essential role in ribosome attachment, as circularisation of pUpG(pA)$_{40-80}$ or of poly(A, U, G) totally abolished ability of the template to bind to wheat-germ or reticulocyte ribosomes. It seems unlikely that the size of the circles restricts their ability to interact with eukaryotic ribosomes. Although the small size of the pUpG(pA)$_{25}$ circle preparation may have precluded interaction with ribosomes, the larger pUpG(pA)$_{40-80}$ circles also failed to bind to wheat-germ or reticulocyte ribosomes. In the case of the circular poly(A, U, G) preparation used in Fig. 5, most of the molecules were more than 80 nucleotides long. Other trivial explanations for the inactivity of circular templates with eukaryotic ribosomes seem to be ruled out by two controls: (1) linear forms of each polymer were able to form initiation complexes in wheat-germ and reticulocyte extracts; and (2) the circular molecules that failed to bind to wheat-germ or reticulocyte ribosomes were able to bind to *E. coli* ribosomes. The striking difference in activity of circular templates with bacterial and eukaryotic ribosomes supports the suggestion[1] that the mechanism by which eukaryotic ribosomes select initiation regions in mRNA seems to be quite different from that of prokaryotes.

If mRNA threads through an aperture on the 40S ribosome, one might anticipate that the 40S subunit would be unable to detach when the ribosome arrives at a termination codon. Following release of the 60S subunit and the nascent polypeptide, the 40S subunit might remain on the message until it reaches the 3′-terminus. An alternative possibility is that the aperture through which the message threads is formed by the junction of the 40S subunit with initiation factor(s), which detach after mediating the early steps of translation.

I thank Dr Joan Steitz for suggesting circular templates for these studies, Joanne Meddick for technical assistance, Dr Aaron Shatkin for comments on the manuscript and Dr W. Szer for providing *E. coli*. This work was supported by NIH grant AI 15242.

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Evidence that *Euglena* chloroplasts do not export tRNAs

THE chloroplastic (Chl) and mitochondrial genomes from various organisms contain 20–30 tRNA cistrons. Several reports have demonstrated the presence of 20–30 tRNA species in these organelles. Thus, both chloroplasts and mitochondria seem to contain at least one tRNA species for each amino acid, plus initiator $tRNA^{Met}$ and a few isoacceptors[1–7]. In the few cases so far examined[1,8], organelle tRNAs have been shown to have primary sequences differing from those of their cytoplasmic isoacceptors. Three laboratories have demonstrated that *Euglena* Chl DNA codes for 22–26 species of $tRNA$[9–11]. In the report of McCrea and Hershberger[11] total cellular tRNA was separated into two fractions (I and II) by diboryl DEAE (DBAE) cellulose chromatography. Fraction I hybridised with both nuclear and Chl (~18 cistrons) DNAs. Fraction II seemed to hybridise only with Chl DNA (7–9 cistrons). Fraction II tRNA was not detected in isolated chloroplasts[11], but was found in either total cellular or cytoplasmic polysomal RNA[12], and contained acceptor activity for eight amino acids (Ala, Arg, Glu, Gly, Leu, Lys, Met and Phe)[12]. As fraction II tRNAs hybridised with Chl DNA but were not found in isolated chloroplasts it was proposed that although they were transcripts of the Chl genome they were selectively exported to the cytoplasm[11,12].

In our laboratory chloroplast-localised tRNAs of *Euglena* have been identified by chromatographic comparisons with either total cellular tRNA or tRNA isolated from mutants lacking Chl DNA[1]. For each of 16 amino acids studied, isolated chloroplasts were found to contain unique chromatographic species of tRNA[1]. tRNA from isolated chloroplasts is complementary to 26 tRNA cistrons and Chl $tRNA^{Phe}$ and $tRNA^{Asp}$ are coded for by one cistron each[9]. In this report, we show that tRNAs obtained from isolated chloroplasts or whole cells hybridise to Chl DNA to the same extent and competition experiments reveal that these two sources of Chl tRNA complement identical cistrons. Furthermore, chromatographic evidence is presented that all the tRNA species represented as being totally exported to the cytoplasm[12] exist in isolated chloroplast preparations (which show no cytoplasmic contamination). These data indicate that there is no detectable transport of Chl tRNAs to the cytoplasm.

The tRNAs from whole cells and isolated chloroplasts were iodinated *in vitro*[9]. The amount of DNA complementary to each tRNA at infinite concentration was determined using double reciprocal plots (Fig. 1). Total cellular tRNA and tRNA from isolated chloroplasts are complementary to essentially the same percentages of Chl DNA (0.73% and 0.69, respectively). These data agree with our previous report[9] that 0.74% of the Chl genomes hybridises with tRNA from isolated chloroplasts. Thus, tRNA from either source is complementary to 24–26 cistrons. Competition experiments were also carried out to verify that both total cellular tRNA and tRNA from isolated chloroplasts hybridised with the same portions of the Chl genome. A constant amount of total cellular ^{125}I-labelled tRNA was hybridised with Chl DNA in the presence of increasing amounts of unlabelled tRNA from either whole cells or isolated chloroplasts (Fig. 2). Both sources of tRNA are equally effective as competitors; over 90% of the hybridised counts could be competed for by the addition of unlabelled tRNAs. On a weight basis, tRNA extracted from isolated chloroplasts is a more effective competitor than tRNA from whole cells, because only ~35% of the total cellular tRNA is chloroplastic[9]. Thus, both whole cells and chloroplasts seem to contain an identical complement of Chl DNA-coded tRNAs.

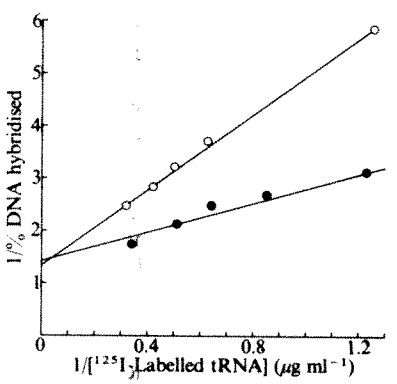


Fig. 1 Double reciprocal plot of the hybridisation of Chl tRNA (○) and total cellular tRNA (●) to Chl DNA. Filters containing 0.25 μg of Chl DNA were hybridised with Chl ^{125}I-labelled tRNA (9.7×10^6 c.p.m. per μg) or total cellular ^{125}I-labelled tRNA (1.4×10^7 c.p.m. per μg) in the presence of a fourfold excess of Chl rRNA as described previously[9]. At infinite concentrations of tRNA, 0.73% and 0.69% of Chl DNA is complemented by total cellular and Chl tRNAs, respectively.

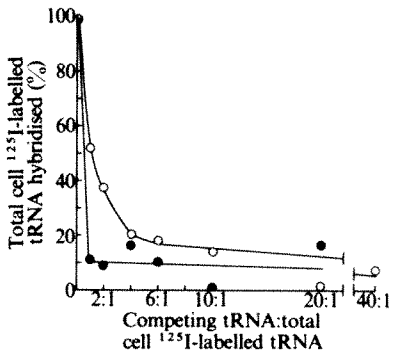


Fig. 2 Competition between Chl tRNA and total cellular tRNA for hybridisation with Chl DNA. Saturating concentrations of total cellular ^{125}I-labelled tRNA (9.4×10^7 c.p.m. per μg) were hybridised with filters containing 0.25 μg Chl DNA and increasing amounts of unlabelled total cellular (○) or Chl (●) tRNAs. All vials contained unlabelled Chl rRNA.

Although McCrea and Hershberger reported [12] that fraction II tRNA contains acceptor activity for eight amino acids, previous reports have demonstrated that chloroplasts contain a unique species of $tRNA^{Phe}$ (refs 13–16) and Kislev *et al.*[17] demonstrated that *Euglena* chloroplasts contain acceptor activities for at least 16 amino acids including all those present in fraction II tRNA. Furthermore, we have been able to demonstrate (using BD-cellulose chromatography) that for each amino acid acceptor activity reported to be present in fraction II $tRNA$[12] a chromatographically unique species is present in isolated chloroplasts. Examples of this are shown in Fig. 3 for $tRNA^{Ala}$ and $tRNA^{Arg}$. Isolated chloroplasts contain only a single symmetrical peak of alanyl and arginyl acceptor activity, whereas tRNA from whole cells has an additional isoacceptor for each of these amino acids. In whole cell preparations, Chl $tRNA^{Ala}$ elutes as a trailing shoulder of the cytoplasmic isoacceptor and Chl $tRNA^{Arg}$ also elutes later than its cytoplasmic counterpart. There is no evidence for the existence of any tRNA species in any case other than those from isolated chloroplasts or from the cytoplasm (nuclear coded).

The results presented here and those in many other reports are inconsistent with the hypothesis that the chloroplasts of *Euglena* selectively export a specific class of Chl-coded tRNAs that may be found in the cytoplasm or cytoplasmic polysomes[11,12]. In the absence of chromatographic characterisation of the tRNA isoacceptors found in their DBAE-cellulose, cytoplasmic polysomal, total cellular or Chl fractions it is difficult to explain the results of McCrea and Hershberger.

Mutants lacking Chl DNA, and thus unable to code for Chl tRNAs, exhibit normal growth and are still able to synthesise all chloroplast-localised (nuclear-coded) enzymes so far examined[18]. Thus, there seems to be no physiological need for

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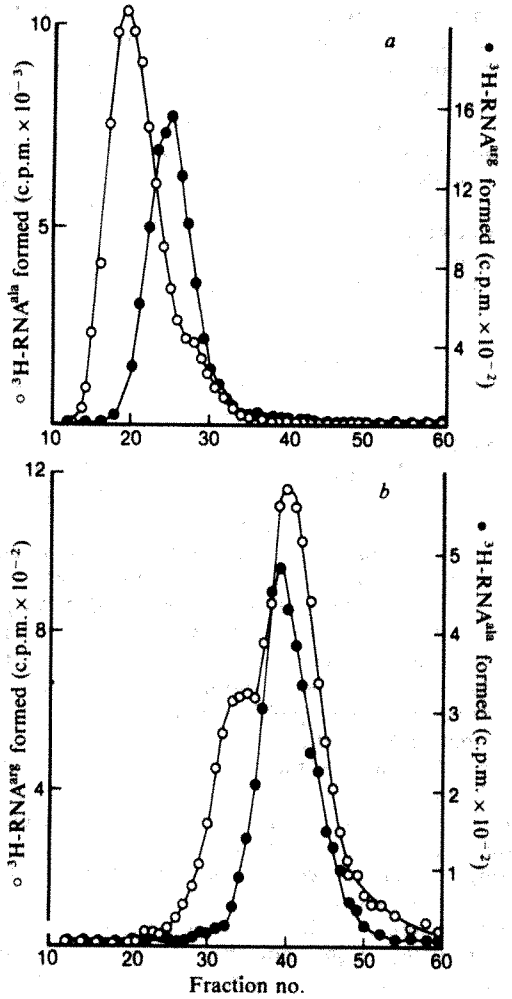


Fig. 3 Benzoylated-DEAE cellulose chromatography of total cellular (○) and Chl (●) deacylated tRNAs. 200 A_{260} units of tRNA from purified chloroplasts or 300 A_{260} units of tRNA from whole cells were applied to a 1 × 21 cm column and eluted with an 800-ml linear gradient from 0.33–1.25 M NaCl (ref. 16). Ala (*a*) and Arg (*b*) acceptor activities were assayed as previously described for Phe[16,18] with the exception that the buffer used was HEPES, *p*H 7.5.

exported tRNAs as either constitutive or regulatory components of the cytoplasmic translation system. Therefore, we conclude that all evidence for the export of Chl DNA-coded tRNAs from chloroplasts is of doubtful significance.

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Molecular basis for proton-dependent anion binding by soybean leghaemoglobin *a*

HAEM proteins are widespread in nature and exhibit diverse biological activities. The properties and function of a given haem protein depend critically on the environment of the haem group. The reactivity of the haem is often influenced by one or more haem-linked ionisable groups. Thus, oxygen affinities of mammalian haemoglobins, for example, exhibit considerable *p*H dependence (the Bohr effect[1]). Anion binding to some haem proteins is also a proton-dependent reaction (for example, peroxidase[2], catalase[2] and leghaemoglobin[3,4]) whereas for others (myoglobin, haemoglobin[1]) it is essentially independent of *p*H. Identification of the ionisable groups which influence the ligand-binding reactions of haem proteins is of fundamental importance in the elucidation of structure/function relationships. Nuclear magnetic resonance (NMR) spectroscopy is one of the most direct methods for observation of titrating groups in proteins. We report here on NMR studies of the nicotinate complex of soybean ferric leghaemoglobin *a*. On the basis of these studies we propose a role for a haem propionic acid group as a mediator of anion binding.

All experiments were carried out with soybean ferric leghaemoglobin *a* prepared by a previously described procedure[5]. Reactions with acetate to form a high-spin complex[3] and nicotinate to form a low-spin ferric complex[4] are proton dependent, with *p*K values of 4.8 and 4.9, respectively. The affinity of leghaemoglobin for these anions increases with decreasing *p*H, suggesting the presence of a haem-linked ionisable group which, when protonated, facilitates access or binding of anions to the haem.

The low field region of the proton NMR spectrum of ferric leghaemoglobin nicotinate is shown in Fig. 1*a*. Resonances in this region of the spectrum are subject to large hyperfine shifts due to the presence of the single unpaired electron of the iron atom and generally arise from protons on the porphyrin ring or axial iron ligands[6]. The resonances are relatively narrow due to the rapid electron spin relaxation characteristic of low-spin ferric haem[6]. The resonances at 25.6 and 18.5 p.p.m. at 45 °C and *p*H 4.5 can be unambiguously assigned to two of the four porphyrin ring methyl groups, on the basis of their intensities and previous NMR studies of haem proteins and model haem complexes[6]. The two single proton resonances at 14.9 and 12.7 p.p.m. at *p*H 4.5 are assigned to the two α-CH_2 protons of one of the haem propionic acid side chains. The resonances shift at acid *p*H, titrating with a *p*K of 4.9 (Fig. 2). The assignment is confirmed by the observation of a large negative nuclear Overhauser effect[7,8] (~40%) on each resonance when the other is irradiated (Fig. 1*b*, *c*). The protons are therefore in close spatial proximity such that each dominates the nuclear relaxation of the other, as would be expected for two inequivalent protons of a propionic acid methylene group. Restriction of the rotational motions of the propionate side chain would give rise to magnetic inequivalence of its α-CH_2 or β-CH_2 protons. Assignment is made to the α-CH_2 rather than the β-CH_2 protons on the basis of the larger contact shift experienced by the former. No other protons on the haem or axial iron ligands can give rise to these

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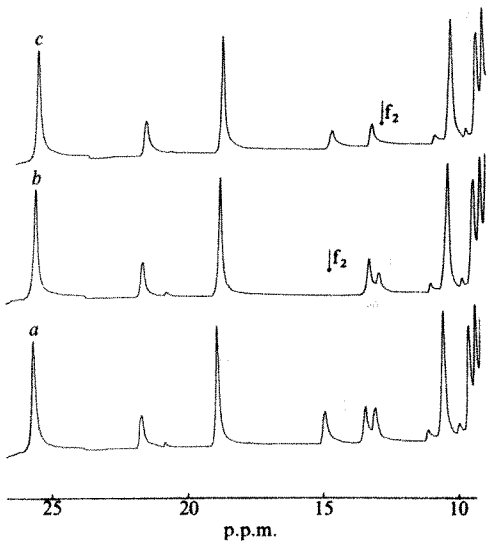


Fig. 1 *a*, Low field region of the 270-MHz proton NMR spectrum of soybean ferric leghaemoglobin *a* nicotinate at 45 °C and *p*H 4.5. *b*, Negative nuclear Overhauser effect (~40%) on resonance at 12.7 p.p.m. due to irradiation at 14.9 p.p.m. *c*, Negative nuclear Overhauser effect (~40%) on resonance at 14.9 p.p.m. due to irradiation at 12.7 p.p.m. Spectra were recorded using a Bruker HX-270 spectrometer operating in the pulsed Fourier transform mode. The residual HOD peak was suppressed by selective gated irradiation. Overhauser effects were measured by selective irradiation which was gated off immediately before data acquisition. Dioxan was used as an internal standard but all peaks are referred to trimethylsilylpropanesulphonic acid. Solutions for NMR were 3–4 mM in leghaemoglobin in phosphate buffer (10 mM, in D_2O). The *p*H was adjusted by careful addition of DCl or NaOD. The *p*H values quoted are uncorrected meter readings.

resonances. We believe this to be the first unequivocal assignment of the α-CH_2 proton resonances of a haem propionic acid side chain in the NMR spectrum of a haem protein. The assignments of the other resonances in the low field region will be published later.

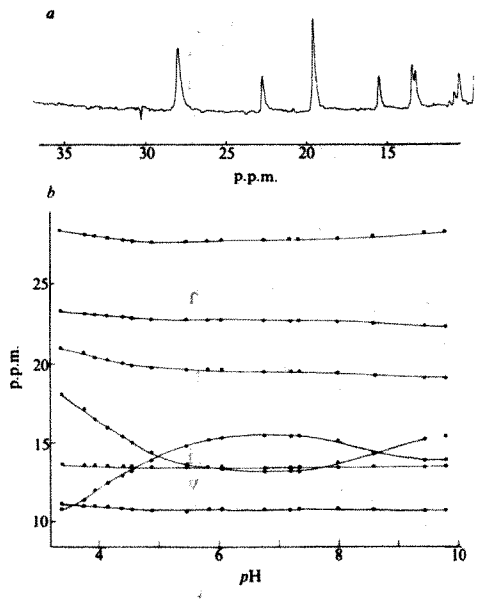


Fig. 2 *a*, Proton NMR spectrum of soybean ferric leghaemoglobin *a* nicotinate at 25 °C and *p*H 7.2. *b*, *p*H dependence of the low field region of the NMR spectrum. Exchange between nicotinate and ferric leghaemoglobin is slow on the NMR time scale. An excess of nicotinate was used such that the leghaemoglobin was fully complexed at *p*H values below ~7.0. At higher *p*H, dissociation of nicotinate leads to a decrease in intensity of the resonances.

Unfortunately, we are unable to assign the resonances of this haem propionic acid side chain in the spectrum of ferric leghaemoglobin acetate. These and the resonances of other protons near the haem are very broad due to the slow electron spin relaxation of the high-spin ferric haem.

The propionic acid resonances in the spectrum of leghaemoglobin nicotinate reflect the titration of two ionisable groups (Fig. 2). As the *p*H is increased above ~7.0 it is evident from the observed decrease in intensity of the low field haem resonances that nicotinate binding becomes weaker. The large perturbations at acid *p*H (Fig. 2) clearly arise from titration of the propionate side chain carboxyl group with *p*K 4.9. A further substantial perturbation is observed above *p*H 7. Its magnitude suggests a close interaction between the haem propionate and a group with *p*K 8.3. We identify this other group as the distal histidine residue, as we can follow the titration of a singlet resonance with *p*K 8.3 as it shifts from 8.1 p.p.m. to ~7.2 p.p.m. with increasing *p*H (unpublished observations). The high *p*K of this histidine is consistent with formation of a salt link or hydrogen bond to the carboxyl group of the nicotinate ligand[9,11].

On the basis of affinity labelling with mesohaem sulphuric anhydride, Ellfolk and Perttila[10] have suggested that one of the haem propionic acid side chains forms a salt bridge to Lys 57 in soybean ferric leghaemoglobin *a* at low temperature. Our NMR studies provide no evidence to support this proposal but indicate an interaction between one of the haem propionates and the distal histidine in leghaemoglobin nicotinate.

The present NMR studies indicate that at least one of the haem propionic acid substituents in soybean ferric leghaemoglobin *a* nicotinate is accessible to the solvent and free to titrate with a *p*K of 4.9. Reactions of the ferric protein with acetate and nicotinate are proton dependent, with *p*K values of 4.8 and 4.9, respectively[3,4]. These *p*Ks correspond closely to that of the free propionic acid side chain, which is thus likely to be the group which mediates anion binding. We thus propose a model for the reactions of soybean leghaemoglobin *a* with anions in which a haem propionic acid group, when deprotonated, functions as an electrostatic 'gate' which restricts the access of anions to the haem pocket. Protonation of this group would facilitate anion binding. In an alternative mechanism access of anions to the haem is sterically hindered as a result of protein conformational changes accompanying deprotonation of the haem propionic acid. This mechanism may be discounted, as reactions of leghaemoglobin with neutral ligands such as pyridine[4] and nicotinamide (C.A.A., unpublished observations) are independent of *p*H.

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reviews

Balanced overview of cancer research

Richard Peto

Cancer, Science and Society. By John Cairns. Pp. 199. (Freeman: San Francisco and Reading, 1979.) Hardback £6.20; paperback £2.90.

THE general public, which provides the money that cancer research consumes, seems to hope for the eventual emergence of the sort of "magic bullet" cancer cure which is as easy, specific and effective as an antibacterial agent can be against infections. In reality, there is little hope of any such thing; indeed, the prospect is so remote that it scarcely enters into the day-to-day conversation of most cancer research workers. Researchers talk of slightly earlier detection, of moderate improvements in current methods of therapy, of the prevention of minor environmental carcinogens, of the identification of life styles (with less meat, less tobacco and lots of vitamins) which would, if only anyone would adopt them, somewhat reduce cancer incidence rates. They talk of developing a greater understanding of tumour antigens or of the nature of the transformed state, but easy and specific cures are not sufficiently realistic to be of interest.

In this book, John Cairns tries to explain the realities of cancer biology and epidemiology in sufficient detail to be interesting and to let the facts themselves give the reader a realistic perspective on "cancer, science and society". In this I feel he succeeds excellently, and anybody with a background in any area of science would enjoy this overview. In style it reminds me of the *Scientific American*; it is clearly written and well illustrated, with separate chapters on each of several completely different areas of research, from demography to biology.

What is perhaps unusual in a book by a professional cancer research worker is his whole-hearted public acceptance of the idea that we cannot expect any "magic bullet" cancer cure to be discovered for at least several decades. Whether this pessimism is justified or not, it is certainly a view which deserves to be more seriously debated than in the past. Obviously, an optimistic claim that understanding viruses, interferon, immunology, and so on, will soon offer specific cures is impossible to refute and, if accompanied by any new finding, will be more newsworthy and therefore more widely published than the pessimistic claim that we are decades away from a detailed enough understanding of cell biology to devise a cancer cure rationally. However, this inevitable emphasis on optimistic perspectives may in the long run produce the sort of distorted hopes of rapid victory that, as with wartime propaganda, is fostered by reporting victories more prominently than defeats. If Cairns is right in his pessimism about the next few decades of cancer therapy, then he is surely right in his inference that *clinical* cancer research nowadays is over-supported, and that the two chief areas of research to foster over the next few decades should be cancer prevention and, at the other extreme, fundamental biological research with no requirement for any obvious connection with cancer. As a strategy, it may well be more successful than seeking vast amounts of money for clinical research with the slogan "Conquer cancer in the seventies".

Whether or not this overall perspective is accepted, the book provides an interesting, readable and balanced overview of cancer research, written for the sort of intelligent general public who perhaps do not know very much about cancer research but who might feel sufficiently interested to read a whole issue of the *Scientific American* devoted to the subject. As an introduction to the subject, it is as good as any I have seen, and would be an excellent textbook for an undergraduate or post-graduate biology course on cancer. However, the people who I chiefly hope will spend a pleasant evening reading it are the cancer research workers who are concentrating on particular details and who need some feel for how (or whether) their activities make sense in a wider context.

The book begins with demography. As a cause of death, cancer was much less important in the nineteenth century, simply because cancer tends to be a disease of old age, and most people never reached old age. However, during the past 100 years life expectancy in developed countries has suddenly doubled, and over 75% of people will now live beyond the age of 65.

Consequently, the present chief cause of death are those which act in old age, the most prominent being vascular disease (half of all deaths), cancer (one-sixth of all deaths) and, in Britain but not America, respiratory disease (another one-sixth). On average, those who die of cancer would only have lived another 12 years if their cancer had been prevented or cured, which is, of course, likely to be a worthwhile gain for the individual if a fair number of those years were passed in reasonable health. However, an average of 12 years extra for one-sixth of the population only represents an additional two years of life expectancy for the population as a whole, and is not comparable with the 30 or more years of extra life expectancy that has been conferred by control of the diseases which used to kill so many children and young adults. (Only 1% of cancers affect children.) In short, even the total prevention or cure of all cancer cannot yield the sort of huge change in the human condition that has followed from adequate nutrition and from the control of the major infectious diseases.

Turning from demography to epidemiology, cancers of different anatomical sites (for example, stomach and lung) behave as different, and largely unrelated, human diseases. Causes of one are unlikely to be causes of the other; a genetic predisposition toward one does not imply an abnormal risk of the other (except in a few people with very unusual genetic defects); and, most importantly, control of one type of cancer does not mean that the age-specific risk from any other cancer need be increased. Thus, there is not one cancer problem but many.

The converse of this rather unwelcome conclusion is that although the total age-specific cancer death rates in different countries tend to be rather similar, this overall similarity is the sum of many widely different parts. Indeed, one of the most encouraging findings in the whole of cancer research is that there exist very great differences between countries in the age-specific risks from particular cancers. (The risk of developing cancer increases so sharply with age that it is often misleading to talk about overall probabilities of cancer, for in poor countries where most people still do not reach old age the net likelihood of cancer is small even if the age-specific cancer risks are normal.) For example, lung cancer is 35 times as common among people of a given age in Britain as among people of that age in

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Nigeria, suggesting that the large majority of our 35,000 annual lung cancers are preventable. Stomach cancer is 25 times commoner in Japan than in Uganda but has been decreasing quite rapidly over recent decades as Japan has adopted a richer, more Western diet. Cancer of the oesophagus is more than 100 times commoner in north-eastern Iran (where it is the major cause of death) than in most other parts of the world, and has apparently been common there for centuries. The general rule is that, although some cancers are rare everywhere, every cancer which is common in one country is rare in another country, and so for every common cancer there presumably exist life-styles which humans would find acceptable that would make it vastly less common, probably without making any other type of cancer much more common. This presumption is supported by what sparse data are available on rapid changes in the age-specific incidence rates of various cancers over the past few decades. In America in 1930, cancer of the stomach was the commonest fatal cancer, whereas cancer of the lung was rare. Nowadays, the opposite is true. Over the same period in Britain, cancer of the tongue has decreased twentyfold, whereas cancer of the lung has increased twentyfold.

Thus, cancers have causes. But what might they be? It is tempting to ascribe them to large-scale chemical pollution of air, food or water, but this is probably mistaken, if only because the widespread dissemination of chemicals (in plastics and agriculture) is too recent for its effects on cancer yet to be apparent. In Britain 30%, and in America 25%, of all cancer deaths can be attributed to smoking, and if those cancers which are closely associated with smoking are omitted there is no upward trend in the overall death rates from the remaining cancers. If smoking accounts for only just over a quarter of all cancer deaths, what preventable causes account for the rest? An unproven but plausible hypothesis ascribes a large role in the genesis of colorectal, breast and endometrial cancer to meat, or fat, consumption; and Cairns presents the visually striking relationship between meat consumption and colon cancer. (Unfortunately, he does not also discuss the interesting hypothesis that certain vegetables or vitamins may be actively protective.)

Cairns then turns his attention to the world of cancer research. It is a rather aristocratic overview ("Biology and cancer research have developed together. Invariably, at each stage the characteristics of the cancer cell have been ascribed to some defect in whatever branch of biology happens at the time to be fashionable and exciting; today it is molecular genetics . . . . "), but tends to be the more interesting for being so.

In order to understand the cancer cell we must be taught something of the chemical basis of heredity, and this is done nicely from first principles, starting with DNA, RNA, the triplet code and the effects of mutations in it. Repair mechanisms are described, and then Cairns is ready to move on to the experimental production of cancers by chemical carcinogens, radiation and viruses. Many of the chemical agents which produce cancers in rodents also turn out to be mutagens for bacteria (or chemicals which can be converted into bacterial mutagens by enzymes which are present in normal mammalian cells). Obviously, this offers the possibility of detecting certain carcinogens quickly by allowing them to act on a mixture of mammalian cell components and bacteria,



Oliver escapes being bound apprentice to the Sweep

Cancer of the scrotum was prevalent in boys not so lucky

and seeing whether they cause mutations in the bacteria. Such methods may, however, be of more use in the prevention of new carcinogens than in detecting those which have already affected us for decades.

The two chapters on the experimental production of cancers are among the best in the book, perhaps because Cairns is here reviewing his own field. Perhaps because I am not, or perhaps because they already represent a greatly condensed account of a great deal of material, I find them difficult to summarise.

The concept of the metabolic activation of relatively inert precursors into electrophilic species that can bind to DNA took a long time to evolve, but it has allowed a grand unification to emerge of the mode of carcinogenic action of a wide class of 'initiating' agents, including chemicals that do and chemicals that do not need metabolic activation, some aspects of radiation carcinogenesis, and even certain viruses which seem simply to cause non-specific damage to DNA. The notable exceptions to this grand unification are the "promoting" agents, which seem to bind only to the surface of the target cell but which can greatly increase the carcinogenic effects of 'initiators', and the specific tumour viruses, which are described nicely in detail.

No virus in its right mind wants to exterminate its host; viruses just want to make sure that host animals continue to excrete enough virus particles throughout their lifespan to make them infect all their acquaintances. The best way to do this is for the virus to insert its genetic material into the DNA of the host's own germ cells or, failing that, the host's tissues, in such a way that copies can be run off whenever necessary. In the process, the virus may by mistake trigger certain host cell functions which make the host cell behave like a cancer cell, but from the virus's point of view that is an expensive mistake, as it kills the host. Alternatively, a really unfortunate virus may when it releases its own genetic material from the host's material inadvertently include a little bit of host DNA containing an instruction which, if expressed without proper host control, could cause uncontrolled proliferation. Without the advent of cancer research workers (who are pleased to discover and to selectively propogate such oddities), such viruses would be at an evolutionary dead end, and their non-sarcomagenic cousins, who could live in hosts without killing them, would meekly inherit the earth.

In short, Cairns is not sure which, if any, of the known tumour viruses are laboratory artefacts, perhaps useful as tools for controlledly manipulating and studying the 'transformed state' *in vitro* but not as models of human carcinogenesis.

Nor is he sure, of course, what the essential attributes of the "transformed state" are; it seems as though the key tricks needed to grow the cells one really wants to study have still not been found. If, as is often done, we define 'transformation' as the ability to grow indefinitely in soft agar, 'transformed' cells will indeed usually develop into a tumour when transplanted into a host. However, cells from real human tumours will usually not grow at all in soft agar or in any other *in vitro* conditions yet tested, and moreover normal human cells growing *in vitro* are almost impossible to 'transform', so most studies of transformation are done with rodent cells. These 'transform' spontaneously, or in response to viruses, chemicals or radiation, but although the biochemistry of transformation is currently being elucidated, its relevance is not yet clear.

Returning to matters which are better understood, the many decades that may elapse between the action of an initiator and the eventual emergence of a carcinoma are discussed in terms of multistage models, emphasising the relevance of such ideas for early detection programmes. The final chapter on prospects for the future is almost too pessimistic about whether preventive measures, even if discovered, will be implemented, and emphasises very strongly the improbability of discovering any simple, effective and specific cancer cure in the foreseeable future. □

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Announcing

Journal of Virological Methods

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The Journal of Virological Methods will publish original papers and invited reviews covering techniques on all aspects of virology. These will include methods for studying the morphology, assembly, replication, composition, function and physiochemical properties of viruses and their components; the purification of viruses and their components; cultivation; properties of viral antigens, production of antibody and techniques for studying the immune response to virions, viral subunits and components; the detection and identification of viruses and viral infections; assay of viruses and viral infectivity and the investigation of transmission and pathogenicity; and methods for investigating the suppression or inhibition of viral growth.

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Discrete charges in quantum mechanics

Gravitation, Electromagnetism and Quantized Charge: The Einstein Insight. By Daniel M. Pisello. Pp. 94. (Ann Arbor Science: Ann Arbor, Michigan, 1979.) Distributed in UK, Africa and Europe by Wiley: Chichester, UK. £7.55.

BY means of dogmatic pronouncements on the limitations of human imagination and language, the quantum mechanical philosophy effectively takes away from the younger physicists their right to question and create. If one finds it necessary to question or theorize beyond the restraints so successfully built into the quantum mechanical rationale, one is then, by fiat, pejoratively considered a revolutionary". Thus ends Mr Pisello's thin treatise, half philosphical, half mathematical, and always bitter. This work will not revolutionise physics, "pejoratively" or otherwise. The book's interest, however, lies in the way its author attempts to link his philosophical and political attitudes with a mathematical search for a purely field theoretical approach to the existence of discrete charges in quantum mechanics. The result is an exercise in topology elevated to the rank of "unified theory" through a casual parallel with Einstein's own epistemological objections to the theory of quantum mechanics.

The first three chapters present the conceptual difficulties arising from scientific breakthroughs like the theories of special relativity, general relativity and quantum mechanics. Old and new concepts which apparently contradict each other are reviewed, the limitations inherent in the use of a language created within an earlier framework are discussed, and the need for a "principle of complementarity" is challenged on philosophical grounds. A rapid analysis of the double slit experiment for which "neither linear wave nor discrete particle interpretation provides an adequate representation of the transfer of energy from source to detector" is used to justify searching for the "basic nature of the field variable". The remainder of the book is devoted to this search in the form of mathematical exercises in homotopy theory and general relativity. It ends with a succinct theory of charge as a counting procedure for "an identifiable number of separate left- and right-hand doughnuts".

Noticing that physical field theories deal only with continuous entities while the physical world displays basic discontinuities (the elementary electric charge of the electron being the one specific case considered here) is not new, but the whole preoccupation with continuity, infinities and singularities is indeed continuing. In this sense Mr Pisello's book is part of the contemporary mood. To try to save continuity by hiding the "inside" of particles into topology has been done with mastery by Professor John Wheeler creating "wormholes" for the delight of many students of general relativity. Mr Pisello attempts something similar but each piece of his puzzle unfortunately remains physically and also mathematically quite disconnected. At the Einstein centennial celebration at UNESCO in Paris a few weeks ago, Professor Dirac stressed his own dis-satisfaction with quantum mechanical calculations for which we "learned to turn a disinterested eye to infinities" and urged that Einstein's objections to quantum mechanics be seriously considered anew. Mr Pisello does just that in so far as he retakes "Einstein's insight" in the direction of unified field theories. He fails, however, to display a satisfactory theory, thus joining many others in this particular domain. The author also clearly shares with Einstein a socialist point of view upon the world; this does not justify the bloated language used by the publishers in the presentation of the book: "Pisello agrees with Einstein that quantum mechanics is a reactionary theory devised to maintain the mechanical view". Trying to get too close to Einstein, you may burn yourself up.

Failing to present clearly physical ideas and political analysis may be a consequence of the deep social frustrations experienced by Mr Pisello's generation of physicists. Trained in high energy particle physics, they got their degrees in America at the time of the Vietnam war. Their belief in 'pure' science was shaken when many of their peers were exposed as direct advisors for the US Department of Defence, and they could not get steady work as physicists in a tight competitive job market. This generation of physicists remains anxiously in search of the social and historical connections of scientific work.

Maurice Bazin

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Sociology of science

Science and the Sociology of Knowledge. Controversies of Sociology. Vol 8. By M. Mulkay. Pp. 132. (George Allen & Unwin: London, 1979.) £7.50; paperback £3.50.

FROM the time of the 'founding fathers' of sociology in the nineteenth century, sociologists have been interested in the way in which bodies of knowledge are influenced by the social and cultural contexts in which they are produced. This is the sociology of knowledge. But scientific knowledge was always seen as something apart, special, and essentially independent of external social influences in a way that was not true of other specialised bodies of thought and knowledge. Thus, when a sociology of science emerged, and developed (quite rapidly from the 1950s), it became something other than the attempt to explain the content of scientific knowledge in terms of social structures and processes. The sociology of science developed as the attempt to understand the social conditions which made scientific progress possible: that is, it was concerned with general social and political conditions (giving rise to the idea of correspondence between science and democracy), as well as with the way in which scientific communities worked. There was a lot of interest in the 'normative structure' of science: the rules or moral code governing the ways in which scientists worked and interacted with each other.

A good deal of work in sociology of science is still being done along these lines, some of the most interesting of it concerned with the emergence and functioning of scientific communities in smaller or less industrialised nations. But Michael Mulkay is concerned with something else. His argument is that both this approach as well as discounting of the possibility of a sociology of scientific *knowledge* are founded upon a particular philosophical view of the nature of science, which he calls "the standard view of scientific knowledge". Roughly speaking, this posits a real, objective, and uniform natural world; it assumes that science, through unbiased and detached observation, can formulate laws of nature which describe this world; that science makes use of entirely objective criteria for evaluating knowledge claims, such that science is ultimately free of bias; and that theoretical laws are of a different status to empirical ones and stand in a quite different relationship to the natural world. He goes on to argue that this philosophy of science is no longer acceptable, and that it is therefore legitimate to try to establish a sociology of scientific knowledge.

Mulkay characterises the "new philosophy of science" as showing the dependence of all observation on theory, of all factual statements on (arbitrary) linguistic frameworks. All meaning in science becomes the product of negotiated agreement and is embeded in some changing framework of values and assumptions. Feyerabend's "epistemological anarchism" is an extreme version of all this.

There have now been a few sociological studies of science which made use of this relativised version of science, and Mulkay describes some of them. It becomes important to try to understand how scientists 'negotiate' 'what counts' as the

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replication of an experiment (for example). Other work has explored, most interestingly, the way in which scientific disputes are conducted, and the way in which all kinds of political resources and non-scientific opinion can be used.

The final part of the book deals with the exchanges between science and other aspects of culture. This includes the use which may be made of theoretical perspectives or forms of explanation available in general thought. Unfortunately there is little discussion of the conditions under which these will be drawn on.

This whole undertaking is relatively new in sociology, and one cannot yet say what it will yield. My own view is that there are more new philosophies of science than Mulkay implies and that there will gradually emerge many attempts at quite different sociologies of scientific knowledge. Certainly the work which has been done so far is interesting, and could give sceptical scientists pause for thought. The book is likely to be of interest mainly to those who have already read into the field of sociology of science. **Stuart S. Blume**

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Mysterious Solar System

Mysterious Universe: A Handbook of Astronomical Anomalies. By W.R. Corliss. Pp. 710. (The Sourcebook Project: P.O. Box 107G, Glen Arm, Maryland 21057, 1979.) $15.95; £8.

MYSTERIES fascinate me and this book contains an abundance. It is a classical "What done it?" — the inanimate scientific relation to the "Who done it?". William R. Corliss has scoured a host of scientific journals for reports of astronomical oddities and has assembled over 500 short articles and extracts, on a multitude of subjects ranging from aurorae in comet tails to zodiacal light-rifts and black and white holes to X-ray bursts.

It is strong meat. Taken at one go the unceasing succession of anomalies, unknowns, mysteries and strange sightings leave the reader mentally punch drunk. You will stagger from the chair to the window to reassure yourself that the Sun and planets and bright stars are still where you thought they should be and have not all capriciously shuffled themselves. But in short bursts the book is a joy and the reader's mind is continually accelerated away in pursuit of ideas and possibilities that may account for the strange events being described.

The timespan covered by the book is reasonably broad, the most up-to-date extracts coming from mid-1978. Approaching from antiquity the number of articles verses time curve increases sharply around the middle of the 19th century, a time which seems to be governed more by the birth of journals such as *Nature* and *Scientific American* than by a sudden onset of mysterious happenings or human curiosity. Our excitement over the 'recent' discovery of the rings of Uranus pales somewhat when we read that Challis and Lassell reported an observation of a ring in 1847. These gentlemen also observed a ring around Neptune.

And things disappear in astronomy too. Vulcan the supposed intra-Mercurial planet sprang into prominence in 1876 only to fizzle from view in the early 1900s. Corliss collects together 14 papers on this subject. We are also introduced to Neith the lost satellite of Venus. The eighteenth-century observations of this object remain enigmatic and simply accumulate distain as they become eroded by time. We can easily smile condescendingly and talk about 'ghosts' in ancient telescope eye pieces but maybe...

Any collection of articles has the character of the collector strongly imprinted on it. This I'm sure cannot be avoided. Corliss shows a strong bias towards the Solar System. For example, the Moon, meteors and meteorites take up 26% of the book, the same amount of space as all the stars, the Sun, galaxies and cosmology put together. A similar percentage is taken up by the planets, leaving the zodiacal light, comets, planetary regularities and the environs of planet Earth to absorb the remainder.

I would be happier if the title of the book was *Mysterious Solar System*. Failing this, there are an enormous number of mysterious star types, stellar origin and evolution problems, interstellar, galactic and intergalactic matter and composition anomalies that could have been expanded to give the book a more 'universal' balance.

However, as a sourcebook of a lot of strange Solar System happenings and a number of stellar and galactic ones, the book is to be highly recommended. It has been well produced, extremely well indexed and is excellent value for money.

David W. Hughes

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Simple classical vibrator

The Physics of Vibration. By A. B. Pippard. Vol. 1. (Cambridge University Press: Cambridge, London, New York and Melbourne, 1978.) £22.50.

THIS is the first volume, devoted to the simple classical vibrator, of a work on the physics of vibration. It is designed to bridge the gap between undergraduate textbooks on the subject and specialist treatises. It ranges widely, discussing not only the obvious examples from mechanics, elementary electronics and acoustics, but also nuclear magnetic resonance, cyclotron resonance and Josephson junctions. The subsequent volume or volumes will deal with specifically quantal pheonomena and with vibrations of complex systems.

There is an introductory chapter describing very clearly the scope of this work; a chapter on the free vibrator; two chapters on applications of complex variables and on Fourier methods; three chapters on spectrum analysis, on the driven harmonic vibrator, and on waves and resonators; then one on volocity dependent forces groups, together with various examples such as whirling, the gyro-pendulum, nuclear magnetic resonance, cyclotron resonance and helicons. This chapter somewhat breaks the flow of the rest of the book, and does not fit in very happily; I am not convinced that this level of treatment of magnetic resonance will be helpful either to the expert or to the inexpert. A chapter on the driven anharmonic vibrator discusses subharmonics and stability. There is a short chapter on parametric excitation and a chapter on maintained oscillators. The final chapter is on coupled vibrators, and includes the Huygens phenomenon, frequency-locking and superconducting weak links.

Although the majority of examples are taken from electric circuit theory and mechanical oscillators, there are many others, some quite intriguing. The level of discussion is what one would expect from a good experimental physicist. The main interest in in common patterns of behaviour rather than in the details of operation, yet the details are worked out for many special cases.

I think that is primarily a book for the teacher rather than for the student. It does not treat any one subject in sufficient detail to be a useful source for someone who really wants to understand it, but it is more detailed than most students who want a general impression of the subject would want. It is, however, full of valuable new insights and analogies which should be helpful to anyone who needs to teach this sort of material. No physics library should be without a copy.

D. J. Thouless

D. J. Thouless is Professor of Applied Science at Yale University, New Haven, Connecticut.

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newly on the market

Pressure transducer. Hewlett-Packard. An extremely durable pressure transducer weighing only 30 g has been developed by Hewlett-Packard. It features quartz crystal sensors to measure patient's blood, gastro-intestinal, intrauterine, intracranial and other physiological pressures. With a flat, rather than cylindrical body the transducer is also easier to attach to a patient's limbs. A disposable dome, which attaches with a simple twist-and-lock motion, provides protection against physical damage. Being constructed from a transparent plastics material, nursing staff can easily check to see and dispel any unwanted bubbles which could distort the measurement results. Standard couplings are used so that the transducer, designated HP129OA, may be used with any Hewlett-Packard pressure amplifier. It measures only 3.5 in by 1.25 in and comes complete with six disposable domes.
Circle No. 77 on Reader Enquiry Card.



Hewlett-Packard pressure transducer

Isoelectric focusing system. Bio-Rad. The Model 1415 horizontal electrophoresis cell from Bio-Rad is designed for electro-focusing applications, immunoelectro-phoresis, DNA electrophoresis, and zone electrophoresis. The cooling platform is 43 cm long and enables the user to perform two preparative electrofocusing runs simultaneously in 20-cm gel trays. The lid of the cell contains a series of small holes to enable the user to check the *p*H along the length of the gel without interrupting the current. A cooling coil attached to the perimeter of the lid ensures preferential condensation on the coil rather than on the lid above the gel tray or plate. The techniques developed by Bio-Rad for electrofocusing introduce new methods for drying the granulated bed gel and locating the protein bands on the completion of the run.
Circle No. 78 on Reader Enquiry Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Enquiry Card bound inside the cover.

Ultramicrotome. Du Pont. Featuring microprocessor control for enhanced operator convenience, the Sorvall MT5000 ultramicrotome has been introduced by Du Pont. It combines reliable cutting precision with operating simplicity for preparation of sections for electron microscopy. Features include: Microprocessor logic for improved accuracy and reproducibility; a Visutrac system for pgrogramming cutting duration; a jog function for precision approach; a built-in anti-vibration system. The MT5000 provides cutting speeds from 0.1 mm s^{-1} to 39.9 mm s^{-1}, with a fast return of 10 mm s^{-1} in the normal sectioning range below 10mm s^{-1}.
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Non-destructive testing system. Elomag. A completely new, optical, non-destructive testing system from Elomag permits a wide variety of measurements to be made in engineering and science. A large freestanding optical tripod supports the main elements of the system including: (1) A double laser speckle camera which is used for non-destructive stress, vibration, and flaw analysis of engineering components. (2) The image derotator, that allows precise, live, continuous measurements to be made on rotating objects and fluids. And (3), a compact pulsed ruby laser, that incorporates a q-switch and is used, in conjunction with the other elements of the system, to study dynamic events. The system is self-contained, easy to operate, and portable.
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Temperature safety controllers. Neslab has developed temperature safety controllers for constant-temperature baths and circulators covering a range of —85°C to +150°C. The safety controller will shut the entire bath system down and sound a buzzer if a pre-set temperature exceeds ±20°C. Simply plug your bath into the TSC, place the sensor in the fluid, and the unit is ready for operation. The Neslab TSC is an independent control system that can be used with all Neslab bath circulators as well as other manufacturers' baths.
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Direct view microscope. Bausch & Lomb. Using the new Bausch & Lomb Balplan Bi*Ocular microscope the magnified specimen image can be viewed in full, natural colour on a 5-inch diameter circular screen. The instrument enables the operator to study specimens in comfort at a magnification of 37.5× to 300× The direct view facility is available on reflected light or transmitted light microscopes.
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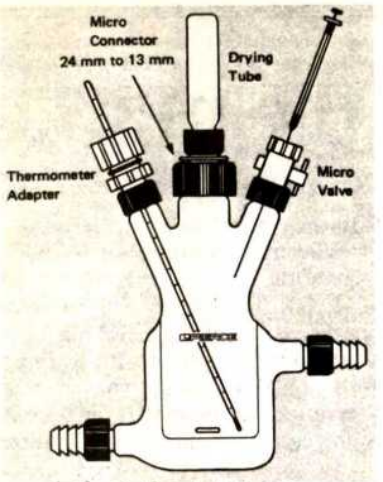
IR Image converter. Wild Heerbrugg. The new Wild image converter allows binocular observation in the near-IR range of 700–1,200 nm. Images can be recorded with the attachable Leica R3 mirror reflex camera in white light or with IR film. The Wild M 520 IR image converter is for observations in the macro range, but can also be used with microscopes and stereo-microscopes
Circle No. 83 on Reader Enquiry Card.

Mycoplasma detection kit. Bioassay Systems. A quick, inexpensive method for detection of mycoplasma contamination has been developed by Bioassay Systems. They have produced a detection kit based on the ability of 4'-6-diamidino-2-phenylindole to form a fluorescent complex with DNA in the mycoplasma. The kit contains a quantity of dye sufficient for 50 assays, a fixed sample of mycoplasma infected cells and a control sample of non-infected cells. The assay requires only 60 min for completion.
Circle No. 84 on Reader Enquiry Card.

Fluorestar microscope. Reichert-Jung. The Fluorestar from Reichert-Jung is a flexible laboratory microscope specifically designed for fluorescence techniques. Facilities are provided for incident light, transmitted light or mixed illumination examinations and all controls are sited to minimise operator fatigue. The incident light fluorescence equipment is very simple to operate. Plug in 'fluo-clusters' are provided with matched exciter filter/dichroic beam splitter/barrier filter combinations. A wide range of optional accessories are available for use with this microscope including camera systems and multi-viewing attachments.
Circle No. 85 on Reader Enquiry Card.



The Reichert-Jung Fluorestar



One of the Pierce jacketed reactors

Multi-purpose jacketed reactors. Pierce. These jacketed reaction vessels offer a wide range of applications in the research laboratory. The reactors feature a central opening to accommodate virtually all combination *p*H electrodes. Two 13-mm side ports permit insertion of a thermometer, delivery of titrant or inert gas and reaction sampling. The angled side ports also simplify transfer of reactants and products. Jacketing of the reaction well allows temperature control of exothermic and enzyme reactions. Stirring is easily achieved with a magnetic stir bar and laboratory stirring unit. These units are completely adaptable to the micro-connectors and accessories of the Reacti-Ware System.
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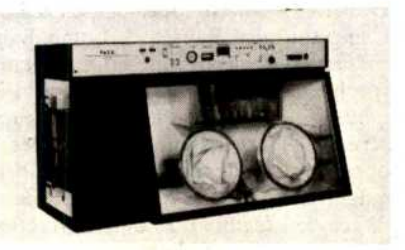
CO_2 Laser attenuators. Rofin announces a new series of CO_2 laser attenuators designed to accommodate a range of input powers from 15 to 500 W. Designated the 7530 series, they are designed to control accurately the power output of any CO_2 laser having linearly polarised output. For unpolarised lasers two attenuators in series can be used to provide a more limited range of attenuation. The range includes both manual and automatic units, the manual units being calibrated at 2° increments. The automatic units are fully computer compatible and may be set to any desired attenuation. For extra safety, the control console may be used remotely from the attenuator.
Circle No. 87 on Reader Enquiry Card.

Affinity absorbents: Immobilised sugars. Pierce. A new line of affinity adsorbents for isolation and purification of lectins, carbohydrate binding proteins, toxins and glycosidases is now available from Pierce. Among the 12 Selectins now offered are immobilised *N*-acetyl-D-glucosamine, lactose, maltose, melibiose, *N*-acetyl-D-galactosamine, D-galactosamine, L-fucose and D-mannose. The Selectins are referenced with preparative applications, binding capacities and ligand-leash structure.
Circle No. 88 on Reader Enquiry Card.

Water bath. Thermolyne announces its new Temptronic 100 water bath, which features selectable temperature set-points, digital readout and stable, uniform bath temperatures. A reset switch allows the operator to dial in a desired temperature to 0.1°C. The Temptronic 100 water bath will heat to the set temperature without further adjustment. It is available in either 120 or 240 V operation and weighs only 14.061 kg. An optional gabled cover is also available. A circulating pump combined with an instant feedback electronic control provides ±0.5°C control to 60°C.
Circle No. 89 on Reader Enquiry Card.

Personnel radiation chirper. Radiation Monitoring Devices. Personnel chirpers are very useful safety instruments but are often not worn because of their bulk and weight. Los Alamos Scientific Laboratories, therefore, have developed a miniature chirper small enough and light enough to be worn on a shirt collar. The device was made possible by the use of a 2 mm^3 chip of CdTe which replaces the much larger Geiger tube sensor. The high sensitivity, reliability and low power requirement (5 V) of the CdTe enabled the new chirper to be quickly accepted by personnel working in nuclear radiation environments.
Circle No. 90 on Reader Enquiry Card.

Microprocessor controlled anaerobic chamber. Lab-Line Instruments has introduced Model 6500 PACE, the microprocessor controlled anaerobic chamber. The microprocessor automatically brings the interchange to anaerobiosis and also controls the locks on the interchange doors to assure opening only in anaerobic conditions. It also continuously monitors all chamber conditions and controls an alarm system. PACE is constructed of steel with a flexible vinyl glove port panel. A radiant warm wall incubator with sliding plexiglas doors is built into the upper right hand side of the chamber to allow the entire 44″ × 26″ floor area free for work space. A digital LED display on the front panel allows visual monitoring of incubator temperature and chamber humidity. Constant circulation of chamber atmosphere through the catalyst and desiccant assures strict anaerobiosis and humidity control.
Circle No. 91 on Reader Enquiry Card.



The Model 6500 PACE

Freeze-dryer. FTS Systems. FTS Dura-Dry freeze-dryers eliminate the effects of constant corrosive attack by using titanium and polypropylene in the critical vapour-contact areas of the system. Further, the Dura-Dry assembly arrangement makes all vital parts of the system accessible for close-up visual examination, removal or substitution. Equipment is housed in a practical, take-apart cabinet that provides a support surface and slip-fit vacuum connection for various manifold arrangements, bulk tray dryers and multiple-shelf *in-vacuo* stoppering units. Options for additional protection include chemical 'up-stream' acid filters; chemically resistant vacuum pumps; manual purge systems; product protection during power failure and sterile air-bleed-back systems. The Dura-Dry has a total freeze-drying capability of 20 l.
Circle No. 92 on Reader Enquiry Card.



The FTS Dura-Dry freeze-dryer

Powermeter. Oriel announce a new addition to their range of photoresist products. The 8427 UV Powermeter is a highly reliable, direct reading and calibrated measurement system. A proprietary technique of system calibration, permits the user to choose from a variety of sensor responses. Each can be plugged into the measurement package and is precalibrated to be direct reading without adjustments of the electronics. Ultra-stable silicon detectors are used with glass absorption filters to shape the spectral response.
Circle No. 93 on Reader Enquiry Card.

Guinea pig complement. Cedarlane Hemo-Lo guinea pig complement is designed specifically for sensitive antibody forming cell plaque assays utilising free or haptenated sheep red blood cells (SRBC) as indicators. This reagent is pre-absorbed with SRBC to reduce background haemolysis. Cedarlane Low-Tox guinea pig complement is a highly potent source of guinea pig complement which has been absorbed with agarose to remove background toxicity to mouse lymphocytes. Each batch is tested for high complement activity and low background toxicity to mouse thymocytes.
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APPOINTMENTS VACANT

KING'S COLLEGE HOSPITAL DENTAL SCHOOL
(University of London)
Denmark Hill,
London SE5 8RX

DEPARTMENT OF ORAL PATHOLOGY
HISTOPATHOLOGY

A vacancy has arisen for a Senior Medical Laboratory Scientific Officer or Medical Laboratory Scientific Officer to participate in routine histopathology.

Good facilities exist for becoming acquainted with a wide range of modern morphological techniques and opportunities are available for the successful candidate to participate in research programmes. Salary on scale £4,347 to £5,769 (SMLSO) or £3,261 to £4,680 (MLSO) & £354 London Weighting (under review) according to age, qualifications and experience.

Applications, giving full curriculum vitae and names and addresses of 2 referees should be sent to the Secretary of the Medical School. Further details available from Professor Garrett's Secretary on 01-274 6222 Ext. 2885. 2432(A)

FACULTY POSITION

The Department of Mechanical Engineering at the University of Southern California has openings for faculty positions at all levels with teaching and research interests in fluid dynamics, heat transfer; thermodynamics and combusion; computer aided design, manufacture and control and biomechanics. Applicants must include an up-to-date resume, including a comprehensive list of publications and supply the names of five references who know of their qualifications intimately and who can be contacted for letters of recommendation. Direct inquiries to Dr Tony Maxworthy, Chairman, Dept. of Mech. Engr., Univ. So. Calif., Los Angeles, CA 90007. EOE/M/F/H. W212(A)

THE POLYTECHNIC OF CENTRAL LONDON

School of Engineering and Science
Applied Microbiology and Biochemistry Research Group

POST-DOCTORAL RESEARCH FELLOW IN ENZYME TECHNOLOGY

Salary:— £4,221 to £4,677 inclusive of London Allowance.

Applications are invited from suitably qualified persons for the above 2-year post. Candidates should have interest/experience in applied enzymology, with particular reference to the use of microbial hydrolytic enzymes for the degradation of natural polysaccharides, under enzyme reactor conditions.

Application form and further details from the Establishment Officer, PCL, 309 Regent Street, London W1R 8AL (Tel. 01-580 2020 ext. 212). 2390(A)

LEICESTER POLYTECHNIC

SCHOOL OF LIFE SCIENCES

Applications are invited from appropriately qualified graduates for the post of:

RESEARCH ASSISTANT

The successful applicant will work with Dr R S Oldham in collaboration with Dr Alistair Ferguson of the Anglian Water Authority on water circulation patterns and biological processes in a large reservoir. The appointee will investigate and model the effect of wind and water currents upon the planktonic and benthic communities of Rutland Water. This is applied research with the objective of improving the efficiency of water abstraction. It will be essentially a physically based project, but with a strong biological component.

The successful applicant will be expected to register for a higher degree and will receive an award equivalent to that paid to NERC/SRC research students (currently £1,610 per annum *tax free*). The award will be renewable annually for a 3 year period subject to satisfactory progress.

Application form and further details are available from: Staffing Officer, Leicester Polytechnic, P O Box 143, Leicester LE1 9BH Tel: (0533) 551551 Extension 2303. 2368(A)

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF CLINICAL BIOCHEMISTRY

Vacancies exist for two Research Technicians in the above Department. The posts are funded by Pharmaceutical Companies and are for 1 year in the first instance. Duties include measurement of metabolic intermediates and hypoglycaemic drugs in patient's serum.

Applicants should have HNC, 1st degree or several years laboratory experience and should send curriculum vitae and names and addresses of 2 referees to Professor C. N. Hales, Department of Clinical Biochemistry, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QR, by 25th July. Starting salary up to £2,930. 2442(A)

UNIVERSITY OF NATAL

DEPARTMENT OF PHYSICS
Durban

PLASMA PHYSICIST

Postdoctoral Research Fellowship available October 1979 for at least one year, for experimental research in plasma waves and beam-plasma interactions. Salary range R8,100 to R11,200 p.a., plus travel allowance each way. Recent postdoc or sabbatical visitor suitable.

Send application, resumé and three referees' reports by September 1st quoting reference ADVD38/79 to Prof. M. A. Hellberg, Plasma Physics Research Institute, University of Natal, Durban, South Africa. W200(A)

THE UNIVERSITY OF MANCHESTER

RESEARCH FELLOW IN PHARMACOLOGY

Applications are invited for this postdoctoral post to work in the field of calcium antagonist drugs. The work will initially involve the determination of the mode of action of novel calcium antagonists on cardiovascular and uterine tissues. The post is tenable for one year and may be extended for a further two years. Initial salary p.a. is £4,232 (£4,333 from October 1979). Particulars and application forms (returnable by July 16th) from Dr. A. H. Weston, Department of Pharmacology, Manchester University, Oxford Road, Manchester M13 9PT. 2378(A)

UNIVERSITY OF DURHAM

DEPARTMENT OF BOTANY

Applications are invited from Botanists, Biologists and Biochemists for several posts of Postdoctoral Research Assistant for two years from 1st September, 1979 or later by arrangement.

For one post, plant physiology or histochemical experience is required and for another, knowledge of recombinant DNA technology.

Initial salary £4,232 or £4,505 (£4,333 per annum and £5,622 per annum from 1st October 1979) within Range 1A plus superannuation.

Applications (3 copies) naming three referees should be sent by 27 July 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. 2376(A)

Senior Research Chemist

We have a vacancy for an Organic Chemist to join our Chemistry Research Department. This post, which is under the scientific direction of the Director of Research, provides an opportunity for taking charge of a programme of work on products of potential interest for medicinal use.

Applicants, male or female, must possess a PhD qualification preferably with some post-Doctoral experience in an academic environment or two to three years industrial experience of innovative research in the Pharmaceutical industry. However, newly qualified PhD's or those expecting this qualification will also be considered, particularly if their research interests involve heterocyclic chemistry.

Roche Products Limited is part of an international Swiss-based pharmaceutical company and the terms and conditions of service are those to be expected from a leader in this industry.

If you would like further information about this post and an application form please write to or telephone quoting reference RC27, Mr. J. G. Bennett, Personnel Manager, Roche Products Limited, P.O. Box 8, Welwyn Garden City, Hertfordshire AL7 3AY, Telephone Welwyn Garden 28128. 2409(A)

ROCHE

Senior Virologist

National Biological Standards Laboratory
Viral Products Section
Parkville, Victoria
Commonwealth Department of Health

A vacancy exists for a senior Virologist in the Viral Products Laboratory. This Laboratory is responsible for the control of the quality of the human and veterinary viral vaccines used in Australia. It is located in a suburban area, close to Melbourne. The capital city of Victoria, Melbourne (population 2.5 million) Australia's second largest city, is located on Australia's south east coast and has a temperate climate.

Duties:
Control and direct a team engaged in the evaluation and testing of human viral vaccines, in the development of standards for these vaccines, and in research into problems associated with the safety and efficacy of the vaccines.

Qualifications:
An appropriate degree or diploma in Science or equivalent qualification. Post-graduate qualifications and extensive experience in virology and immunology desirable.

Salary:
Within the range $20,443-21,702 (Australian) (At present exchange rate £1 = $A1.90 approx.).

Conditions include four weeks annual recreation leave, long service leave, cumulative sick leave and superannuation benefits. Overseas appointees may receive assistance with fares, removal expenses, and temporary accommodation costs.

Applications to be submitted to: Director of Health, Commonwealth Government Centre, Cnr Spring and Latrobe Streets, Melbourne, Victoria, Australia 3000.

Enquiries in Britain may be made with the Chief Medical Officer at Canberra House, Maltravers Street, Strand, London WC2R 3EH. Applications close on July 26 1979. 2430(A)



SENIOR PHARMACOLOGIST
St. Albans

WEDDEL PHARMACEUTICALS LTD. are seeking an experienced Pharmacologist to fill a key post as Section Leader in the Biological Services Department at the Union International Research Centre in St. Albans, Herts.

The successful candidate will lead a team engaged in pharmacological research and development of naturally-occurring substances active on the cardiovascular system. Ideally, we are seeking someone with a PhD in Pharmacology or a related subject and it would be advantageous if the candidate has several years' experience of the pharmaceutical industry, perhaps working in cardiovascular or biochemical pharmacology. Applications, which will be treated in confidence, with full curriculum vitae should be made to:

Mr J. E. Dawes (AD 6088),
The Union International Company Ltd.,
14 West Smithfield,
London,
EC1A 9JN

2440(A)

THE POLYTECHNIC OF NORTH LONDON
DEPARTMENT OF GEOGRAPHY
TECHNICIAN GRADE 2B

The post involves supervision of the Departmental Map Library and general assistance in the Laboratories.

The post offers an excellent opportunity to gain a first-hand experience in a large and developing Department which includes over 250 students and 18 full-time academic staff. The work is at degree and post-graduate level.

Education level and experience (inclusive of training period) ONC or OND or 2 'A' levels, or Ordinary City & Guilds or equivalent. Three years' experience.

Salary Scale: £3282 to £3663 inclusive of London Weighting.

Application form can be obtained from the Establishment Officer, The Polytechnic of North London, Holloway Road, N7 8DB. 2388(A)

POSTDOCTORAL POSITION

Postdoctoral position available for a biochemist or chemist on isolation and characterization of components and receptors from blood platelet membranes. Regular salary of the Weizmann Institute for this grade. Available from August 1, 1979. Write to: Dr Avner Rotman, Department of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel. W206(A)

UNIVERSITY OF SUSSEX
School of Biological Sciences
LABORATORY OF EXPERIMENTAL PSYCHOLOGY
Lectureship in Psychology

Applications are invited from candidates in Human Experimental Psychology, with an interest in memory, attention and performance. Special consideration will be given to those capable of teaching statistics.

Salary scale: £4,333 to £8,992 (under review)

Further information may be obtained from Professor N. J. Macintosh, Laboratory of Experimental Psychology, University of Sussex, Falmer, Brighton BN1 9QG.

Candidates should apply to: Secretary of Science, Science Office (E), University of Sussex, Falmer, Brighton BN1 9RH.

Closing date for applications 27 July 1979. 2434(A)

UNIVERSITY OF WARWICK
RESEARCH TECHNICIANS

The Department of Biological Sciences has vacancies in its four Research Groups, namely Chloroplast development, Developmental Biology (Molecular Genetics), Microbiology and Virology. The posts involve working under the direction of a senior scientist on a specific research project using the modern experimental techniques and instrumentation of molecular biology. The posts are for contract periods of one to three years and are available from October, 1979. Applications are invited from graduates and others with appropriate qualifications in biochemistry and/or microbiology. Salary either on Technician Grade 2B scale £2,759 to £3,138 p.a., or Grade 3 scale £2,931 to £3,336 p.a. Applications by letter giving full details of qualifications, experience etc to the Personnel Office, University of Warwick, Coventry CV4 7AL, quoting Ref. No: 47/D/79 by 20th July, 1979. 2396(A)

UNIVERSITY OF LEICESTER
DEPARTMENT OF ZOOLOGY
RESEARCH TECHNICIAN

Applications are invited for the post of Research Technician Grade 5 to work with a group of investigators who are studying various aspects of the structure and function of chromosomes in amphibians and other animals. Preference will be given to applicants who have some experience of technologies associated with biochemistry of nucleic acids or those involved in advanced light and electron microscopy. The appointment will be for 3 years, in the first instance, commencing 1st September 1979. Salary will be on the range £3,474 to £4,056. Applications, together with the names of 2 referees, should be sent to Professor H. C. Macgregor, Zoology Department, University of Leicester, Leicester LE1 7RH, before 14th July 1979. 2404(A)

INSTITUTE OF CANCER RESEARCH: Junior Technician required for the Department of Molecular Biology, Chester Beatty Research Institute, Fulham Road, London, SW3, in the general field of protein chemistry. The successful candidate will assist in the isolation and characterisation of proteins and peptides. Applicants should have at least 2 'A' levels in Maths., Physics or Chemistry. Salary in scale £2,370 to £3,015 p.a. plus London Allowance of £354 p.a. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref. 301/B/76. 2373(A)

ORGANIC/ PHYSICAL CHEMIST

We have a vacancy for an organic or physical chemist for multidisciplinary work on organic electro-optical display materials and their applications.

Applicants should possess a good degree and/or membership of the Royal Institute of Chemistry and should have experience in Research and Development, including knowledge of physical and electronic measurement techniques. Experience in the field of display devices would be an added advantage.

This position offers interesting work, with an opportunity to contribute to the expanding technology of electro-optical devices.

We offer all the normal benefits associated with a major progressive company, including a competitive salary, a 37 hour week, contributory pension scheme, hospital fund and a discount on certain GEC products. Relocation expenses will be paid where appropriate.

Please write to: Mrs. S. A. Fox, Marconi Research Laboratories, West Hanningfield Road, Great Baddow, Chelmsford, Essex, CM2 8HN.

The above vacancy is open to both men and women.

Marconi Research Laboratories

GEC-Marconi Electronics Ltd.

2425(A)

UNIVERSITY OF ESSEX

FELLOW in the DEPARTMENT OF PHYSICS

(Laser-Photoconductivity in Semiconductors)

Applications are invited for a post of Fellow (starting salary up to £4,776 on Range IA) tenable for 3 years, to carry out SRC — funded experimental research into deep-level impurities in semiconductors in collaboration with Dr B. K. Ridley. Candidates should have a PhD or equivalent qualification. Experience of infrared lasers, semiconductors and low-temperature techniques would be useful but not essential. The appointment will run from 1 September 1979 or as soon as possible thereafter.

Application should be made before 16 July 1979 to Mrs D. A. Weatherall, Department of Physics, University of Essex, Wivenhoe Park, Colchester CO4 3SQ (Tel. 0206 862286).

2436(A)

ASSISTANT PROFESSORS

Applications are invited for two positions at the level of Assistant Professor in the Department of Pharmacology of Yale University School of Medicine. Applicants must have a doctoral degree and postdoctoral experience. Preference will be given to individuals with experience and research skills in the neural sciences, particularly at the molecular and cellular levels. Demonstrated potential to initiate a strong independent research program and to engage in the teaching activities of the department are expected. Interested candidates should send curriculum vitae, reprints, a brief description of research interests and the names of three possible references by August 15, 1979 to: Dr. Alan C. Sartorelli, Chairman, Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510. An Affirmative Action/Equal Opportunity Employer. W211(A)

UNIVERSITY OF ABERDEEN

TECHNICIAN

Required to look after the electron-microscope unit in the Department of Botany and to undertake a range of work associated with transmission and scanning electron microscopes. Applicants should hold an ONC (or equivalent qualification), have had at least 4 years' laboratory experience and have an interest in electronics and photography; training will be given in electron microscopy.

For suitably qualified candidate, salary on scale £2,931 to £3,336. Applications from recent graduates interested in training in electron microscopy will be considered for appointment against this post; salary for such a candidate during initial training period would be on scale £2,556 to £2,724.

Applications giving date of birth, educational qualifications and previous experience should reach the Secretary, University Office, Regent Walk, Aberdeen AB9 1FX, by 27 July, and quote Ref. No. 142/79. 2398(A)

UNIVERSITY OF LEEDS. Departments of Biochemistry and Genetics. Applications are invited for a Research Technician, Grade 5, to assist with projects based on an inter-departmental protei sequencing unit. The appointment, financed by a grant from the Science Research Council to Dr. J. B. C. Findlay (Biochemistry) and Dr. J. C. Wootton (Genetics). The work will include operation of an amino acid analyser with computer integration and of a solid phase sequencer. Applicants should have a Degree, an HNC or equivalent, or proven experience and expertise in this field. Experience in biochemical methods, instrumentation and in the processing of quantitative data would be an advantage. Salary on the scale £3,474 to £4,056 pa (under review). Applications in writing, stating date of birth, qualifications and career details, and the names of 2 referees to Dr. J. B. C. Findlay, Department of Biochemistry, 9 Hyde Terrace, Leeds LS2 9LS. 2428(A)

JOHN INNES INSTITUTE

DEPARTMENT OF APPLIED GENETICS

THREE YEAR FELLOWSHIP FUNDED BY THE JOHN INNES CHARITY

Applications are invited from molecular biologists/geneticists to study the basis and manipulation of cytoplasmic male sterility in sugar beet. Further particulars may be obtained from Professor D. Roy Davies.

Appointment will be as Higher Scientific Officer (£4,101 to £5,448) (pay increase pending), starting salary depending on qualifications and experience. Non-contributory superannuation. Minimum qualifications: First or Upper second class degree in molecular biology, genetics or virology with at least two years' relevant experience. Preference will be given to candidates with a PhD.

Applications, quoting reference AG/42, giving full particulars and the names of two referees, should be sent to the Secretary, John Innes Institute, Colney Lane, Norwich, NR4 7UH, not later than 30th July, 1979. 2393(E)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF BIO-MEDICAL PHYSICS AND BIO-ENGINEERING

LECTURER/ASSISTANT RADIATION PROTECTION OFFICER

Applications are invited from suitably qualified Physicists or Radio Chemists for the post of Lecturer in the Department of Bio-Medical Physics and Bio-Engineering. The person will also act as Assistant to the Radiation Protection Officer who has the responsibility for radiation protection in the University, and the Hospitals of the Grampian, Orkney and Shetland Health Boards.

Salary on scale £4,232 to £8,452 per annum (under review), with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by 27 July 1979. 2391(A)

ROTHAMSTED EXPERIMENTAL STATION HARPENDEN, HERTS AL5 2JQ

BIOLOGIST

to join a small group working on the taxonomy of root-knot nematodes. The person appointed will be expected to take a major role in initiating and developing the work. Previous experience in nematology and/or an interest in taxonomy preferred. The position is funded by the Overseas Development Ministry and the appointment will be for three years only.

Qualifications: First or Upper Second Class Honours Degree in a biological subject. Preference given to candidates with a PhD degree.

Appointment in grade of Scientific Officer (£2,839 to £4,415) or Higher Scientific Officer (£4,101 to £5,448). (Pay award pending). At least two years' relevant post-graduate experience is required for appointment as HSO. Non-contributory superannuation.

Apply in writing to the Secretary giving names and addresses of two referees and quoting Ref. 402 by 8th August, 1979. Further details on request. 2443(A)

UNIVERSITY OF LIVERPOOL

TECHNICIAN (GRADE 5)

DEPARTMENT OF BIOCHEMISTRY

For the Electron Microscope unit to carry out work in association with several research groups.

Applicants should possess HNC or equivalent as minimum qualification and minimum of ten years appropriate experience. Salary range £3,474 to £4,056 p.a. Application forms may be obtained from the Registrar, The University, PO Box 147, Liverpool L69 3BX. Quote Ref: RV/683. 2418(A)

Analytical Research in Drug Development

We have a number of vacancies for male and female scientists and technicians to work on various aspects of drug analyses.

Analytical Development

We seek a scientist able to work with minimal supervision on the development of methods for the analysis of new drugs in biological fluids. The ideal candidate will hold a PhD in a relevant science (chemistry, analytical chemistry, biochemistry) with 2-3 years experience in drug research.

Mass Spectrometry

This vacancy is for an experienced technician (HNC or degree) to assist in the operation and maintenance of our MS 30 mass spectrometer (with GC and data system). The work of this unit is varied including characterisation of newly synthesised compounds, identification of drug metabolites isolated from biological samples, and the use of the GC-MS system for quantitative drug analysis. Although we are looking for experience, training would be given to a candidate with suitable qualities.

Technicians

We also have vacancies at various levels of seniority (A levels to HNC) for technical staff to assist in the work of the department. As indicated above this involves the development of methods for the analysis of drugs (both new and marketed) in biological fluids using chromatography and spectrophotometry, and the application of such methods to interesting areas of drug research (pharmacokinetics, clinical trials, toxicology, etc). Applications are invited from technicians with an interest in drug research or analysis as a career and training will be given to suitable candidates.

Conditions include four weeks holiday, flexible working hours, subsidised staff restaurant, free private health scheme and excellent sports and social facilities including an open air heated swimming pool.

Please write or telephone for an application form to:
Adrian Forrest, Personnel Department, Hoechst UK Limited, Walton Manor, Walton, Milton Keynes, Bucks.
Tel: Pineham 5068

Hoechst

2412(A)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified persons for appointment to the following positions:

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES

RESEARCH FELLOWS IN MOLECULAR GENETICS — GENETICS DEPARTMENT
(two posts)

Applicants should preferably have experience in the areas of microbial or plasmid genetics and recombinant DNA techniques. The successful candidates will be strongly encouraged to collaborate in joint projects with existing groups in the Genetics Department. These include: Professor J. A. Pateman, with broad interests in the carbon and nitrogen regulation of gene expression in simple eucaryotes and particular emphasis on the glutamate dehydrogenases, glutamine synthetase and amino acid catabolism; Dr. G. D. Clark-Walker, working on mitochondrial and plasmid genetics in yeast; Dr. B. Rolfe and Dr. J. Shine, working on nitrogen fixation genes in Rhizobia and the structure and expression of eucaryote genes.

Applications should be lodged as soon as possible.

FACULTY OF SCIENCE
DEPARTMENT OF MICROBIOLOGY

SENIOR TUTOR IN MICROBIOLOGY

Applicants should have a higher degree in microbiology with experience in medical microbiology. The department is developing teaching in areas of microbial pathogenesis and immunology, and applicants able to contribute to course development and teaching in microbial pathogenesis in particular will be preferred.

The successful applicant will be required to assist in preparations, supervision and assessment of practical classes in second year general microbiology, to participate in training PhD and Honours students, and to contribute to the departmental research programme.

Closing date: 27 July, 1979.

Salary will be in accordance with qualifications and experience within the range: Research Fellow $A15,786 to $20,606; Senior Tutor $A13,606 to $15,586 per annum. Present exchange rates: $A1 = UK53p; $US1.10.

Terms of Appointment: Research Fellow — three years in the first instance with the possibility of extension to five years; Senior Tutor — one year in the first instance with the possibility of yearly renewal up to five years.

Other Conditions: Reasonable appointment expenses are paid. Travel expenses are paid for Senior Tutors appointed from Australia and New Zealand only. Superannuation benefits are available for applicants who are eligible to contribute. Assistance with finding accommodation is provided for an appointee from outside Canberra.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should first obtain further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H0PF. 2438(A)

To place your advertisement in these pages

Phone: 01-831-6901

British Museum (Natural History)

The British Museum (Natural History) is an institution for taxonomic research and an education focal point for the public. The main aim of the research is to produce definitive accounts of the world's animals, plants and minerals as well as the preservation of an extensive collection of fossils. There are unrivalled opportunities for research, aided by comprehensive library and technical services.

HEAD OF FRESHWATER ALGAE SECTION

. . . to supervise the work of junior staff, conduct research on the taxonomy of freshwater Chlorophyta and/or Rhodophyta and organise the SEction's curatorial and advisory service.

Candidates, normally aged under 32, should have a good honours degree or equivalent in an appropriate discipline and at least 2 years' postgraduate experience. Experience in algal taxonomy is highly desirable. Appointment as Senior Scientific Officer or Higher Scientific Officer. *Ref: SB/55/DK*

PALAEONTOLOGISTS

. . . to work on either Fossil Bryozoa or Palaeozoic Plants and take responsibility for the curation and conservation of the appropriate collection. The work will also involve dealing with scientific and general enquiries, and developing and maintaining relationships with scientists and research institutions throughout the world. The successful candidates will each undertake approved research in the appropriate field which in Fossil Bryozoa is expected to centre on palaeozoic forms and in Palaeozoic Plants will be on aspects of the gross morphology of plants, not palynology.



Applicants must have a good honours degree in Geology or Botany, and at least 2 years' relevant postgraduate experience. A strong interest in fossil bryozoa or plant taxonomy and gross morphology, and a knowledge of modern curatorial methods would be an advantage. Appointment as Senior Scientific Officer or Higher Scientific Officer. *Ref: SB/56/DK.*

ENTOMOLOGISTS

Taxonomic Research on Caraboidea/ Scarabaeoidea.

The successful candidate will work in the section dealing with Caraboidea, Scarabaeoidea and Staphylinoidea and will undertake taxonomic research on either Caraboidea or Scarabaeoidea, including their immature stages. The work will include curatorial duties aimed at the improvement of the National Insect Collection through exchange, collecting and rearing. Departmental staff are encouraged not only to collect in the field themselves, but also to take part in expeditions abroad.

Medical Insect Studies

The successful candidate will supervise and participate in taxonomic research on one or more families of medically important Diptera and become a specialist adviser on mosquitoes. The work will also involve improving and maintaining the national collection of medical insects.

Candidates, normally aged under 32, should have a good honours degree or equivalent in zoology, preferably with entomology. At least 2 years' relevant postgraduate experience is essential. Appointment as Senior Scientific Officer or Higher Scientific Officer.

Taxonomic Research on Coccoidea

The successful candidate will participate in taxonomic research studies on scale insects and mealy bugs, and maintain and improve the Department's collection through exchanges with other specialists and by collecting and rearing. The work will involve a great deal of microscope slide preparation, and some supervision of junior staff. In addition, the entomologist will deal with enquiries from other institutions and members of the public.

Candidates, normally aged under 30, should have a good honours degree or equivalent in zoology, preferably with entomology. Relevant postgraduate experience is advantageous; at least 2 years is essential for Higher Scientific Officer level. Appointment as Higher Scientific Officer or Scientific Officer. *Ref: SB/54/DK*

Salary for Senior Scientific Officer in the range £5675-£7420; Higher Scientific Officer £4620-£5970; and Scientific Officer £3360-£4910. Level of appointment dependant on qualifications and experience.

All salaries are under review. Promotion prospects. Non-contributory pension scheme.

These posts are exempted from the Government's ban on recruitment.

For further information and an application form (to be returned by 27 July 1979), write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote appropriate reference.*

2453(A)

NATIONAL INSTITUTE OF AGRICULTURAL BOTANY

TECHNICAL OFFICER

quired in the Official Seed Testing tion, principally for the development of ining methods and programmes for seed alysts, preparing and maintaining ormation on seed analysis techniques d providing specialist advice. Applicants ould have a degree in botany, icultural botany or a related subject and least 2 years' post-graduate experience. qualification in, or experience of, ching is an advantage.

The appointment would be on the gher Scientific Officer grade, salary der review) £4,101 to £5,448 per annum cording to qualifications and experience. Application form and further particulars m the Establishments Officer, NIAB, ntingdon Road, Cambridge CB3 0LE. : Cambridge 76381 Ext 234 (quoting ref. /378) Closing date: 24 July 1979.

2394(A)

INTERNATIONAL LABORATORY FOR RESEARCH ON ANIMAL DISEASES (ILRAD)
Nairobi, Kenya

IMMUNOLOGIST TO WORK ON PARASITIC DISEASES

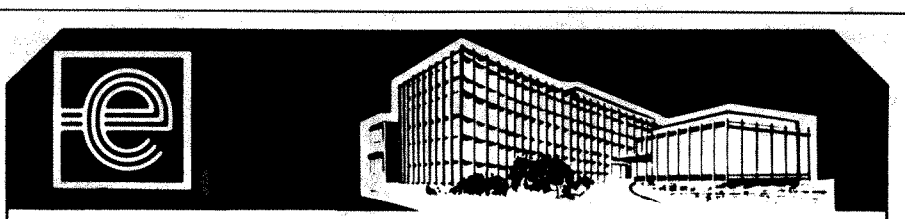
There are in the laboratory of the Director of ILRAD two vacancies for immunologists to work on theileriosis. The research will include the infection and transformation of bovine lymphocytes and other cell types by theileria parasites and analysis of cell-mediated immune responses to the parasites and parasitized cells.

Applicants should have experience of cellular immunology and of parasitic or other infectious diseases.

Salary will depend on age and experience. Perquisites include moving allowance, housing and commutation allowances, medical and retirement benefits.

Applications, with *curriculum vitae*, list of publications and the names of three referees, should be submitted to the Director, ILRAD, PO Box 30709, Nairobi, Kenya by 12th August 1979.

W203(A)



RESEARCH OFFICER

CHEMISTRY DIVISION – CORROSION RESEARCH

A scientist with PhD or good honours degree and relevant experience is required for research on corrosion problems in power plant. The successful applicant will work with other scientists concerned with a wide range of corrosion problems involving ferrous and non-ferrous metals in aqueous and high temperature environments. He or she will be expected to contribute to fundamental understanding of corrosion mechanisms and to advise on corrosion problems in plant. Excellent facilities are available for corrosion measurements and the examination of corroded specimens.

According to age, experience and qualifications the appointment will be made within incremental salary ranges rising to £6035 or £7050 plus a payment under a Self Financing Productivity Scheme ranging between £8 and £17 per month.

The Laboratories are situated in a pleasant part of Surrey and offer attractive conditions of service, and facilities for the total of 800 Research and Support Staff engaged in a broad spectrum of research into the materials, technologies, and plant performance problems of the Central Electricity Generating Board.

Application forms are available from the Head of Personnel Development & Services Section, Central Electricity Research Laboratories, Kelvin Avenue, Leatherhead, Surrey KT22 7SE or telephone Leatherhead 74488 Ext 363, quoting reference number **RL/39/NS.** Closing date is: **Friday 20 July 1979.** 2457(A)

CENTRAL ELECTRICITY RESEARCH LABORATORIES

INSTITUTE OF CANCER RESEARCH: Applications invited for appointment as a TECHNICIAN in the Physics Department, Royal Marsden Hospital, Sutton, Surrey. Work includes the dosimetry and planning of radiation treatments, with linear accelerators and other equipment, and involves the use of a digital computer system. Previous experience not required. For applicants with a degree in Physics or allied science, appointment on scale £3,261 to £4,680 p.a. (under review) plus London Allowance of £354 p.a., starting salary depending on age and experience. Applications in duplicate (within two weeks of advertisement) giving full details of education and qualifications and with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 301/B/77. For further details telephone Dr. N. G. Trott, 01-642 6011, Ext. 250. 2379(A)

A RESEARCH ASSISTANT as a staff member is required by the Faculty of Medicine of Memorial University of Newfoundland. The incumbent must have academic training at the masters level or equivalent with experience to enable him to participate in research on human physiological circadian rhythms. The incumbent will be responsible for the modification of research procedures and computer analyses. There will be some field work. This is an established post and carries a commencing salary of $13,292. Applications or enquiries should be directed to Dr. Mary Lobban, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, A1B 3V6. W201(A)

BREWING RESEARCH FOUNDATION
NUTFIELD, SURREY

A biochemist or microbial physiologist is required to study the regulation of the enzymic synthesis of ethyl acetate by Saccharomyces cerevisiae.

Experience of lipid metabolism and with membrane bound enzymes would be helpful. The post seems likely to suit a graduate with several years' relevant experience but would possibly be suitable for a new graduate with in-course experience on the appropriate topics.

The appointment will be made on either the scientist (£4979-£6882) or experimental officer (£4486-£6198) scales, in the Microbiology Section.

Applications, including curriculum vitae and names of two referees should be sent to:—

Assistant Director, Brewing Research Foundation, Lyttel Hall, Nutfield, REDHILL, Surrey, RH1 4HY.

2448(A)

ROYAL FREE HOSPITAL

POST DOCTORAL BIOCHEMIST

A position is available immediately to join a group working on the interactions of blood coagulation factors and platelets. The work is financed by the M.R.C. and involves the purification of factors viii, ix and x from normal and abnormal blood, leading to a detailed study of the interaction of these factors with each other and with platelets. An individual with experience in protein biochemistry and/or haemostasis would be preferred. The appointment is for three years in the first instance.

Salary from £5,342 to £5,817. Send curriculum vitae and names of three referees to Doctor E. G. Tuddenham, Haemophilia Centre, Royal Free Hospital, Pond Street, London NW3 2QG. Tel: 01-794 0500 ext. 3806. 2429(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the post of post-doctoral Senior Research Assistant in the Department of Biochemistry. The post is funded by a grant from the North West Cancer Research Fund to study the hormone responsiveness of a line of human breast cancer cells. Experience of tissue culture would be an advantage.

The initial salary will be within the range £4,232 to £5,321 per annum (Senior Research Assistant Ia). The appointment will be for one year in the first instance.

Applications, together with the names of two referees, should be received not later than 27th July, 1979, by The Registrar, The University, Liverpool, L69 3BX. Quote Ref RV/682. 2419(A)

UNIVERSITY OF OXFORD

INORGANIC CHEMISTRY LABORATORY

Appointment of

POSTDOCTORAL RESEARCH ASSISTANT

with a Chemistry, Materials Science, Physics Degree.

A postdoctoral Research Assistant required for research on the developmer of new solid electrolytes and solid-solutio electrodes for secondary batteries of hig specific energy and power. The post is fc one year with good prospects for contra renewal for upwards to 5 years. Experienc in solid state synthesis and x-ray structur characterization is highly desired; the wor also involves electrochemical characteriz tion. Starting salary in range £4,232 t £4,776.

Applications, including names of tw referees, should be sent to Professor Joh B. Goodenough, Inorganic Chemistr Laboratory, South Parks Road, Oxfor OX1 3QR. 2400(A)

THE AUSTRALIAN NATIONAL UNIVERSITY

PhD SCHOLARSHIP IN BIOCHEMISTRY

The Australian National University invite applications for a PhD Scholarship i Biochemistry funded by the Australia Meat Research Committee under th supervision of Dr. K. C. Reed, to study th biochemical aspects of primary sexu differentiation. The main area of work i anticipated to be the development an application of hybridoma cell lines whic secrete antibody directed specificall against the testis-differentiating H antigen. Applicants should have a goo honours degree (or equivalent) in Lif Sciences, preferably with theoretical an practical experience in one or more of th following techniques; molecular genetics immunology and tissue culture. Th scholarship provides a living allowance c $A4,200 per annum plus additiona allowances for dependants and incidenta expenses. (Current exchange rate i $A1 = 53p). Assistance with the cost o travel within Australia to take up th scholarship will be provided. Furthe details and application forms are availabl from Dr. K. C. Reed, Department o Biochemistry, Faculty of Science, Th Australian National University, Pos Office Box 4, Canberra ACT 2600 Australia. Closing date: 31 July 1979.

2439(A)

LAMBETH SOUTHWARK & LEWISHAM AREA HEALTH AUTHORITY (T)

GUY'S HOSPITAL

Come and work at our modern Denta Hospital, in Guy's Tower — as a

MEDICAL LABORATORY SCIENTIFIC OFFICER

In the Department of Oral Immunolog and Microbiology. This post is combination of Clinical Diagnosti Microbiology, Serology and Associate Research.

We are looking for an enthusiasti person who has the AIMLT and preferabl at least 2 years experience in routin Clinical Microbiology.

Salary is on the scale of £3,261 to £4,68 plus £354 London Weighting.

Please telephone or write to Th Personnel Department on 01-407 7600 Ext 3462 at Guy's Hospital, St Thomas Street London SE1 9RT. 2385(A)

UNIVERSITY OF EDINBURGH

CENTRE FOR TROPICAL VETERINARY MEDICINE

LECTURER IN TROPICAL ANIMAL PRODUCTION

Applications are invited for the post of Lecturer in Tropical Animal Production. The appointment will be for two years.

The successful candidate will contribute to the M.Sc./Diploma course in Tropical Animal Production and Health run jointly by the Centre for Tropical Veterinary Medicine and the School of Agriculture.

The person appointed would become a member of staff of the Department of Tropical Animal Health. Preference will be given to graduates with experience of animal production in the tropics and specialist knowledge of an aspect of cattle production would be an advantage.

The salary offered would be in the range £3,689 to £8,452 (£3,775 to £8,992 from 1st October 1979).

Applications should be sent to the Director, Centre for Tropical Veterinary Medicine, Easter Bush, Roslin, from whom further particulars can be obtained. Closing date 16th July. Please quote reference 1065.

2405(A)

MEDICAL RESEARCH COUNCIL VIROLOGY UNIT

ENZYMOLOGIST PROTEIN BIOCHEMIST and NUCLEIC ACID BIOCHEMIST

wo vacancies exist for postdoctoral search scientists with experience in nzymology/protein biochemistry or ucleic acid biochemistry to work on a riety of molecular biological problems volving Herpes Simplex Virus and other ammalian virus systems.

Previous experience with virus systems is lvantageous but not essential.

The appointment will be for three years. lary on an appropriate point on Grade 2 the non-clinical scientific staff scale. pplicants should send a Curriculum vitae ving full details of qualifications and perience and the names and addresses of ree professional referees to: Professor J. . Subak-Sharpe, Institute of Virology, niversity of Glasgow, Church Street, LASGOW, G11 5JR, by 1st August, '79. 2454(A)

PROFESSOR OF ASTRONOMY University of Victoria Victoria B.C. Canada

he Department of Physics at the niversity of Victoria anticipates the tablishment of an endowed position for a stinguished astronomer at the Full ofessor rank to begin on or after July 1, '80. Candidates should possess an tensive research record and have an ternational reputation. They should be lling to interact with faculty, research rsonnel and students at the University d with others in the community of tronomers in Victoria. Preference will be en to an observational astronomer with oretical interests. In addition to the ailability of research facilities at the iversity and at the nearby Dominion trophysical Observatory, opportunities ll exist for observing time on the 3.6m nada-France-Hawaii telescope which is w nearing completion in Hawaii.

Expressions of interest, enquiries and plications are invited. Please reply to: airman, Department of Physics, iversity of Victoria, Victoria, B.C. V8W 2, Canada.

The closing date for receipt of plications is January 1, 1980.

W204(A)

Experimental Support Staff

Neutron Scattering Instrumentation

A new intense source of pulsed neutron beams, the spallation neutron source, is being built at the Rutherford Laboratory for use in research programmes of UK universities and polytechnics. A substantial number of neutron scattering instruments will be provided based on the time-of-flight method and outline designs have already been completed for the first seven. A wide range of equipment and techniques is involved; for example, position sensitive detectors for epithermal neutrons; high resolution choppers; sample environments controlling parameters such as temperature, pressure, magnetic field; neutron guides; neutron polarisation systems.

There are vacancies for support staff to participate in the development and provision of this equipment in the Neutron Beam Research Programme, which is expanding to meet the requirements for efficient utilisation of the SNS. When fully operational, in the 1980's, the SNS will be a world class facility serving a very widely based scientific community. As part of their development activities those appointed will have opportunities to work with research scientists using instruments at Harwell and the Institut Laue-Langevin, Grenoble. In addition, close collaboration is maintained with laboratories in the USA and elsewhere where spallation sources are being built. Applications should have HNC or HND and relevant experience or degree in physics or applied physics. Some experience in fields such as electronics, high vacuum, or cryogenics would be an advantage. Appointments will be made to permanent positions in the Science Group at the level of Scientific Officer or Higher Scientific Officer. Salary scales (at present under review).

Scientific Officer £3,037-£4,724.
Higher Scientific Officer £4,388-£5,820.
Please apply to
Jane Griffiths in the Personnel Group on Abingdon (0235) 21900 Ext. 510 or write to her quoting Reference No. VN. 818.
Closing date for applications, July 20th 1979.

RUTHERFORD Science Research Council
Rutherford Laboratory, Chilton, Didcot, Oxfordshire OX11 0QX. Tel Abingdon 21900

2452(A)

CHEMICAL NEWS and CURRENT AWARENESS MAGAZINE requires ASSISTANT EDITOR

to edit contributed scientific and professional articles and to write original material. Some experience of magazine editorial work desirable and acquaintance with Chemistry would be helpful. Please write giving full details to the Personnel Officer, The Chemical Society, 30, Russell Square, London WC1B 5DT. 2399(A)

UNIVERSITY OF OXFORD

DEPARTMENT OF METALLURGY AND SCIENCE OF MATERIALS

Two research appointments to study interfacial segregation and precipitation reactions during liquid-phase sintering and subsequent heat-treatment of tungsten-based alloys are available from 1st October, 1979.

The posts may be filled at either postdoctoral level (for 2 years) or predoctoral level (for 3 years) and applications are invited from suitably qualified candidates who have recently completed their D.Phil. or who wish to register for a D.Phil. at the university.

Applications, including a curriculum vitae, and the names and addresses of two referees, should reach the Administrator, Department of Metallurgy and Science of Materials, University of Oxford, Parks Road, Oxford OX1 3PH, not later than the 23rd July, 1979. 2451(A)

Pharmacologist
Gastroenterology

Smith Kline & French Laboratories Limited is the UK subsidiary of the international SmithKline Corporation whose interests include the research and manufacture of pharmaceuticals.

The Research Institute presently operates in Welwyn Garden City, but over the next few years it will be relocating a few miles away to newly-built laboratories in a rural setting.

An opportunity has arisen in our Pharmacology Department for a Graduate Pharmacologist/Physiologist with experience in the field of gastroenterology. This position involves heading a small group conducting basic research into the Gastrointestinal function.

It is envisaged that the successful man or woman will have recently obtained a PhD in a relevant area of research. Experience in the electrophysiology of the gastrointestinal tract would be advantageous. Benefits include free life assurance, discretionary annual bonus, four weeks annual holiday, subsidised cafeteria and a growing Sports and Social Club. Assistance with relocation is available where necessary.

To apply please telephone or write for an Application Form quoting Reference Number NAT 142 to:

Kate Viscardi, Recruitment Administration Officer,

SMITH KLINE &FRENCH LABORATORIES LIMITED

Mundells, Welwyn Garden City, Hertfordshire, AL7 1EY. Telephone: Welwyn Garden 25111, Extension 26.

1978 1979

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2455(A)

INTERNATIONAL LABORATORY FOR RESEARCH ON ANIMAL DISEASES (ILRAD)
Nairobi, Kenya

IMMUNOLOGIST TO WORK ON PARASITIC DISEASES

There are in the laboratory of the Director of ILRAD two vacancies for immunologists to work on theileriosis. The research will include the infection and transformation of bovine lymphocytes and other cell types by theileria parasites and analysis of cell-mediated immune responses to the parasites and parasitized cells.

Applicants should have experience of cellular immunology and of parasitic or other infectious diseases.

Salary will depend on age and experience. Perquisites include moving allowance, housing and commutation allowances, medical and retirement benefits.

Applications, with *curriculum vitae*, list of publications and the names of three referees, should be submitted to the Director, ILRAD, PO Box 30709, Nairobi, Kenya by 12th August, 1979.

W210(A)

AUSTRALIA
VICTORIAN PUBLIC SERVICE

DEPARTMENT OF AGRICULTURE

Applications are invited from suitably qualified and experienced persons for appointment to the following position:

DIRECTOR
(PASTORAL RESEARCH INSTITUTE, HAMILTON)
$A 23,718

Duties: To administer and lead the Institute; to be responsible for the overall development and direction of research programmes; to undertake personal research and to advise management on research initiatives and general developments in the Institute's area of work.

Qualifications: A degree in an appropriate branch of Science or approved equivalent with appropriate post graduate training to PhD level or approved equivalent; proven research ability and achievement and capacity to administer the research organization.

Note: 1. Hamilton is situated in the Western District of Victoria, approximately 291km from Melbourne.
2. An official residence is provided for the successful applicant, if required, and a rental of 6 per cent of standard salary, plus $234 a year, will be charged.

The successful applicant will, subject to passing a prescribed medical examination, be appointed as a permanent officer of the Victorian Public Service. Appointment will be as Class "SO-11", Second Division. Conditions of service include four weeks annual recreation leave with holiday pay loading, cumulative sick leave, three months long service leave on completion of ten years continuous service and a comprehensive contributory superannuation scheme.

Written applications, quoting Position No A 05/05/1307/3 and stating personal particulars, qualifications and experience, must reach the Secretary, Public Service Board, 1 Treasury Place, Melbourne, 3002, by no later than 9.30am on Wednesday, 8th August, 1979. W208(A)

UNIVERSITY COLLEGE LONDON
HUMAN SCIENCE LECTURESHIP

Application invited for a lectureship in Human Science in the Department of Anthropology. Tenable from 1st October 1979 or later by arrangement. The lecturer will have responsibilities for teaching in the multi-departmental Human Science BSc. Preference will be given to candidates with a background in Biology, Psychology or Geography with interests which would reinforce the inter-disciplinary nature of the degree; for example evolutionary aspects of reproductive and social strategies, genetical and environmental aspects of human behaviour, human population studies especially in an ecological context.

Applicants should have completed post graduate studies for a Higher Degree in these or related fields. The post will be situated in the Department of the Anthropology and the lecturer's duties determined in consultation with the Head of the Anthropology Department and the Chairman of the Human Science Committee. It will be filled at a salary according to the experience and age of the successful applicant.

Applications together with names of two referees and full curriculum vitae to Assistant Secretary (Personnel) University College London, Gower St, London WC1E 6BT. 2369(A)

UNIVERSITY OF MANCHESTER
DEPARTMENT OF MEDICAL BIOCHEMISTRY
POST-DOCTORAL RESEARCH ASSOCIATE

Applications invited for this post (availat up to three years from October) to wo with Dr. C. A. Shuttleworth and Dr. M. Grant on "The Role of Non-Collageno proteins in the Periodontal Ligaments Some experience in the fields of connecti tissue biochemistry, protein chemistı tissue culture or embryogenesis would an advantage. Starting salary in the ran £4,333 to £4,910 p.a. Superannuatio Applications (three copies) includi curriculum vitae and the names of tv referees should be sent to Dr. C. Shuttleworth, Department of Medic Biochemistry, University of Manchest Medical School, Manchester M13 9PT soon as possible. 2375(A)

FORINTEK CANADA CORPORATION
WESTERN FOREST PRODUCTS LABORATORY

Wood Biodeterioration and Protection a PROFESSIONAL MYCOLOGIST required immediately to join a high qualified research team in Vancouv Canada. Applicants should have a Ph. plus several years experience with woo inhabiting fungi. A Knowledge of dec problems in buildings would be desirab A demonstrated ability to do appli research and to communicate both to i dustry and the general public will be quired. Salary negotiable from 25,0 dollars, depending on qualifications a experience. Applications, with two ref ences, to Dr R. S. Smith, Forint Canada Corp., Western Forest Produ Laboratory, 6620 N.W. Marine Driv Vancouver, B.C., Canada V6T 1X2. W199(

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

POSTDOCTORAL RESEARCH FELLOW IMMUNOLOGY/ BIOCHEMISTRY

Applications are invited for a post-docto Research Fellow in a group involved in t biochemical and immunologic characterisation of the cell surface cultured human pancreatic and otl cancer cells. The appointment is for thı years with the possibility of extension anc supported by a grant from the Can Research Campaign. Salary in Univers Scale IA. Applications to Dr. D. Gra lecturer in Biochemistry, Department Surgery, St. George's Hospital Medi School, Cranmer Terrace, London SW 0RE, (01-672 1255 Ext. 4717) from wh further particulars may be obtained. 2414(A)

NATIONAL VEGETABLE RESEARCH STATION
ASSISTANT NEMATOLOGIST

A SCIENTIFIC OFFICER is required assist with work on the nematode pests vegetables.

The appointment will be in the grade Scientific Officer salary scale £2,839 p annum rising by annual increments £4,415. (Salary review 1 April 19 pending).

Minimum qualifications required is degree or equivalent in Biological Scien and experience in Nematology would be advantage.

Non-contributory employers' additior pension scheme.

Full particulars and application form (be returned by 18 July 1979) from t Secretary, National Vegetable Resear Station, Wellesbourne, Warwick CV 9EF. 2316(A)

UNIVERSITY OF STRATHCLYDE

Applications are invited from Honours graduates in PHARMACY or other appropriate disciplines for a

LECTURESHIP in PHARMACEUTICAL CHEMISTRY/FORENSIC SCIENCE.

Applicants should have a good general knowledge of the chemistry, properties and fate of natural and synthetic drugs in regard to their abuse and/or legitimate use. The appointee should be prepared to participate in teaching in courses in both disciplines, but will be primarily associated with one or other.

Salary scale from 1st October 1979 £4,333 to £8,992 with placing according to qualifications and experience. Superannuation benefit.

Application forms and further particulars (quoting 33/79) and enclosing a self-addressed envelope (9" × 4") may be obtained from the Academic Appointments Officer, University of Strathclyde, Royal College Building, 204 George Street, Glasgow, G1 1XW with whom applications should be lodged by 14th July 1979.

2422(A)

SUNDERLAND POLYTECHNIC

FACULTY OF SCIENCE
DEPARTMENT OF BIOLOGY

RESEARCH ASSISTANTS (3 Posts)

Applications are invited for the ollowing posts: to work with Dr J. A. ooke on the dynamics of fluorine in ontaminated ecosystems (joint project ith the University of Liverpool); with Dr B. Mitchell on the anthelminthic tivity of eosinophils in fishes and nphibians; and with Mr A. Peat on the trastructual aspects of differentiation in okaryotic organisms.

Salary for this post which is tenable for o years from 1st September, 1979, with possible extension up to three years is ,778 to £3,054 per annum (under view).

Applicants, who will be expected to gister for a higher degree of the CNAA, ould have a good honours degree or an uivalent qualification. Students taking gree examinations in 1979 may apply.

An application form and further details ay be obtained from the Personnel fficer, Sunderland Polytechnic, ester Road, Sunderland, SR1 3SD, and ould be returned as soon as possible.

2445(A)

MEDICAL RESEARCH COUNCIL

Clinical and Population Cytogenetics Unit,
Western General Hospital,
Crewe Road, Edinburgh.

SCIENTIST

he Medical Research Council has a acancy at the above Unit for a Scientist to ork in the Experimental Studies Section n the genetics of human lymphoid cells in ulture.

Emphasis will be placed on studies of mmunoglobulin production and on chniques for the separation of bpopulations of lymphoid cells. The ccessful applicant will collaborate with embers of the pattern-recognition section the above Unit in the development and plication of a fluorescence activated cell rter in relation to these studies.

It is anticipated that the appointment ill be a 3 year temporary one, suitable for recent PhD graduate with relevant perience.

Salary will be on the scale £4,232 to ,627.

Applications containing full curriculum tae and the names of two referees should sent to the Administrative Officer at the ove address before 16th July, 1979.

2423(A)

KOMT 4 ALLES M·N TERG

Martinus Nijhoff Publishers bv

Lange Voorhout 9 - The Hague

Martinus Nijhoff Publishers specialise in the publication of English-language books of academic standard for the international market.

Martinus Nijhoff Publishers B.V. has an immediate opening for a full-time experienced

DESK EDITOR

The job involves the preparation of manuscripts (both books and journals) for printing, the correction of proofs, and communication with authors and editors on points of publishing practice. The successful applicants will work as members of a small team.
Candidates should have an excellent grasp of the English language and appreciation of good English style. Knowledge of additional languages is preferred. A meticulous eye for details is also essential.
Preference will be given to applicants with experience in applied sciences and/or medical desk editing.

As a subsidiary of the Kluwer Academic Publishers Group, the group's excellent fringe benefits will apply to the post, in addition to a competitive salary, dependent on the applicant's qualifications.

Letters of application with full C.V., to:
The Personnel Department Martinus Nijhoff Publishers B.V.
P.O. Box 269 . 2501 AX The Hague . The Netherlands
Telephone 070-469460

Martinus Nijhoff Publishers bv is a division of the Kluwer Academic Publishers Group

W205(A)

ROTHAMSTED EXPERIMENTAL STATION

Harpenden, Herts. AL5 2JQ

The Soil Survey of England and Wales South-East Regional Office, Alice Holt, Hampshire, requires a scientist to map soils, prepare soil and land use capability maps for publication, and write explanatory texts describing soils, their distribution and their relationship to agriculture.

Qualifications: 1st or upper 2nd class honours degree in Chemistry, Geography, Geology, or Soil, Environmental or Agricultural Science.

Appointment in grade of Scientific Officer (£2,839-£4,415). (Pay Award pending). Non-contributory superannuation.

Apply in writing to the Secretary giving names and addresses of two referees and quoting Ref. 400 by 27th July. Further particulars on request. 2449(A)

AGRICULTURAL RESEARCH COUNCIL

INSTITUTE OF ANIMAL PHYSIOLOGY
Babraham, Cambridge CB2 4AT

SCIENTIFIC OFFICER

required in the Endocrine Laboratory of the Department of Applied Biology. The research involves biochemical analytical methods; experience with such techniques and an interest in electronics would be an advantage. Applicants should have a pass degree, HNC or equivalent in a relevant biological science. Salary in scale which is under review, £2,839 to £4,415 per annum, according to qualifications and relevant experience. Non-contributory superannuation scheme. Application form and further details may be obtained from the Secretary of the Institute. Quote Ref. D.R.B. 2.

Closing date July 19th 1979.

2446(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL (University of London) Denmark Hill, London, SE5 8RX

DEPARTMENT OF OBSTETRICS & GYNAECOLOGY

Applications are invited from graduates or suitably qualified people for the post of Research Technician on a project involving tissue culture and cytogenetics and some histological and histochemical techniques in the area of pre-natal diagnosis of inherited diseases.

Starting salary £3,669 inclusive (under review).

For informal enquiries please contact Dr. J. Singer on 01-274 6222 extension 2349. Applications in writing giving full details of qualifications and experience should be sent to the Secretary of the Medical School at the above address by 19 July 1979. 2413(A)

Division of Hormones

Research Officer

Required to work on a project as part of a team engaged on studies on the mechanism of action and secretion of hormones; for studies on the mechanism of coupling between hormone receptors and adenylate cyclase systems in cell membranes, using cell fusion techniques.

A knowledge of general techniques used in biochemical endocrinology, with particular reference to isolated cell and membrane preparations, adenylate cyclase-cyclic AMP assays, tissue culture and/or cell fusion methods would be an advantage. Applicants should have or expect to gain this year a first degree in an appropriate subject.

Salary in accordance with age and experience on the scale £3,715 to £5,259 (under review) including London weighting plus superannuation benefits.

Please write or telephone for an application form and further details quoting reference SU/HO/290 to the Personnel Officer, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB Telephone: 01-435 2232, ext. 200.

NIBSC

National Institute for Biological Standards and Control

2384(A)

CHELSEA HOSPITAL FOR WOMEN DOVEHOUSE STREET, LONDON SW3

BASIC GRADE BIOCHEMIST required to

work at Chelsea Hospital for Women (acute gynaecology). The Successful applicant will be mainly concerned with investigations of disorders of fertility. The Hospital is situated in a pleasant part of Chelsea within easy reach of tube and bus routes. Whitley Council conditions of service. Further details and application forms from the Administrative Assistant at Queen Charlottes Hospital. Tel: 01-748 4666 ext. 204.

2401(A)

RESEARCH OFFICER

LABORATORY ANIMALS CENTRE

The Director's research programme is primarily concerned with an evaluation of the advantages which accrue from the use in research of specific pathogen free animals, in particular the cat. A person in the Research Officer category is required to assist the Director in this programme. Opportunities will occur for collaborative work with other departments in the Centre, which cover the whole spectrum of Laboratory animal science.
The successful applicant will have a degree or equivalent in a biological science, and at least two years relevant research experience. Salary will be related to age, qualification and experience and is supplemented by £354 London Weighting. Contributory pension scheme.
Applications, together with a copy of curriculum vitae and the names of two professional referees, should be addressed to The Director, MRC Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey, SM5 4EF.

MRC Medical Research Council

2417(A)

BIOCHEMISTS — SWEDEN

Pharmacia Fine Chemicals AB manufacturers of Sephadex[R] and other products for biochemical separations require two biochemists to work at our head office in Uppsala, Sweden.
The successful applicant for the first post will join the Scientific Services group of our Export Department, which is responsible for communicating technical information to research workers using chromatography electrophoresis and other biochemical separation methods. This work includes:
- Developing, organising and taking part in courses and symposia
- Scientific information
- Planning and participation in exhibitions

Applicants for this post should have research experience in biochemistry, cell biology or a closely related field and should be prepared to travel for approximately three months per year, throughout the world.

The second successful applicant will join our Scientific and Technical Services group responsible for chromatographic separation methods. The group's activities include:
- The preparation of all kinds of scientific information material
- Work with new products
- Lecturing

but applicants should be prepared for extremely varied work in close co-operation with our research departments and a certain amount of travelling, chiefly within Europe.
Applicants for this position should have research experience in biochemistry or a closely related area.
For both of these positions a good knowledge of a major European language is advantageous but clear and accurate expression in the written word with English as the mother tongue, is essential.
These are responsible positions and carry appropriate salaries. Preferred age is between 25 and 30.
Write in confidence to:

**Mr. David S. Sweetman
Pharmacia (GB) Limited,
Prince Regent Road,
Hounslow, Middlesex
TW3 1NE, England.
Or telephone, during office hours, (01) 572 7321.**

Pharmacia

2416(A)

UNIVERSITY OF REGINA
(Canada)

ASSISTANT PROFESSOR
DEVELOPMENTAL
BIOLOGY

Applications are invited from candidates who have training and experience in the biology of development at the molecular, cellular and organismic level. Expertise in plant development and regeneration from somatic cells is desirable, but not essential. Applicants must have at least two years of postdoctoral experience.
The appointee will share in the teaching of undergraduate and graduate students and is expected to develop an active research programme. Minimum salary: $18,829. Starting date: as soon as possible.
Applications including a curriculum vitae, list of publications, a statement of research interests, and the names of at least three referees should be sent to Dr. H. Bertrand, Department of Biology, University of Regina, Regina, Saskatchewan S4S 0A2, Canada before August 20, 1979. W207A

THE UNIVERSITY
OF SHEFFIELD

Postgraduate School of Librarianship and Information Science

LECTURESHIP IN
INFORMATION SCIENCE

Applications are invited from men a women for the above post. Candidat should have a good honours degree science or technology and a qualification substantial experience in informatic science; they should also present eviden of experience in research. Teaching duti will involve various aspects of compute based bibliographic information system computer programming, and statistic methods as applied to library ar information work. The School has wi research interests among which a research in computer-based informatic retrieval systems, including those involvi chemical structure information. Initi salary in the range £4,333 to £5,199 a ye on scale rising to £8,992 a year. Particula from the Registrar and Secretary, t University, Sheffield S10 2TN to who applications (5 copies) should be sent by August 1979. Quote ref: R334/G.

2420(A)

To place your advertisement in these pages

Phone: 01-831-6901

Geophysics Research

We are looking for graduate scientists, preferably with a Ph.D., to join our Geophysics Research Branch. This is an important opportunity to join in leading research and specialised service work in all aspects of seismic prospecting except instrument development.

Applicants will have a strong mathematics background and be skilled computer users. We need people who study problems from the viewpoint of an earth scientist rather than that of a mathematician. You will be experienced in using large quantities of experimental results to validate hypothetical models and experience related to seismic prospecting will be an advantage.

Our current salary levels, together with a non-contributory pension scheme, subsidised lunches and excellent sports and social facilities now represent outstanding conditions of employment.

Please write giving brief details of age, qualifications and experience quoting reference U.670, to:

Sue Tickle,
Central Recruitment,
The British Petroleum Company Limited,
Britannic House,
Moor Lane,
London EC2Y 9BU.

2458(A)

Process Technology

Antibiotic Fermentation

Merseyside

Dista Products — a leading company in the antibiotics field and a subsidiary of The Eli Lilly International Corporation — invites applications from candidates — for 2 new appointments:

Fermentation Technologist

Where the main objective will be to advise on methods of maintaining and improving productivity and yields through the application of biological expertise in the optimisation of critical process parameters for various fermentation processes.

Candidates for this appointment will have as a minimum a good Honours Degree in one of the Biological Sciences together with specialised knowledge of fermentation processes gained in either an academic or industrial environment. Experience in the use of computer process control techniques would be an advantage.

Process Technologist/ Biochemical Engineer

The main objective will be to increase operating efficiency through the modification and development of new and existing process plant. Candidates will have as a minimum a good Honours Degree in Chemical Engineering with additional experience in: Biochemical Engineering, the recovery of active compounds from fermentation processes together with the application of modern process control techniques.

An excellent salary — commensurate with level of experience is offered for both these challenging opportunities together with BUPA, non-contributory pension scheme and relocation expenses.

PER Professional & Executive Recruitment

If you are interested in these appointments telephone Howell Williams on 051-227 4111 Ext 551 or write to PER, 3rd Floor, Graeme House, Derby Square, Liverpool L2 7SP.

Applications are welcome from both men and women

2408(A)

ROTHAMSTED EXPERIMENTAL STATION

Harpenden, Herts. AL5 2JQ

DATA BANK OFFICER

to assemble and maintain an automated bank of data recorded by field and laboratory staff of the Soil Survey of England and Wales, and to be responsible for computing resources assigned to the Survey.

Qualifications: Degree in a science discipline or HNC with computer programming experience for appointment as SO. Degree or HNC with at least 5 years' computer programming experience for HSO.

Appointment in grade of Scientific Officer £2,839-£4,415, or Higher Scientific Officer £4,101-£5,448. (Pay Award pending). Non-contributory superannuation.

Apply in writing to the Secretary giving names and addresses of two referees and quoting Ref. 401 by 27th July. Further particulars on request. 2450(A)

UMIST

PAPER SCIENCE SECTION

Prof. H. W. Kropholler

Post-Graduate Research

The Paper Science Section undertakes a variety of research into physical chemical and technological aspects of paper and its manufacture. Current topics include the use of automatic image analysis for studying cellulose fibres, drying and pressing studies, microprocessor applications, pigment coating, waste paper recycling, hemicellulose chemistry and environmental studies.

A variety of grants, up to £3,000 p.a., are available for suitably qualified graduates, and the work can be submitted for a higher degree. Please write for further information and application form to Dr J. C. Roberts, Paper Science Building, UMIST, P.O. Box 88, Manchester M60 1QD. 2447(A)

STUDENTSHIPS and FELLOWSHIPS

UNIVERSITY OF BRISTOL

DEPARTMENT OF CLINICAL BACTERIOLOGY

RESEARCH STUDENTSHIP

Applications are invited from graduates with a first or upper second class honours degree in a biological science for a research studentship sponsored by Janssen Pharmaceutical Ltd, tenable from 1st October 1979. The investigation is concerned with the development of methods for the differentiation of strains of the pathogenic fungus *Candida albicans* and their application to different epidemiological problems, and will be carried out under the supervision of Dr D. W. Warnock. The successful applicant will be expected to register for a PhD degree.

Applications, together with the names of two referees, should be sent as soon as possible to Professor D. C. E. Speller, Bristol Royal Infirmary, Bristol BS2 8HW, from whom further information may be obtained. 2402F

UNIVERSITY OF KENT AT CANTERBURY

BIOLOGICAL LABORATORY

PROTOZOOLOGY RESEARCH STUDENTSHIPS

Applications are invited from candidates holding a good Honours degree in Biochemistry or a related subject for the following research studentships:

(1) an SRC CASE Studentship in collaboration with Pfizer Inc., Sandwich, Kent and jointly supervised by Drs. R. B. Freedman and W. E. Gutteridge to work on ribosome structure and function in *Trichomonas vaginalis*;

(2) a University Studentship, supervised by Dr. W. E. Gutteridge, to work on ribonucleotide reductase in *Trypanosoma cruzi*.

Applications should be submitted to the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH by 16 July 1979. Please quote Ref: PG20/79. 2387(F)

LIVERPOOL POLYTECHNIC
DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY

The following research posts are available in the Department for suitably qualified candidates to undertake research work towards a PhD degree in October, 1979.

SRC RESEARCH STUDENTSHIP
Five positions are available.
Candidates should have or expect to obtain a First or Upper Second Class Honours degree, or equivalent, in a relevant discipline.
SRC salary rates apply. For CASE awards the collaborating body can also provide up to £735 per annum without reduction in the SRC contribution.

Penetration of Soluble Herbicides into Plants
Drs. R. J. Hamilton and P. A. Sewell (Liverpool Polytechnic) and Dr. T. Merrall (Shell Limited).
CASE Award with Shell Research Limited, Sittingbourne, Kent. The work will involve cultivation of plants, studies of their surface constituents and the development of an assay for water soluble herbicides.
Informal enquiries can be made to Drs. R. J. Hamilton or P. A. Sewell, Tel. 050-207 3581, Extension 31/146.

Insect Attractants and Repellants
Drs. R. J. Hamilton (Liverpool Polytechnic) and Dr. E. A. Bernays (London)
CASE Award with the London Centre for Overseas Post Research. To study the constituents in the *Sorghum* species which attract and repel stem-boring insects. The work will include extraction of plant chemicals, their fractionation using TL, GC, and HPL chromatography, and their identification by ir, nmr, and ms. An interest in developing bioassays with insects is needed.
Technical enquiries should be made to Dr. R. J. Hamilton. Tel: 051-207 3581, Extension 31.

Bacterial Degradation of Steroids
Dr. R. F. Bilton (Liverpool Polytechnic) and Dr. A. Atkinson (Porton Down).
CASE Award with CAMR (PHLS) Porton Down (formerly MRE).
The project will involve a study of the enzymology and genetics involved in the degradation of steroids by bacteria.
Informal enquiries can be made to Dr. R. F. Bilton, Tel: 051-207 3581, Extension 26, or Dr. A. Atkinson (Porton), Tel. 0980-610291.

Studies of Potential Polyhalogenoaromatic Pesticides
Dr. C. A. Heaton. The project will consist of a study of the chemistry and biological (ie pesticidal activity) or polyhalogenodinitrobenzenes and related compounds.
Informal enquiries to Dr. C. A. Heaton, Tel. 051-207 3581, Extension 117.

Glow Discharge Electrolysis (gde)
Drs. P. A. Owens and A. Wood.
In gde an electric discharge is struck, under reduced pressure, between an electrode and the surface of a solution. Charged particles formed in the discharge initiate reactions analogous to those found in radiolysis. The study will involve the elucidation of the mechanism and kinetics of the process. The project will embrace vacuum techniques, electronics and a wide range of analytical methods.
Further information may be obtained from Drs Owen and Wood, Tel. 051-207 3581, Extensions 28/31.

LOCAL EDUCATION AUTHORITY RESEARCH ASSISTANTSHIPS
Two posts are available. Candidates should have or expect to obtain a good Honours degree or its equivalent in Chemistry, Biochemistry or Biology.
Salary: £2,823 to £3,009 per annum.

Metal Oxide Chemistry
(Drs J. Fenerty and J. Pearce)
This project entails the preparation and characterisation of novel metal oxide-hydroxide systems of catalytic importance. Thermal methods of analysis (DTA, TGA) and X-ray powder diffraction will be used extensively in the investigation.
Technical details from Drs. Fenerty and Pearce, Tel. 051-207 3581, Exts. 145/28.

Gut Biochemistry
(Dr. A. N. Mason)
Studies of the effects of dietary fibre on bacterial transformations implicated in diseases of the human intestine.
Informal enquiries to Dr. A. N. Mason, Tel. 051-207 3581, Ext. 144.
Applications, with curriculum vitae, and the names and addresses of two referees, should be sent to the appropriate supervisors named above. The address for all correspondence is—:

Department of Chemistry and Biochemistry
Liverpool Polytechnic
Byrom Street
Liverpool
L33AF **2441(F)**

THE UNIVERSITY OF LEEDS

DEPARTMENT OF BIOCHEMISTRY
(in association with the Department of Dermatology, Leeds General Infirmary)

SRC (CASE) RESEARCH STUDENTSHIP

Applications are invited from students having a good honours degree in Biochemistry or a related subject for a three-year research studentship awarded by SRC available from 1 October 1979.

The proposed project is a study of normal and abnormal keratinisation in relation to various skin diseases (principally acne and psoriasis) and will involve the purification and characterisation of epidermal proteins and a study of the synthesis in normal and diseased skin. The value and conditions of the award will be those of SRC studentships. The successful candidate will be required to register for a PhD.

Applications giving curriculum vitae and the names and addresses of two referees, should be sent to Professor D S Robinson, Department of Biochemistry, (Telephone Leeds (0532) 36171). Closing date for applications 20 July 1979. 2421(F)

DEPARTMENT OF PHARMACOLOGY
INSTITUTE OF BASIC MEDICAL SCIENCES
ROYAL COLLEGE OF SURGEONS OF ENGLAND
Lincolns Inn Fields
London WC2A 3PN

Investigation of Inflammatory Mediators

Applications are invited from any discipline for a

POSTDOCTORAL RESEARCH FELLOWSHIP

to study chemical mediators of inflammation. The department has developed radio-isotopic techniques to measure plasma exudation and blood flow changes in animal models of inflammation. This is supported by other techniques such as radioimmunoassay, smooth muscle bioassay, isolated tissue perfusion and cell culture. Of special interest is the interaction between the prostaglandin system and the complement system. The position will be supported by a three-year MRC project grant awarded to Professor G. P. Lewis and Dr T. J. Williams. Salary on scale to £7,145 plus £502 London Allowance.

Enquiries and applications (two referees) as soon as possible to Mr W. F. Davis, Secretary, Institute of Basic Medical Sciences. 2411(E)

THE UNIVERSITY OF BIRMINGHAM
FACULTY OF MEDICINE AND DENTISTRY
DEPARTMENT OF IMMUNOLOGY (RHEUMATISM RESEARCH)

SRC CASE STUDENTSHIP

Applications are invited from graduates with a Hons. BSc degree (1st or 2 I) in Biochemistry or Biological Sciences for a 3 year research post starting on 1st October 1979, leading to a PhD degree. The project, in collaboration with the Boots Company Limited of Nottingham, will be concerned with the development and application of a rabbit model of arthritis.

Applications, together with the names of two referees, should be sent as soon as possible to Dr. D. R. Stanworth, Department of Immunology, University of Birmingham, Birmingham B15 2TJ. 2386(F)

UNIVERSITY OF STIRLING
BIOLOGY DEPARTMENT

SRC STUDENTSHIP IN ANIMAL OR PLANT BIOLOGY

tenable for 3 years leading to a PhD degree.

Applications giving two referees should be sent to Head of Department of Biology, Stirling University, Stirling, FK9 4LA as soon as possible. 2406(F)

UNIVERSITY OF ALBERTA
BIOCHEMISTRY DEPARTMENT

POST-DOCTORAL FELLOWSHIP

Apply to Dr A. R. Morgan, Biochemistry Department, University of Alberta, Edmonton, Alberta, T6G 2H Canada. Candidates must b knowledgeable in physico-chemical an metabolic aspects of DNA. The two mai areas in which the successful candidat would have a choice of doing researc would be multi-stranded nucleic acids an their possible biological roles (nucleoids chromosome condensation, tertiary structures) and DNA replication. Th position is available immediately. W213(E)

LANCHESTER POLYTECHNIC
At Coventry
FACULTY OF APPLIED SCIENCE
Department of Biological Sciences

NERC CASE RESEARCH STUDENTSHIP

The job: to work on the respirator physiology and energetics of Antarcti protozoa. The project will be jointl supervised by the Department of Biologica Sciences at the Polytechnic and the Lif Sciences Division of the British Antarcti Survey.

Required: applicants should have, o expect to obtain, a first or upper secon class honours degree in biological scienc and an interest in ecology, so microbiology or invertebrate zoology.

For application form and jo specification please apply in writin enclosing a large stamped and addresse envelope to the Personnel Officer Lanchester Polytechnic, Priory Stree Coventry. (Informal enquiries to Dr. H. G Smith, telephone (0203) 24166 Ext. 629 o to Dr. W. Block, British Antarctic Survey telephone Cambridge (0223) 61188 (Ext 256) returnable by Monday 16th July 1979. 2382(F)

THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS
Hallamshire Hospital, Sheffield

SRC CASE STUDENTSHIP

Applications are invited from men an women graduates with a good honour degree for an SRC CASE Studentship to b held in the above Department i conjunction with Reckitt and Colman Lt commencing in October 1979. The projec will involve an investigation of the factor affecting the systemic availability of som drugs administered via the oral route Suitable for individuals interested in th fields of drug metabolism and kinetics Applications should be sent to Professo H. F. Woods or Dr. G. T. Tucker at th Department of Therapeutics, The Hallamshire Hospital, Sheffield S10 2JE by 16 July 1979. Quote ref: R 332/G. 2403(F)

ICI PHARMACEUTICALS DIVISION
Alderley Park Cheshire

Case Research Studentships

The Science Research Council is offering SRC (CASE) studentships, tenable from 1 October 1979, to persons who already hold, or expect to obtain a First or Upper Second Class Degree. Applications are invited for the following projects which will lead to the degree of PhD:

The synthesis of stable analogues of thromboxane A_2	Prof. J.K. Sutherland, Chemistry Department, University of Manchester.
Metalloporphyrins as diamagnetic shift reagents	Dr. R.J. Abraham, Department of Organic Chemistry, University of Liverpool.
Organoiron complexes in organic synthesis	Dr. A.J. Pearson, University Chemical Laboratory, University of Cambridge.
Isolation and characterisation of drug conjugates	Dr. J. Caldwell, Department of Biochemical and Experimental Pharmacology, St. Mary's Medical School, London.
Alkylborane — amine complexes as reducing agents	Prof. A. Pelter, Department of Chemistry, University College, Swansea.
Routes to hydroxy 3-and 4-amino acids	Dr. C.M.C. Drey, School of Chemistry, Robert Gordon's Institute of Technology, Aberdeen.
Physiological and biochemical mechanisms in the development of pulmonary interstitial fibrosis	Dr. M. Griffin, Department of Biochemistry, Trent Polytechnic, Nottingham.
Aspects of gastric mucosal protection	Dr. A. Allen, Department of Physiology, The Medical School, University of Newcastle-upon-Tyne.
The effects of 5-hydroxytryptamine (5-HT) agonists and antagonists on brain 5-HT metabolism and release	Dr. C.A. Marsden, Department of Physiology and Pharmacology, University of Nottingham Medical School.
5-hydroxytryptamine receptors in cortical neurones	Dr. E. Szabadi, Department of Psychiatry, University Hospital of South Manchester.

The successful applicants will carry out their research under the principal direction of the Academic Supervisor indicated. In addition they will spend a minimum of 3 months during the tenure of the studentship working in ICI Pharmaceuticals Division laboratories at Alderley Park.

Applications, indicating the particular project of interest, and accompanied by a brief curriculum vitae and the names of two referees, should be sent as soon as possible either to the named Academic Supervisor or to:

Dr. N.F. Elmore, Research Collaborations Coordinator, Imperial Chemical Industries Limited, Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG. Tel: (0625) 582828.

2410(F)

THE INTERNATIONAL INSTITUTE OF TROPICAL AGRICULTURE

PMB 5320, OYO ROAD, IBADAN, NIGERIA

POSTDOCTORAL FELLOWSHIPS

The International Institute of Tropical Agriculture, one of ten institutes supported through the Consultative Group for International Agricultural Research, conducts research to improve the quantity and quality of the principal food crops of the humid and sub-humid tropics, such as cowpeas and other grain legumes, rice and maize and the root crops of the tropics, cassava, yam, sweet potato and cocoyam. Research is also conducted on the farming systems of the humid tropics and covers the areas of soils, economics, mechanisation and storage.

IITA awards as many as fifteen postdoctoral fellowships yearly to selected persons who have newly-acquired degrees of the level of the degree, Doctor of Philosophy. Through these fellowships, the Institute seeks to increase and strengthen the body of agricultural scientists with experience in dealing with problems of food crop production in the world's humid and sub-humid tropics.

Postdoctoral fellows are salaried and receive such benefits as subsidised housing, the use of a small car, medical and accident insurance, sick leave accrual and vacation leave accrual of two working days per month of service. Travel is provided for the fellow and his family from the place of residence to the Institute and return. Fellowships are for an initial period of one year with the possibility of extending for a second year subject to the results of a mutual review conducted around the eighth month of the fellowship. Because of housing considerations, the Institute is not able to award fellowships to applicants with families of more than one very small child.

GRAIN LEGUME IMPROVEMENT PROGRAM

Crop Physiology

Qualification desired: Recent PhD in Crop Physiology.

Research: To develop and validate in co-operation with program scientists from collaborating institutions a heuristic model to simulate the growth and yield of Cowpeas (*Vigna unguiculata*) in relation to variations in the environment.

The model will be based on the extensive data available from field and controlled environment studies conducted over the past five or six years. The objective will be to elucidate further the interactions between crop response and environment; to provide additional understanding of how the information now available can be used most effectively towards crop improvement; an equally important aim will be to highlight areas where further investigation is required.

Plant Breeding

Qualifications desired: Recent PhD in Plant Breeding.

Research: 1. Plan and implement a program to develop grain type cowpea (*Vigna unguiculata*) cultivars adapted for use in intercrop and relay crop systems with maize and cassava, suitable for different ecological regions of West Africa.

2. Develop a program for selection and testing of green-pod vegetable cowpea cultivars for use in the more humid environments.

CEREALS IMPROVEMENT PROGRAM

Entomology

Qualifications desired: Recent PhD in Entomology with strong background in crop pest resistance research. A knowledge of insect ecology and plant breeding would be an advantage.

Research: Integrated team research leading to development of resistance in maize and rice to their major African insect pests most of which are species of stem borers.

TUBER AND ROOT IMPROVEMENT PROGRAM

Sweet Potato Breeding

Qualifications desired: Recent PhD in Plant Breeding. Experience in breeding root crops will be an added advantage.

Research: Breeding and selection for drought tolerance, weevil and virus resistance, good storability and high yield.

Agronomy/Physiology

Qualifications desired: Recent PhD in Agronomy with strong background in Crop Physiology.

Research: Development of improved technological packages suitable for small farmer adoption for such crops as cassava, sweet potato, yams and cocoyams.

Entomology (two positions)

1. **Qualifications desired:** Recent PhD in Entomology with strong background in Host Plant Resistance. Experience with biological control of insect pests would be an advantage.

Research: Screening for host plant resistance in the field. Some laboratory work required. The place of principal residence will be Tanzania.

2. **Qualifications desired:** Recent PhD in Entomology with strong background in biological control of insect pests. A knowledge of spoken and written French would be an advantage.

Research: Biological control of insect pests of tropical root crops. Field and laboratory work requiring artificial rearing of insects, release and recapturing. The place of principal residence will be Zaire.

Pathology

Qualifications desired: Recent PhD in Plant Pathology with strong background in diseases of plants caused by fungus pathogens. Some background in the physiology of parasitism would be an advantage.

Research: Investigations on the Cassava (*Manihot esculenta*) Anthracnose Disease caused by *Colletotrichum spp.*, including the development of a screening program for host plant resistance to the disease.

FARMING SYSTEMS PROGRAM

Soil Fertility

Qualifications desired: Recent PhD in Soil Science with strong background in Soil Fertility and Analytical Chemistry.

Research: The effects of various methods of crop residue management and tillage systems on fertiliser use efficiency in the humid and sub-humid tropics.

Soil Physics

Qualifications desired: Recent PhD in Soil Physics, Agricultural Engineering (Water Management) or Hydrology.

Research: 1. Root growth and development in relation to soil profile characteristics and tillage systems.

2. Computer stimulation of hydrological balance over an agricultural watershed in the tropics. A strong background in hydrology (Agricultural Engineering) and capability of writing computer programs required for this research.

2444(E)

Athrofa Gogledd-dd Cymru
The North E Wales Institute
of higher education

KELSTERTON COLLEGE, CONNAH'S QUAY, DEESIDE, CLWYD

SRC CASE STUDENTSHIPS

Applications are invited for two SRC CASE Studentships, tenable from 1st October, 1979 for work on the following projects:

1. "Mechanism of lipid oxidation in aqueous emulsions", in collaboration with Unigate Ltd. Supervisor: Dr. J. C. Allen.
2. "Radioimmunoassay of polyamines", in collaboration with Ortho Diagnostics, Ltd. Supervisors: Drs. J. C. Allen and C. J. Smith.

The co-operating bodies will supplement the SRC Studentships by £250 p.a. Applicants should possess or expect to obtain 1st or upper 2nd class Honours, and should write as soon as possible, with curriculum vitae, to the Institute Registrar, North E Wales Institute, Kelsterton College, Connah's Quay, Deeside, Clwyd. CH5 4BR.

2426(F)

UNIVERSITY OF STRATHCLYDE

Applications are invited for

TWO POSTDOCTORAL RESEARCH FELLOWSHIPS IN POLYMER SCIENCE

in the

DEPARTMENT OF PURE AND APPLIED CHEMISTRY

to examine the synthesis and physical properties of materials encapsulated in various polymer matrices. One post will be concerned with the investigation of the suspension polymerisation of various monomers in the presence of encapsulation materials. The other post will deal with the investigation of the electrical properties of these and related materials.

Applicants should have either a background in synthetic chemistry, or polymer science or experience in dielectric or related physical measurements.

The appointments will be from 1st October 1979 and will be on Range IA of the national salary structure for research and analogous staff; Commencing salary within the Range £4,333 to £5,488 dependant upon age and experience.

The appointments are for one year with probable extension to three years.

Applications accompanied by a Curriculum Vitae and the names of two referees (quoting R25/79) should be sent to Dr. R. A. Pethrick, Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow, G1 1XL.

2389(E)

UNIVERSITY OF WARWICK

SRC (CASE) Chemistry Research Studentship New Cationic Photopolymerisation Initiators

Applications are invited for a three year SRC (CASE) Research Studentship in the Department of Chemistry and Molecular Sciences, starting 1st October, 1979, and supervised by Dr. T. J. Kemp in collaboration with Ozalid (UK) Ltd.

The project involves development of new compounds releasing initiating centres for polymerisation on photoirradiation in an image-wise manner. Applicants should hold, or expect to hold, at least an upper second class honours degree in chemistry. Further details may be obtained from Dr. Kemp, Department of Chemistry and Molecular Sciences. Applications, with the names of two referees, should be sent as soon as possible to the Academic Registrar, University of Warwick, Coventry CV4 7AL. Please quote Ref. No. PG/42/79.

2435(F)

UNIVERSITY OF LONDON KING'S COLLEGE

DEPARTMENT OF GEOLOGY

NERC CASE STUDENTSHIP

Applications are invited for a NERC CASE Studentship for research into the mineralogical properties of clays which are of importance in the development of colour in fired building products.

The successful applicant will spend two months each year at the Redland Technology Laboratories at Horsham in Sussex — additional payments will be made for this.

Applicants should have a good honours degree in Geology/Mineralogy/Ceramics or a related discipline.

Further information may be obtained from Professor J. E. Prentice, Dept of Geology, King's College, Strand, London WC2R 2LS, to whom applications should be sent as soon as possible.

2370(F)

MIDHURST MEDICAL RESEARCH INSTITUTE

West Sussex GU29 0BL

Applications are invited for a C.A.S.E. studentship, to work on the Histrochemistry and Biochemistry of Atherosclerosis.

The work can lead to a higher degree, will be carried out at the Institute, and the May and Baker Laboratories.

Applications should be addressed to Dr R. Butcher at the above address. 2415(F)

UNIVERSITY OF NEWCASTLE UPON TYNE

MRC STUDENTSHIP IN GENETICS

Applications are invited for an MRC studentship on a project concerned with plasmid-coded restriction and modification systems. The work will involve both genetic and physical techniques in the study of bacterial plasmids.

Applicants who have an upper second or first class degree should apply *as soon as possible* to Professor S W Glover, Department of Genetics, Ridley Building, Claremont Place, The University, Newcastle upon Tyne NE1 7RU. 2395(F)

Commonwealth of Australia

"QUEEN'S FELLOWSHIPS IN MARINE SCIENCE"

To commemorate the Royal visit to Australia in 1970 the Australian Government established the Queen's Fellowships Scheme. Under this scheme up to five fellowships may be awarded each year for full-time research by young scientists of exceptional promise and proved capacity for original work. These awards are tenable at an Australian university or approved research institution normally for two years. Tenure of a Fellowship is expected to commence within nine months of the announcement of the award.

QUALIFICATIONS: Queen's Fellows should have a Ph.D., or equivalent qualifications, or equivalent research or professional experience, in a discipline applicable in marine science such as coastal engineering. Awards will, in general, be restricted to applicants who are not more than 30 years of age on the date when applications close.

STIPEND: $17131 (Australian) per annum – increased to $18403 per annum at age 28 years.

ALLOWANCES: Allowances are payable in respect of a Fellow's dependent spouse ($500 p.a.), each dependent child ($200), superannuation payments (up to 10 per cent of stipend), appropriate insurance coverage and necessary travel expenses.

APPLICATIONS: Persons interested in applying for the above fellowships should obtain application forms and a statement of the conditions of award from the Secretary, Queen's Fellowships Committee, Department of Science and the Environment, P.O. Box 449, Woden. A.C.T. 2606 Australia; The Minister (Scientific), Canberra House, 10–16 Maltravers Street, London WC2R 3EH; or the Australian Embassy, 1601 Massachusetts Avenue, Washington D.C. 20036, U.S.A. Applications for the next round of awards, which will be announced in December 1979, close at the Canberra address on 17 August 1979.

2383(E)

UNIVERSITY OF GLASGOW

CHEMISTRY DEPARTMENT AND ICI ORGANICS DIVISION

Applications are invited for an SRC CASE Research Studentship tenable from October 1st, 1979, to study the synthesis and applications of polycyclic organo-sulphur compounds. Applicants, who must hold, or expect to obtain by October, a First or Upper Second Class Honours Degree (or equivalent qualification) in chemistry, should send a curriculum vitae and the names of two referees to Dr P. H. McCabe, Department of Chemistry, University of Glasgow, Glasgow, G12 8QQ. 2424(F)

UNIVERISTY OF NOTTINGHAM

FACULTY OF AGRICULTURAL SCIENCE

SRC (CASE) RESEARCH STUDENTSHIP

Applications are invited for a Research Studentship to investigate the relationship between the structure and the texture and appearance of a new type of food gelling agent. The project is in conjunction with a leading food manufacturer and will be aimed at obtaining increased understanding of this novel material. The successful candidate will be required to adopt a multidisciplinary approach and work in close collaboration with the Company concerned.

The successful applicant will be expected to register for the degree of PhD. Candidates holding a degree in Chemistry, Physics, Food Science or Biochemistry should apply to Dr. J. R. Mitchell, Department of Applied Biochemistry & Nutrition, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD, enclosing a brief curriculum vitae. 2372(F)

UNIVERSITY OF TORONTO CLIMATE-RELATED RESEARCH

Applications are invited for two post doctoral fellowships at the University of Toronto. One position is for a climate modeller interested in the CO_2 problem; the other is for a social scientist interested in the impact of climate change on society. Application deadline: 1 August, 1979. Salary range and conditions of employment are those of the Canadian NRC. Apply to Prof. F. K. Hare, Director, Institute for Environmental Studies, The University of Toronto, Toronto, M5S 1A4, Canada. W202(E)

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF NEUROLOGY

A Postgraduate Research Studentship is available for three years from October 1st, 1979 at the Regional Neurological Centre. The work will be concerned with the enzymology of protein breakdown in muscle. There is a large unit studying various aspects of muscle diseases at the Regional Neurological Centre. Applicants should have or expect to obtain a good Honours degree with biochemistry or chemistry as a major subject, and the successful candidate will register for a PhD degree. Remuneration will be the same as that of postgraduate students supported by the Medical Research Council.

Applications, including the names and addresses of two referees should be sent, as soon as possible to Dr R. J. T. Pennington, Neurochemistry Department, Regional Neurological Centre, General Hospital, Newcastle upon Tyne NE4 6BE. (0632-738811.) 2343(F)

INSTITUTE MARINE BIOCHEMISTRY

Applications are invited from recent Graduates in Biochemistry with at least an upper 2nd class honours degree for a NERC Research studentship. The work will be concerned with the normal Biochemical mechanisms — minimise the peroxidation of membrane lipids in wild and cultured fish and with the effects of peroxidized lipids in the diets of cultured fish.

It is envisaged that the work will form part of the requirements for the degree PhD from the University of Aberdeen.

Apply with curriculum vitae including the names of two academic referees to:

The Director
Institute of Marine Biochemistry
St Fitticks Road
Aberdeen AB1 3RA.

By Monday 23rd July, 1979. 2431(F)

BRUNEL UNIVERSITY

DEPARTMENT OF PHYSICS

Applications are invited from scientists and engineers for a

RESEARCH STUDENTSHIP

in the lively and friendly electro-optics group of this department. The studentship is funded by the S.R.C. for a period of three years, leading to the PhD degree. It is a CASE studentship, in collaboration with Unilever Research Ltd, for the study of the electro-optical properties of

LYOTROPIC LIQUID CRYSTALS

Applicants should be British citizens and have a first class or upper second class degree. Please contact Professor B. R. Jennings, Electro-Optics Group, Physics Department, Brunel University, Uxbridge, Middlesex UB8 3PH. Telephone Uxbridge 37188. 2433(F)

LINCOLN COLLEGE
(University College of Agriculture)
New Zealand

POST-DOCTORAL FELLOWSHIP

The Council of Lincoln College invites applications for a Post-Doctoral Fellowship, tenable in any of the following Departments:
Agricultural Economics and Marketing; Agricultural Engineering; Agricultural Microbiology; Animal Science; Biochemistry; Entomology; Farm Management and Rural valuation; Horticulture, Landscape & Parks; Plant Science; Range Management; Soil Science; Veterinary Science; Wool Science.

The object of the award is to contribute to the research programme at Lincoln College. In addition to carrying out research, the successful applicant will be expected to participate in limited teaching activities without further emolument.

The Fellowship will have a total value of NZ$10,000, part of which will be available for fares. Tenure will be for one year in the first instance, but may be extended at the discretion of Council.

Conditions of Appointment may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications for the Fellowship close on 15 August 1979. 2437(E)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF MICROBIOLOGY

RESEARCH FELLOWSHIP

Applications are invited for a three-year MRC-financed research fellowship. Salary will be on the RF IA scale £4,333 to £7,521 (to operate from 1st October 1979) plus superannuation. Starting salary will be up to £5,488 according to age and previous (not necessarily related) postdoctoral experience.

The project is to study the chemical changes in virus-infected cells both *in vitro* and *in vivo* by X-ray microanalysis of frozen, freeze-dried unstained sections of cells. Observed changes will be correlated with virus-induced changes in patterns of polypeptide synthesis analysed by conventional techniques. The work will be carried out in two laboratories under the supervision of Dr. J. Stephen (Department of Microbiology, Birmingham University) and Dr. T. A. Appleton (Department of Physiology, Cambridge University). Full provision has been made for travel between the two institutions and stays of short duration in Cambridge.

Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham, P O Box 363, Birmingham B15 2TT, to whom applications (three copies) including full curriculum vitae and naming three referees, should be sent by Friday 27 July, 1979. Please quote ref NM6. 2397(E)

THE UNIVERSITY OF MANCHESTER

A research studentship is available for candidates holding or expecting to gain a good honours degree in a Biological Science (including Pharmacology and Immunology) for an investigation of experimental arthritis in primates and other mammals. The programme, under the direction of Professor D. L. Gardner, is intended to provide the opportunity to study for a PhD of this University. Applications and enquiries should be addressed to Professor D. L. Gardner, Department of Pathology, University of Manchester, Stopford Building, Oxford Road, Manchester, M13 9PT. 2377(F)

UNIVERSITY OF NOTTINGHAM
MEDICAL SCHOOL

RESEARCH STUDENTSHIP IN MICROBIOLOGY

Applications are invited for a Research Studentship in Microbiology leading to the degree of PhD for work on the continuous monitoring of the action of antibiotics on dense bacterial cultures.

Applications with the names and addresses of two referees to Professor F. O'Grady, Department of Microbiology, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH. 2374(F)

UNIVERSITY COLLEGE LONDON
DEPARTMENT OF BIOCHEMISTRY

Applications are invited for an SRC CASE Studentship for three years from 1 October 1979 under the supervison of Dr M. A. Rosemeyer in collaboration with G.D. Searle and Co. Ltd. (High Wycombe). The project is to develop agents to prevent polymerisation of haemoglobin in sickle-cell disease.

Applicants should have a good honours degree and will be expected to register for a PhD.

Further details can be obtained from Dr M. A. Rosemeyer, Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, to whom applications with curriculum vitae and the names of two academic referees should be sent as soon as possible. 2371(F)

THE UNIVERSITY OF MANCHESTER
DEPARTMENT OF BACTERIOLOGY AND VIROLOGY

The North West Water Authority is funding a three-year postgraduate studentship in microbiology which will be primarily concerned with the development of standard methods for the quantitative estimation of salmonellae strains in sewage sludge. Maintenance grant payable: around £2,000 p.a. The successful candidate will register for a higher degree, probably at MSc level for one year but renewable for a further two years for a PhD, subject to satisfactory academic progress. The award is tenable from October 1st, 1979. Particulars from Professor P. Collard, Department of Bacteriology and Virology, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT. 2380(F)

THE UNIVERSITY OF SHEFFIELD

SRC RESEARCH STUDENTSHIPS IN SPACE PHYSICS

Applications are invited from men and women with a class 1 or 2:1 Honours Degree in Physics, Engineering or Maths. The vacancies are in the field of Magnetospheric Physics involving ground based, rocket and satellite observations. Please reply immediately to Professor T. R. Kaiser, Department of Physics, the University, Sheffield S10 2TN giving full curriculum vitae and names of two referees. Further details may be obtained by telephoning 0742 78555 Ext. 4277. Quote ref: R335/G. 2427(F)

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LEARNED AND SCIENTIFIC Journals and Reference Works wanted. Walter H. Gardner Ltd., Booksellers, 74-80 Stamford Hill, LONDON N16 Tel: 01-806 1981. 2392(L)

ASSISTANTSHIPS

DEPARTMENT OF GEOLOGY
POST-DOCTORAL RESEARCH ASSISTANTSHIP

Applications from suitably qualified Geologists, Materials Scientists, Physicists or Chemists, are invited for a NERC-funded Research Assistantship to be filled at post-doctoral level. The position is with a group, directed by Dr. S. White and K. Brodie, studying the effects of deformation on metamorphic reactions and on mineral chemistry. The successful applicant will work on the micro-analysis, using TEM and STEM, of naturally deformed minerals. Particular attention will be paid to the development of metamorphic foliations during deformation. Previous micro-probe or TEM experience is required.

The appointment is tenable for three years and would start as soon as possible but preferably not later than January 1980. Salary in range £4762-£7647 inclusive according to age and experience.

Further particulars can be obtained from Dr. S. White, Department of Geology, Imperial College of Science and Technology, London SW7, to whom 2 copies of applications, giving a curriculum vitae and the names of two referees should be sent as soon as possible.

2456(P)

POSITIONS WANTED

MICROBIOLOGIST
(PhD, England 1978)/Pharm. D

Strong background in lipid biochemistry (discovery and characterisation of sphingalipids in bacteria, temperature effect on lipids), experience in fungal viruses, electron microscopy, centrifugation techniques, seeks research position in US or Canada. Box 2407(B) Nature 2407(B)

CONFERENCES

THE CITY UNIVERSITY
DEPARTMENT OF CHEMISTRY

POST-DOCTORAL RESEARCH ASSISTANTSHIP

Organic Chemists with interests in biological chemistry are invited to reply for a Post Doctoral Research Assistantship to work in collaboration with Dr J B Hobbs on the synthesis of novel nucleosides with potential antiviral activity. The appointment is tenable for one year from 1st October 1979. The salary will be £4,333 p.a.; (under review) + £502 p.a. London Allowance and USS Superannuation Benefits.

Applicants should submit a curriculum vitae and the names of 2 referees to Dr J. B. Hobbs, Department of Chemistry, The City University, Northampton Square, London EC1V 0HB, as soon as possible and not later than 31st July 1979. 2381(P)

Attachment

NATO ADVANCED RESEARCH INSTITUTE *IN VITRO* TOXICITY TESTING: CURRENT AND FUTURE POSSIBILITIES

Monte Carlo, September 22-28 inclusive, 1979

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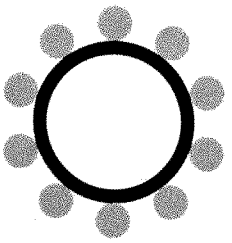
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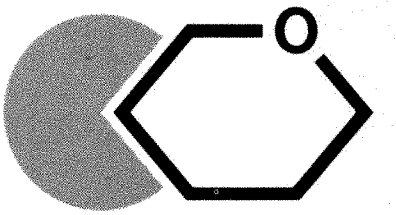
Applications should reach *A. Kolber, Chairman,* Organizing Committee, Research Triangle Institute, Research Triangle Park, North Carolina 27709, USA, before August 10, 1979. Please include a curriculum vitae, brief summary of applicant's background, current interests, and reasons for attendance. A small number of fellowships are available.

W209(C)

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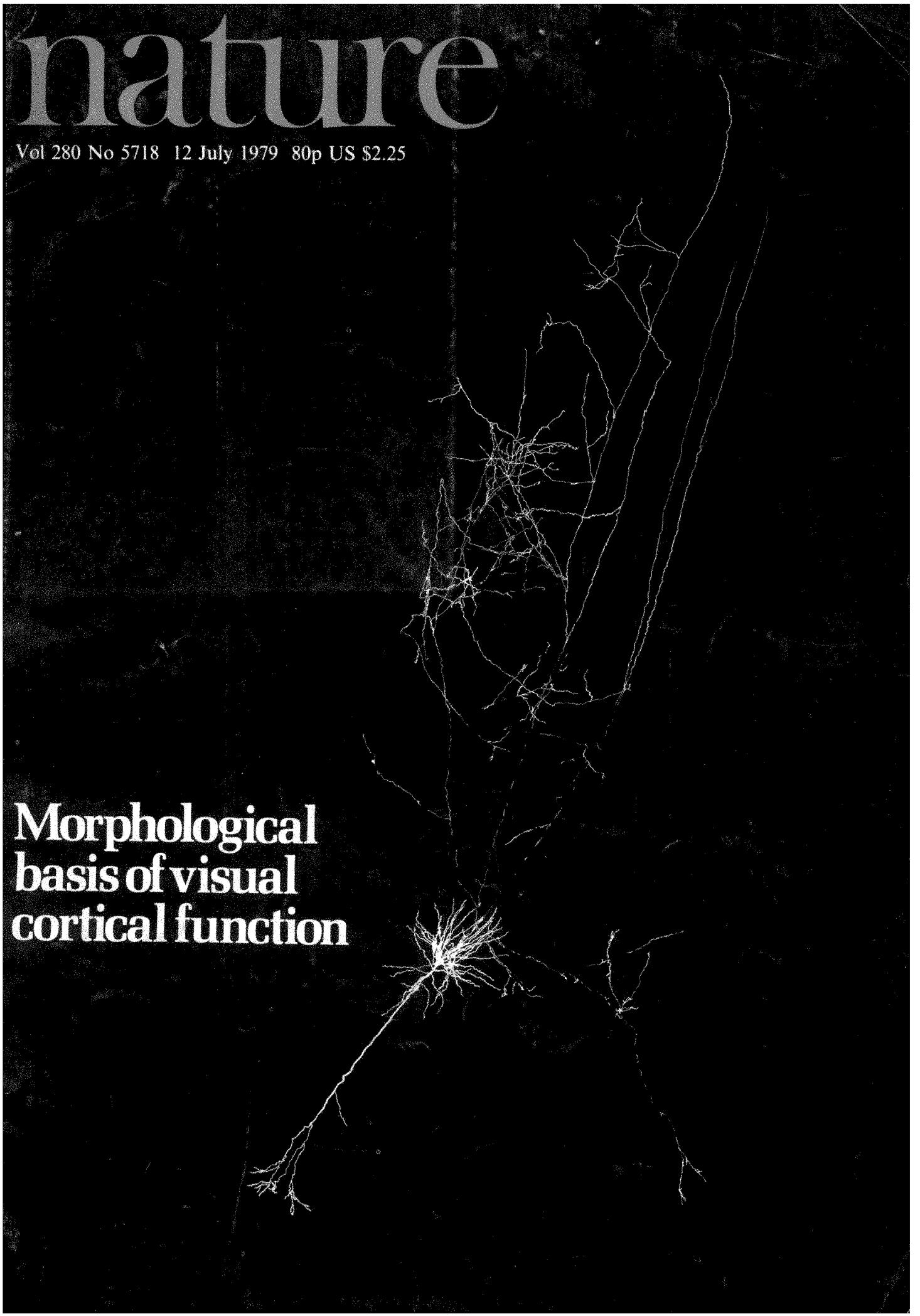
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Cover picture

The course of a pyramidal cell projection into the visual cortex. Gilbert and Wiesel have visualised functionally identified neurones in the cat visual cortex by making intracellular recordings with a micropipette filled with a histochemical marker. The marker was injected into the cells after their receptive fields had been characterised. See p.120.

Vol.280 No.5718 12 July 1979

nature

Volume 280 12 July 1979

The Skylab syndrome	93
UK research council hits back at 'horror lab' allegations	94
Polish science writers go too far	94
UNCSTD success still hangs in the balance	96
In brief	97
France puts science under the microscope	98
Will China be tainted by western science?	100
Mexico: fight against impossible odds	101
All at sea — Thomas H. Jukes	102

NEWS AND VIEWS

Peptides in profusion/Atmospheric arsenic/Anomalous antigens on tumour cells/ Glueballs without gullibility/Incest re-assessed/Polymers for blood replacement	103

REVIEW ARTICLE

Hormonal regulation of peptide receptors and target cell responses	*K.J. Catt, J.P. Harwood, G. Aguilera and M.L. Dufau*	109

ARTICLES

Accretion temperature of the Tieschitz, H3, chondritic meteorite	*R. Hutchinson, A.W.R. Bevan, S.O. Agrell and J.R. Ashworth*	116
Morphology and intracortical projections of functionally characterised neurones in the cat visual cortex	*C.D. Gilbert and T.N. Wiesel*	120

LETTERS

The 78.4-day period of Cygnus XR-1	*J.F. Dolan, P. Caraveo, M.J. Coe, C.J. Crannell, B.R. Dennis, K.J. Frost and L.E. Orwig*	126
Simultaneous effects of CO_2 and chlorofluoromethanes on stratospheric ozone	*K.S. Groves and A.F. Tuck*	127
Geochemical and geophysical evidence on the geothermal potential of Caledonian granites in Britain	*G.C. Brown, J. Plant and M.K. Lee*	129
Pliocene eustasy and the onset of sand barrier formation in Gippsland, Victoria	*A.N. Carter*	131
Holocene submergence of southern Long Island, New York	*M.R. Rampino*	132
Mössbauer spectral studies of the diagensis of iron in a sulphide-rich sediment core	*P.G. Manning, J.D.H. Williams M.N. Charlton, L.A. Ash and T. Birchall*	134
A new 14 Å mineral of the birnessite group in deep-sea micronodules	*F.V. Chukhrov, A.I. Gorshkov, A.V. Sivtsov and V.V. Berezovskaya*	136
New fossil finds from the Libyan Upper Neogene site of Sahabi	*N.T. Boaz, A. W. Gaziry and Ali el-El-Arnauti*	137
Insecticide-resistant *Myzus persicae as* as an example of evoluion by gene duplication	*A.L. Devonshire and R.M. Sawicki*	140
Nerve growth factor alters the fate of embryonic neuroblasts	*J.A. Kessler, P. Cochard and I.B. Black*	141
Appearance of H-W(H-Y) antigen in the gonads of oestradiol sex-reversed male chicken embryos	*U. Muller, M.T. Zenzes, U. Wolf, W. Engel and J.P. Weniger*	142
Preimplantation lethality of monosomy for mouse chromosome 19	*C.J. Epstein and B. Travis*	144

Guide to authors

● Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.

● Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.

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Selective suppression of IgE antibody responsiveness by maternal influence	*E. Jarrett and E. Hall*	145
T cells primed by influenza virion internal components can cooperate in the antibody response to haemagglutinin	*S.M. Russell and F.Y. Liew*	147
Use of cyclosporin A in allogeneic bone marrow transplantation in the rat	*P.J. Tutschka, W.E. Beschorner, A.C. Allison, W.H. Burns and G.W. Santos*	148
Degranulation capacity of cultured mast cells in the presence of histamine releaser	*H. Ginsburg, D. Ben-Shahar, I. Hammel and E. Ben-David*	151
Orderly compression of the retinotectal projection following partial tectal ablation in the newborn hamster	*B.L. Finlay, S.E. Schneps and G.E. Schneider*	153
A rich VIP nerve supply is characteristic of sphincters	*J. Alumets, J. Fahrenkrug, R. Håkanson, U. S. de Muckadell, F. Sundler and R. Uddman*	155
Increased sensitivity to (+)amphetamine self-administered by rats following meso-cortico-limbic dopamine neurone destruction	*M. Le Moal, L. Stinus and H. Simon*	156
Extracellular Ca^{2+} and excitation-contraction coupling	*W. Spiecker, W. Melzer and H.C. Lüttgau*	158
Parallel regulation of acetylcholinesterase and pseudocholinesterase in normal, denervated and dystrophic chicken skeletal muscle	*I. Silman, L. Di Giamberardino, J. Lyles, J.Y. Couraud and E.A. Barnard*	160
Human embryonic haemoglobins Gower 1 and Gower 2	*R.E. Gale, J.B. Clegg and E.R. Huehns*	162
K562 human leukaemic cells synthesise embryonic haemoglobin in response to haemin	*T.R. Rutherford, J.B. Clegg and D.J. Weatherall*	164
Induction of sporulation in *Bacillus brevis* by peptide antibiotics	*H. Ristow, W. Pschorn, J. Hansen and U. Winkel*	165
Bacillus megaterium resistance to cloxacillin accompanied by a compensatory change in penicillin binding proteins	*A.F. Giles and P.E. Reynolds*	167
Influence of leaves on sporophore production by fungi forming sheathing mycorrhizas with *Betula* spp.	F.T. Last, J. Pelham, *P.A. Mason and K. Ingleby*	168

MATTERS ARISING

Upper limits on production rate of NO per ion pair	*C.H. Jackman, H.S. Porter and J.E. Frederick*	170
Amount is not supply rate in energy intake control	*D.A. Booth*	170
	S.S. Alpert	171
Replies	*N.J. Rothwell and M.J. Stock*	170,172
Rothwell and Stock's paradox and multiple controls of feeding	*N. Mrosovsky*	172
Reply	*N.J. Rothwell and M.J. Stock*	172
Is the benzodiazepine receptor coupled to a chloride anion channel?	*J.M. Candy and I.L. Martin*	172
Reply	*D. Rodbard, T. Costa and C.B. Pert*	173
Another interpretation of communal breeding in green woodhoopoes	*J.L. Brown*	174
Reply	*J.D. Ligon and S.H. Ligon*	174

BOOK REVIEWS

Le Pouvoir et la Science en France (Pierre Papon)	*R.W. Cahn*	175
Biogeography and Adaptation: Patterns of Marine Life (G.J. Vermeij)	*R.C. Newell*	175
Radiation Effects in Semiconductors and Semiconductor Apparatuses (V.S. Vavilov and N.A. Ukhin)	*Andrew Holmes-Siedle*	176
Mechanical Behaviour of Ceramics (R.W. Davidge)	*J.E. Bailey*	176
Chlorophyll Organization and Energy Transfer in Photosynthesis (G. Wolstenholme and D.W. Fitzsomons, editors)	*Richard Cammack*	177
Electrical Properties of Polymers (A.R. Blythe)	*B.E. Read*	177
The Scientific Papers of C.V. Raman (Indian Academy of Sciences)	*G.R. Wilkinson*	178
Marine Production Mechanisms (M.J. Dunbar, editor)	*G.E. Fogg*	178

Announcements xix

nature 12 July 1979

The Skylab syndrome

There has been something deeply symbolic about Skylab's ungainly descent back down to earth. After all, it is almost ten years to the day since the US successfully carried out what many consider to represent the pinnacle of its scientific and technological achievement: placing a man on the surface of the moon. The deed did, indeed, turn out to be a turning point, but perhaps not in the way that those responsible for the lunar programme intended. For it took place on the eve of a decade in which mankind has turned its technological concerns away from the stars and back to its own planet, and to the more mundane, but no less complex, concerns such as health, energy and natural resources that have become the new prime targets for research. Skylab's demise comes on the eve of a new decade; and its message, which seems likely to become a dominant political theme in the years ahead, is that high technology frequently carries a built-in risk factor which, if not adequately planned for, will return to haunt us with its own particular form of revenge.

The whole incident could not have occurred at a worse time for the National Aeronautics and Space Administration. The agency's public image is currently being seriously tarnished by a set of technical problems, and related cost over-runs, in the space shuttle development programme. These problems have forced the agency, for the first time in many years, to go back to Congress cap in hand to request a substantial increase in funding over what had previously been agreed. With Congress already doing what it can to reduce expenditure across the whole federal budget, NASA's case has not been helped by an incident which links the agency directly to other areas of public concern over the dangers of high technology, in particular the Three Mile Island accident and the DC-10 aircrash. And although it seems likely that the extra money will be forthcoming — largely because of the military importance of space shuttle for launching satellites and carrying out surveillance tasks — other areas of NASA's activity may be made to suffer as a result.

At one level, both the problems with Skylab and the space shuttle can be attributed to the consequences of working with tight financial budgets. Skylab's designers saved $10 to $20 million by not adding engines that would have boosted the space station in its orbit when it began to slip back to earth, enabling it to be kept in orbit until it could be reached by space shuttle (the shuttle was initially expected to be launched this year, and Skylab to stay in orbit until 1983). And in the case of the shuttle itself, delays have been caused largely as a result of the development programme being planned according to a success-oriented strategy, with minimum back-up to allow for problems.

But it has not only been a question of money; there is also the question of control, and the associated attitude towards risk-taking. With space shuttle, the main contractor for the engine development has admitted to a certain laxity in overseeing the development of the programme, and has announced moves to remedy this situation by establishing close links between the technical and the financial staff. In Skylab's case that lack of adequate control, manifest in a more direct sense, has given rise to problems far too late for anything to be done.

The lesson of both would, in different ways, appear to be obvious: that in developing high technology with a potentially significant social impact, mechanisms to ensure adequate control of the technology should be developed at the same pace as the technology itself. The problem is that although this prognosis has logic on its side, it runs directly into conflict with the ideology of a system which rewards technological ingenuity rather than social responsibility. The market-place, rather than the political process, has been treated as the proper forum for weighing risks against benefits; and those who have introduced new technologies have, as a result, felt no responsibility for the way that purchasers choose to make use of them. Indeed a surfeit of regulation — society's attempt to control the various non-market impacts of technology — has been increasingly criticised in recent years for its impact on the innovation process; and pressures are growing both in the US and, in line with the new government's current ideology, in the UK to return to the market place in its purest form as the prime regulatory agency.

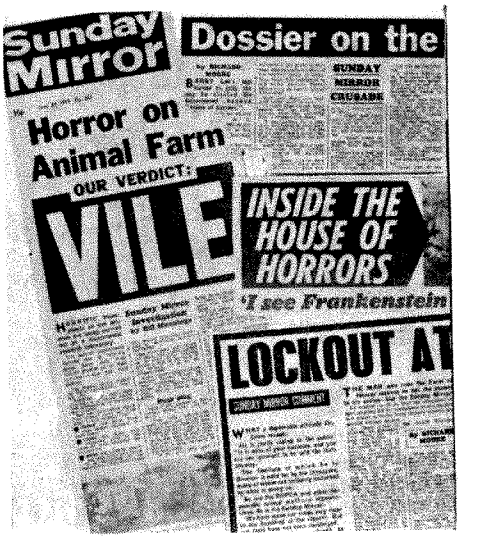
Such programmes are, as their supporters point out, intentionally risky ones; only, they would argue, by encouraging people to take risks — and subsequently to reward those whose risks pay off — can vitality be reintroduced into the economic system. But such an approach is also predicated on the basis that the losers will suffer; and the particular concern generated by high technology is that the economic gains generated by the risk-taking of some will be outweighed, not so much by the losses of unsuccessful risk takers, but by the broader impact of failure upon those excluded from the decision-making on the risk in the first place.

What has concerned people most about Skylab is not so much the fact of its re-entry as the virtual total lack of control over its descent. Just as in the case of the Three Mile Island incident, it was the involuntary aspect of exposure to radiation emitted from the plant, rather than the actual level of exposure (compared, for example, to normal background radiation), that generated the fiercest complaints. Equally control cannot be reduced simply to a managerial or technical function, but must be seen to reflect a broad sense of responsibility to the whole community.

It would be a tragedy if the dominant message left by the Skylab incident, following as closely as it does the other high technology accidents that have occurred this year, merely frightened people away from such technology on principle. However if what comes through is that the control of technology is as important as the technology itself, then the lesson will have been worth-while. Perhaps this is the most significant theme to have emerged from recent debates about the concept of "appropriate" technologies, both in developed and developing countries; perhaps, too, it is one that we can learn to put into action in the decade ahead. □

Nature Vol. 280 12 July 1979

UK research council hits back at 'horror lab' allegations



THE UK Agricultural Research Council last week held a press conference to present journalists with a defence of its use of animal experiments, after the *Sunday Mirror* had published 'shock' accounts (see right) of experiments at the ARC's principal laboratory, the Institute of Animal Physiology at Babraham near Cambridge. "Scientists transplant udders!" said the *Sunday Mirror*, and "Our verdict: vile".

But last week the director of the centre, Dr Barry Cross, countered that the story was "a statement about human reaction, not about animal cruelty". He questioned the credentials of the *Sunday Mirror*'s two witnesses of what went on at the institute. The first, Dr Cross said, "was a grade EW6 technician — the lowliest form of human life — no O-levels, no CSEs", and he was remembering 10 years after the event. The second "was a window cleaner or an asphalter who strayed from his proper job". To an expert, their reports "indicate pain and distress on the part of the unqualified observer".

Dr Cross's response was perhaps not calculated to enlist the sympathy of the journalists from the popular press (the *Star*, the *Express*, the *Sun*, the *Daily Mirror* and the *Sunday Mirror* were present); but the statement that followed was.

"I'm not here to defend cruel experiments on animals", Dr Cross said. "I was the first student president of the Union for Animal Welfare". Cruel experiments "would frustrate all our work — because we are studying healthy animals".

The experiments questioned by the *Sunday Mirror* involved udder transplants in goats and cows, to investigate secretions into the milk; permanent access to the animal's stomach, to investigate the digestion of the rumen; and an operation to bring a vein in the neck closer to the surface, to allow less painful injections to be made.

The ARC scientists have developed techniques, involving skilled surgery such as would "shame the National Health Service", which induces only mild post-operative pain while the wounds are healing, said Dr Cross. After that, the animals enjoy "a life of 5-10 years of cossetted living".

Stress levels in the animals are remarkably low. A researcher attempting to measure cortisol levels — a stress indicator — in Babraham cattle was shocked to discover amounts well below textbook levels. At first he thought that the Babraham cattle must be of a particularly phlegmatic strain, thus disqualifying many previous experiments, but then the cortisol shot up to the standard level when a noisy visitor entered the room. "Animals are more stressed on a farm than in our laboratory", said Dr Cross. "Our animals are the friendliest I know. They don't expect hostile people."

Moreover, said Dr Cross, referring to the case of a goat udder transplant, which appears to have caused most public reaction, a new-born kid actually prefers the transplanted teat, "and he must be considered unbiassed".

"We have never been refused a Home Office licence" said Dr Cross. (The Home Office controls animal experiments through the 1876 Cruelty to Animals Act.) "I've only been a director at Babraham for five years; previously I've worked in seven laboratories in the US, Scandinavia, and the UK, and I've visited many hundreds of others, and I have never seen a standard of care that surpasses or even equals Babraham's."

Dr Cross also defended the Babraham work on scientific, economic, and medical grounds. "One in seventeen women suffer from breast cancer", said Dr Cross, "but ruminants do not. Our experiments may give a clue to what predisposes women to this." Tranquillisers derived from experiments in the 1940s and 1950s on the effects of drugs on animal brains. The contraceptive pill based on hormones secreted by the ovary, a lot of work on which was done at Babraham. Furthermore, "there is a lot of evidence" that the brain is altered by sex hormones at a very early period, and there is a question of injurious effects from the mother's milk if the mother is on the pill. The basic work on the udder at Babraham may shed light on this, said Dr Cross.

More direct agricultural benefits have been: pregnancy tests, synchronisation of oestrus in herds of cows to save veterinary labour, the use of prostaglandin to induce pigs to give birth in daylight (which reduces mortality), the improvement of animal welfare in intensive production, a reduction in methane production in the digestion of cattle (so lowering energy costs), and the protection of plant protein in the rumen (so increasing the conversion efficiency of plant to animal food). "If you lock up Babraham, you lock up these advances", said Dr Cross.

It was also "a complete myth" that Babraham concealed its work. The institute has produced 2,700 scientific publications, and nine biennial reports — with photographs. Large numbers of the public have been admitted — 12 parties a month of students, farmers, foreign correspondents "and all sorts of layman" pass through. In the past 18 months there have been four visits from journalists; four TV teams; and three broadcasts.

Nevertheless Dr Cross refused to allow *Sunday Mirror* journalists, whom he called "propagandists", or photographers into the laboratory; but there was an agreement to allow an observer acceptable to both sides. Subsequently (8 July) the *Sunday Mirror* commented: "He is really saying to the public: 'It's none of your business, and you are too ignorant to be told the facts anyway' ".

Meanwhile, Jean Pink, organiser of the anti-vivisection group 'Animal Aid', has claimed that the *Sunday Mirror* is prepared to give further press coverage to the "diabolical tests", and will photograph demonstrators outside the Institute on Saturday.

Robert Walgate

Polish science writers go too far

Last month the Polish media celebrated the first anniversary of the flight of Cosmonaut Miraslaw Hermaszewski. Ironically, their special features coincided with a growing disillusion among Polish scientists not so much with the space programme itself but with what they see as its propaganda exploitation — notably in the case of the controversial 'Syrena' results.

'Syrena' was a Polish-designed experiment, carried out aboard the Salyut-6 space station, in order to obtain semi-conductor triple crystals of mercury, cadmium, and tellurium using a gradual cooling method and of mercury, cadmium and selenium, using gas phase sublimation. In order to maintain controlled conditions of weightlessness, the production of the crystals, using the Soviet-built 'Splav' furnace, was carried out during the cosmonauts' rest period, lest undue motion disturb the space-craft.

Nevertheless, last autumn, Hermaszewski intimated to *Nature* that he had got up several times during the 'night' to ensure that the experiment was working properly, since the project, named after the symbol of Warsaw, had considerable national significance.

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Not surprisingly, initial reports in the Polish media claimed the experiment as an unqualified success. Monocrystals of high homogeneity were it was said, obtained, such as would be totally impossible to produce under conditions on Earth. Then in February 1979, a meeting was organised in Krakow by the Japan Electro-Optics Laboratory for Polish users of its equipment.

A paper presented here by Warminski and Zahorowski of the Institute of Physics of the Polish Academy of sciences indicated that 'Syrena' had simply yielded a mixture of crystals of various compositions, in which the degree of homogeneity and the composition of the specimens depended on experimental parameters such as temperature gradient.

This would not in itself have disconcerted the scientists — a negative result is also of scientific value — had they not felt the results had been deliberately exaggerated by the media, which had claimed the alleged hyper-homogeneous triple crystals as a triumph for Polish science within the framework of "Interkosmos" (the Comecon space programme). "Science has become a state prostitute!" one young physics graduate put it.

Nor, apparently, was the disillusion confined to the lower echelons. According to a circumstantial report published by the "opposition" (i.e. underground) *Biuletyn Informacyjny KOR*, Professor Adam Gierek, the son of the party leader, remarked to a prestigious gathering of specialists that 'Syrena' has brought us back from space to Earth with a bump!"

The disappointment with 'Syrena' does not, however, extend to the Polish Academy of Sciences. Dr R. Galazka, of the Institute of Physics, who presented the results of the 'Syrena' experiments at the European Space Agency Conference in Grenoble in April, told *Nature* that "it was not our aim to produce crystals for some specific purpose, but to check the process of crystallization in space. We were able to use a diffusion controlled technique, impossible on Earth, to get crystals with some parts up to 50% homogeneous. Also we were able to study the problem of cavitation under pressure. We never aimed at getting a single crystal — indeed the technique of the experiment made this impossible. But we did get some 5mm single crystal blocks".

Professor Stanislaw Grzedzielski, of the Academy's Institute of Space Research, had just returned from a discussion with Dr Jan Kaczmarek, Academic Secretary of the Academy (a post which carries Ministerial rank) on the future of the space programme. He told *Nature* that plans for 1981-85 would include projects taking into account the results of Syrena. These results, he said, were not strictly speaking a surprise "because we simply did not know what to expect. Had we known, we need not have done the experiments."

Any exaggerated claims in the media, he said (and he himself always avoided popular science features), must have been due to patriotic zeal on the part of the reporters and/or their inability to understand the nature of the experiment. Even in a "controlled" press, he intimated, such divergencies can and do occur.

He himself, although not an expert in materials science, was not unhappy with the results. "Had they simply obtained perfect monocrystals every time", he said, "then there would be nothing more to find out, and the matter would no longer be one of space science but only of space technology. As it is, we can do a lot more experiments. Indeed it is expected that the Americans will try to replicate our work."

"Now that the work has started," he added, "it is far too valuable to stop. And it is very cheap, too. You see, it is not true that it involves 'cosmic sums of money' as some people would like one to believe. We don't pay for the 'ride' at all — the place on the rocket, the launch, ground control systems and so on. We only pay for the apparatus produced in Poland, and the associated data processing etc."

The Soviet Union, he explained, were so far prepared to provide launch facilities free. "Fraternal solidarity is very important to them. If you are a big power and want your neighbours as friends you have to give them something they wouldn't have had otherwise".

This assurance, if passed on to the Polish public, would doubtless allay the rumours that 'Syrena' cost "several tens of millions of dollars" (from Poland's scanty hard-currency reserves) — if, of course the public were to believe it. Disillusion with the reporting of 'Syrena' however, bids fair to percolate from scientific circles to the wider public — carrying with it a disillusion with all forms of science reportage. It is perhaps not without significance that at this year's meeting of the Polish Academy of Sciences, a determined effort was made to bring forward a resolution calling for an end to press censorship — a motion which was thrown out by an amendment proposed from the chair. **Vera Rich**

Mystery beams affect UK satellite

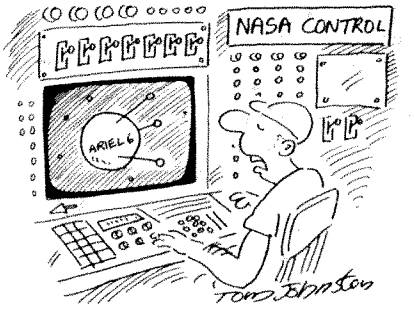
BRITISH scientists are mystified by signals emanating from regions around British Columbia and the Caspian Sea that are turning off high voltage supplies in the UK research satellite, Ariel 6. After each satellite orbit over the areas, the high voltage of two experiments is switched off while a third supply is completely unaffected. The commands controlling the switching consist of a 148.25MHz carrier with a 5kHz subcarrier carrying the command signal so the interfence has to be highly specific in order to affect two supplies but not the third. Dr Len Culhane Chief Project Scientist in Mullard Space Science Laboratory's X-ray astronomy group has no explanation. "We think the signals are being generated spuriously but we can't figure out how."

Five additional NASA ground control stations have been brought into play to turn the switches on after the satellite passes through the regions. Culhane is hopeful that eventually the satellite can be brought up to 80% of its operational capacity by further interventions.

Two other features of the interference puzzle the experimenters. First the interference occurs only when the Sun is shining on British Columbia or the Caspian. And second the experimenters have discovered that the satellite can be "immunised" against being switched off by beaming pure carrier frequency at it just before it enters the mysterious zones. The satellite orbits are such however that the satellite passes close enough to a neighboring ground station only 30% of the time — thus it cannot be immunised completely. Alan Wells of the Mullard group thinks that the likely cause is a jolt effect — a ground based radio signal combines with electrical noise in the space craft to produce coding signals that activate the equipment switches. The possibility that the interference has been caused by vhf blasting signals coming from a dam construction site has been ruled out as has the possibility that interference is random.

In spite of the mystery the spacecraft is taking useful data. A Bristol University experiment on ultra heavy cosmic ray nuclei ($26<Z<92$) using a gas Cerenkov counter has been unaffected. The two X-ray experiments, one by Leicester University and the other a Mullard-Birmingham University collaboration has been fortunate enough to observe an X-ray burst from the X-ray source 1735-444 which was one of the hoped for observations of the mission. However a solar panel experiment designed by the Royal Aircraft Establishment at Farnborough has been unable to operate.

But short of some unexpected revelation it is likely that the reason for the interference will not be found, leaving the possibility that a space variant of the Bermuda triangle legend may be in the making. **Joe Schwartz**



"As it's a British satellite, it's probably just taking a tea-break!"

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UNCSTD success still hangs in the balance

David Dickson reports from New York on final preparations for the UN Conference on Science and Technology for Development

With less than six weeks to go before official proceedings open in Vienna, a cloud of uncertainty still hangs over the chances of any major achievements coming out of the United Nations conference on Science and Technology for Development.

Delegates attending the fifth and final meeting of the conference's preparatory meeting in New York last week managed to reach agreement on a number of proposals that will be put to the conference as part of a 'plan of action' which the conference will be urged to adopt. However there are still major differences in three of the key areas to be discussed — the transfer of technology, and the institutional and financial procedures necessary to implement the decisions of the conference — and the stage has now been set for some hard bargaining over these in Vienna.

In general, the developing countries are seeking ways to improve their bargaining position over acquiring technology from the industrialised countries, to gain additional funds from these countries to build up their scientific and technological efforts, and to make the UN system more responsive to their needs in these areas by increasing their control over the relevant decision-making processes. In contrast, the industrialised countries, quoting domestic, economic, and political problems of their own, are keen to discuss specific areas of scientific and technological assistance, but have so far been digging their heels in over agreeing to any major procedural changes or financial commitments.

Most of the two-week session was spent by delegates in closed drafting meetings discussing, paragraph by paragraph, proposals for a new plan of action. The plan has been compiled by the Group of 77 (to which over 120 developing countries now belong) as an alternative to proposals put forward by the conference secretariat.

Agreement was reached on a number of proposals, mostly on relatively uncontroversial areas dealing with the internal arrangements that developing countries should make in order to increase their scientific and technological capabilities.

There were difficulties, however, whenever a paragraph arose that involved — or seemed to involve — the developed countries making any type of commitment to change existing arrangements. The developing countries have essentially used the proposed plan of action as the occasion to make a detailed statement of principle on what, they feel, should be done by developed countries and the United Nations bodies in the area of science and technology for development. In contrast, the developed countries — with a few notable exceptions, particularly the Nordic countries — claim that such statements imply definite commitments of the type which many of the negotiators said they were unable to make.

The result is that when delegations arrive in Vienna, they will be faced with a series of documents liberally peppered with square brackets containing alternative formulations of key proposals. For example, where the Group of 77 suggests that the plan of action say "developing countries should be able to play a more effective role at the decision-making level in international organisations", the EEC suggests the same paragraph should read "all countries should . . . ". Similarly, where the developing countries are demanding "full access" to western technology, the developed countries want this changed to "freest and fullest possible" access, a qualifier that dilutes the initial intent.

Three areas of disagreement remain. The first is the general area of technology transfer. Here the developing countries, in line with the stance taken in other UN forums, are demanding substantial concessions in their terms of access to the technology of the developed world. Industrialised countries, in contrast, are refusing to make any significant concessions, arguing partly that this is the responsibility of the private sector over which they have no control, and also that the issues are already being dealt with sufficiently elsewhere. The second area of disagreement is over the financial support needed to carry out the conference's recommendations. The Group of 77 argue that this can only be done with a considerable increase in the transfer of funds from the developed countries. They have suggested setting up a new financing system, largely financed by the industrial world, with a provisional target of $2 billion by 1985 and $4 billion by 1990.

The industrialised countries, however, have made clear their reluctance to commit themselves to any increase in such contributions. They are particularly critical of the suggestions that they be required to make "automatic" contributions to such a fund on the basis of, for example, their trade surplus in manufactured goods with the developing world.

Financial issues are also linked to the third area of disagreement, namely what changes should be carried out in the UN system to implement the conference proposals. The developed countries would like to see the strengthening of existing bodies, in particular the UN's economic and social council, to do this, although accepting that the status of science and technology should be raised within general development strategies.

In contrast, the developing countries, distrusting what they consider to be the undue dominance of the industrialised countries in ECOSOC, want a new intergovernmental body to be set up. This might report directly to the General Assembly, with an executive general secretariat possibly established under the recently created UN post of Director-General for Development and International Co-operation.

A critical factor in this debate is likely to be the United Nations Development Programme. Although it has so far done relatively little explicitly concerned with science, UNDP has both wide experience of development issues and a high credibility among third world countries, and it may well be given operational responsibility, with an increase in funds, for decisions made by the intergovernmental committee in line with UNCSTD's final recommendations.

One phenomenon noted by a number of delegates at the meeting was an apparent softening of the US negotiating tactics. According to those who attended the closed drafting sessions, whereas the US had previously been pushing a relatively hard line in negotiations, disputing even relatively small points, they have subsequently slightly relaxed their stance, leaving the hard line more to the EEC countries ("and the British were the toughest of the lot" said one South American delegate).

Some have suggested that this shift in tactics may partly reflect a change of strategy by the transnational companies, eager to maintain their links with third world markets, but coming under increasing pressure from third world governments (particularly after the failures of UNCTAD V in Manila), and being forced to be more conciliatory.

Given the uncertainties that still remain, delegates leaving New York expressed views ranging from "concerned pessimism" to "cautious optimism" about chances of a significant agreement on these issues being made in Vienna. Much will depend on what happens in the negotiations between now and the end of August. The Group of 77 is meeting in Bucharest to discuss its negotiating strategy at the conference and others are meeting elsewhere.

On one point, however, the secretariat had its way. On the first day of the conference, the initial proceedings will include a performance of Beethoven's Fidelio overture. Some had complained that this would be time-wasting; others that, as western music, it might not be appreciated. The secretariat has defended its choice by pointing to the music's radical theme — and after all, you can't go to Vienna without listening to music. □

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news in brief

UK government scientists step up strikes: British scientists are calling a further series of half-day strikes and demonstrations in support of their pay claim of 36%-47%. The government has offered increases ranging between 15% and 24%. The Institution of Professional Civil Servants is calling out its 20,000 scientists, 40,000 technicians and 10,000 related staff in a series of Tuesday and Thursday actions that will last until 7 August. Government establishments at Southampton, Leeds, Bath, Reading, Newcastle, Birmingham, Bedford, Cardiff and Glasgow will be involved successively. "This campaign will stop everything in these localities", said an IPCS official. The half-day actions add to the small number of volunteer indefinite strikers who are seriously affecting the country's communications and power supplies. In addition, IPCS has announced withdrawal of "good will" from operations at the Ministry of Defence, Departments of Environment and Industry and the Atomic Energy Authority. Support for further action is strong among union members.

King's College principal says UK universities should be cut: Sir Richard Way, principal of Kings College, London University has called for a reconsideration of the number of universities in the UK. Speaking at the opening of the 150th anniversary exhibition of King's College, Sir Richard gave his support to government economies. "Your government has been elected to try to establish some control over public expenditure and this is an aim which I very strongly support. I don't personally believe that the university system as a whole is necessarily short of money." Sir Richard told *Nature* that university expansion in the last 15 years did not mean that the expansion should be defended. "Just because you have 45 universities doesn't mean that you need 45 universities" he said. "I am an advocate of pruning." But such a measure need not compromise the UK's research capability. "It would be desperately wrong to compromise research", he said.

Benn calls for UN energy organisation: Tony Benn, former Energy Secretary in the Labour Government, has called for the creation of a United Nations Energy Agency to help avoid "confrontational energy policies". Although OPEC has previously resisted this proposal because of their fears of price control, Benn feels that the world is slowly moving in that direction. The important thing is to bring OPEC, the West, the Communist countries (who export oil to the West) and the non-oil producing countries into an arena that can prevent confrontation, he said.

Speaking at the annual conference of the National Union of Mineworkers, Benn also called for a public debate on nuclear power development in the UK. Previous growth of nuclear power has been predicated on overall growth, he said. But with a forecast of negative UK growth and with the events of Harrisburg in mind, big nuclear plants, particularly pressurised water reactors, need full public discussion. "We need absolute satisfaction that expansion is necessary, that expansion must be nuclear and that leaks and waste disposal problems are solved before going ahead."

UK stand on whaling: The UK government will support the proposal for a moratorium on commercial whaling. Mr Alick Buchanan-Smith, Fishing Minister at the Ministry of Agriculture, Fisheries and Food, announced this on Monday at the opening of the 31st annual meeting of the International Whaling Commission. "Resumption of whaling", he said, "should only be reconsidered if evidence of recovery of stocks and improvements in the methods of killing justifies it." He also announced government proposal for immediate discussions with the European Community calling for a Community-wide ban on sperm whale imports. This greatly disappointed environmentalists who had expected the government to announce a ban at the meeting. Czech Conroy, for Friends of the Earth, said although they welcomed the government's support for a moratorium on commercial whaling, they found it "totally illogical and unsatisfactory" that sperm whale imports would continue to be allowed. "The proposed discussions between the government and our partners in the EEC to achieve a community-wide ban on sperm whale imports will permit thousands more whales to be killed." Friends of the Earth, he said, would continue to press the government "hard and fast" to follow up these discussions.

France opens new information centre: A new data centre, with the purpose of providing French scientists with bibliographic information produced by the world's major documentation services, was opened at Valbonne near Nice at the end of last month. The capacity of the computerised system will be 10^{10} bytes which will allow access on-line to about 10 million references.

The Centre was built in the record time of six months at the special request of Giscard d'Estaing, the French President. The authorities had been concerned about the rapid development of data banks in the US, UK and Germany, which according to Pierre Aigrain, Secretary of State for Research, could lead to French "cultural alienation". There was also concern that French research is not properly represented in foreign data banks. The official reason for the creation of the new centre, however, is economic. French scientists have been making about 60,000 inquiries to US data banks each year at a cost of 15 million FF.

— From the staff of La Recherche

Chinese synchrotron construction slows: China's 50 GeV/c alternate gradient proton synchrotron, originally planned for completion by 1982, will not be finished until 1985. the announcement appeared in the Beijing (Peking) People's Daily in an article by Zhao Dongwan, a vice-chair-person of the State Scientific and Technological Commission, who appears to be in charge of the administrative side of particle physics in China. He wrote that work is proceeding on schedule for the experimental centre housing the accelerator, but the target date of 1982 announced at last year's National Science Conference has been put back to 1985 because China's national economy is being adjusted. "This means a higher standard" Zhao emphasised. Zhao also stressed the need to continue the implementation of the principle of self-reliance. The basic approach he wrote would be to send people abroad to study advanced accelerator technology while designing and building the equipment at home. China should not import equipment that she herself could produce.

In the meantime the Sino-American Joint Committee on High Energy Physics held its first meeting in Beijing on 11-12 June. This joint committee is the first of a kind which came into being after the signing of the Scientific and Technological Agreements between China and the US earlier this year. The two-day meeting was jointly chaired by Professor Zhang Wenyu, director of the High Energy Institute under Academia Sinica and by Dr J.E. Leiss, associate director of the Office of Energy Research in Washington.

— From T.B. Tang

Education in Chile in decline: The long term educational policy of the Chilean junta aims to eliminate entirely universal education. In a statement made in March, President Pinochet stated that the government intends to reduce educational opportunities to ensure that no more than a handful of children, whose parents can afford it, go to secondary school. In 1978, less than 70% of all children received an education compared to 92% under the Allende government. The education budget has been reduced from 17% of the total to 13.5%. In addition, the administration of many schools has been given over to business consortia who hire the teachers and plan the curriculum. The consortia receive tax benefits for providing "resources for educational purposes". Operation of all schools from primary to university level is under the final authority of the Commando de Institutos Militares.

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France puts science under the microscope

Pierre Aigrain talks about his first year as France's science minister, which has culminated in a blockbusting survey of the whole of French science. **Judy Redfearn** reports

AT THE END of last month, a report entitled "L'état des sciences et des techniques Francaises', was circulated to French scientists, administrators and politicians for their information and comments. A slim document, it is the summary of a study conducted by the Délégation Generale à la Récherche Scientifique et Technique and the Comité Consultatif de la Récherche Scientifique et Technique (*comité des sages)* under the auspices of the science minister, Pierre Aigrain. Two thicker tomes, one for physics and one for life sciences are to be published later. "As far as I know, it is the first time that any country or any government has tried to do a study of this kind", according to Pierre Aigrain.

Previous studies, he says, have compared the level of funding of a particular area of research in France with that of other countries. But "that's not what we have tried to do here". The aim has been to identify the strengths and weaknesses of each branch of research in France: to compare it internationally and assess its relevance to national economic and social objectives. "This is a critical study which is necessarily subjective. I expect that it is going to make us very unpopular". But it was felt necessary as a basis for formulating future science policies. The exercise is to be repeated annually, to begin with, and then at longer intervals as the methodology improves.

Good research, explained M. Aigrain, poses few problems. Even if it is not particularly relevant to national needs, provided that it is not too expensive, it should be supported and encouraged. Problems arise, however, "with sciences in which we are weak. There are those in which we are weak compared to the rest of the world and those in which we are weak but the rest of the world is no better". An example of the latter is human nutrition. France may be in the front row, but "that row is moving much too slowly compared to needs".

The greatest difficulties arise, however, when France lags well behind international competitors in a particular field. Sometimes the best decision will be to forget about those areas. But in some cases, a weakness can have "unfortunate, sometimes ctastrophic consequences", not only on that field but also on neighbouring ones. An example is clinical pharmacology which is important for good pharmaceutical research.

Pierre Aigrain, together with the DGRST and the *comité des sages*, is now faced with incorporating the main findings of the study into the eighth five year plan for 1980-85 and a ten-year research and development strategy, both currently under preparation. France has been running five year plans for science and technology since the end of the war: the idea is to plan fairly precisely in advance what research projects should be done during the next five years. The ten year strategy, however, is more of an attempt to formulate basic guidelines for government R&D "in a ten year perspective".

One problem the ten year strategy will address is that of providing enough jobs. After the expansion of the universities in the 1960s, France, like many industrialised countries, now faces the problem of continuing to provide work for young scientists. With only a modest growth in the real value of the science budget, it has had the choice of either freezing the number of posts available and providing each scientist with a good working budget, or increasing the number of posts and consequently reducing the money available for equipment and research. As the lesser of two evils,it has chosen the latter.

In 1975, the government decided to increase the number of research scientists employed in its laboratories by 3% per annum and to keep the level of the science budget in line with the average rate of government investment. "The rules for the next ten years will be of a similar kind, although more elaborate", says M. Aigrain.

"The training of scientists is a long term problem", he explained. "Not only does it take a long time to train them, it takes even longer to establish good training systems. We feel that although creating new jobs only makes our problem worse, from the point of view of the amount of money each scientist has to work with, it is a less serious problem and more easily reversible than the one we would have if we let the training systems die out".

A good budget could greatly improve the remaining problem. The 1980 budget, for example, is expected to be appreciably more than the one for this year. If it is, then the shortfall in funds per research worker could be remedied in "one or two years". A new training system, however, would take 15 years to establish "if we let the present one die out".

The 3% growth rate has by no means solved the jobs problem. The French universities system is suffering particularly badly from stagnation as a result of the earlier expansion — a situation made worse by the fact that very few scientists, even compared to most other countries, take jobs outside academia. "Our mobility rate is very low. If a greater proportion of scientists employed in government laboratories moved to other sectors, including research in private industry, we could have our cake and eat it: we could increase the means at the disposal of each scientist and at the same time maintain our training system".



Minister of Science, Aigrain: "it's a rather special sort of job."

Mobility, however, is a problem of psychology — not only of scientists but also of prospective employers. "You can't change behaviour by law — at least not in a democractic society. Actions can only be indirect". The indirect actions France is currently taking involve giving advantages to scientists who move into industry and their employers.

It is also trying to revamp an old system whereby scientists can be put on "detached duty" in industry for a few years. "Even this has not been as successful as we would have expected because while the scientists are on detached duty they have not been getting promotion from their government employers. This is something which we are going to try to change."

Moving research scientists into industry, however, not only benefits the universities and government research laboratories; it can also bring valuable expertise and new ideas to industry which badly needs more contacts of this type. "On the whole French science and technology is holding its own", according to M. Aigrain, "but the contacts between it and the people who can apply it are very poor. In my opinion, it is a matter of personal contacts, which are insufficiently developed."

The solution does not lie with creating more government labs for applied research, however. "I've been in industry in charge of R&D in a major company and I know that doing industrial research efficiently implies an industrial environment".

Knowing how best to act to stimulate

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greater mobility of scientists or greater cross-fertilisation of ideas with industry, involves judgement of human behaviour and a bit of applied psychology. Psychology, however, is not one of the hard sciences, says Aigrain, who was initially trained as a solid state physicist. But since he became science minister one year ago it has assumed a greater role in his working life.

Aigrain came to the post after a varied career in both science and administration. As a young physicist he was appointed by General de Gaulle in 1958 to the newly created *comité de sages* (CCRST) — de Gaulle had wanted some young blood on the committee — and later through earlier connections as a naval officer, he was appointed head of an advisory committee concerned with defence research.

After a spell with responsibility for higher education and a five year term as head of the DGRST (1968-73), he moved into private industry to become general technical manager of Thomson. "I had planned that the rest of my active life would be spent as general technical manager of an industrial company. I have never been involved in active politics, so it was a very great surprise to me when the Prime Minister, Raymond Barre, asked me to enter the government in April 1978."

Pierre Aigrain is one of a handful of French ministers who have never run for election. Another is André Giraud, the industry minister, under whose department science and technology officially come. The job of the science minister, however, is unique in the French government.

"I operate by delegation from the Prime Minister on inter-ministerial matters. My work mostly involves coordination, strategy, planning and budgeting of civilian R&D in all ministerial departments. This is a rather different job from that of a minister who has to run a department. What I have to do is convince my colleagues or the Prime Minister of what should be done in matters of science and technology. It's a rather special sort of job." The DGRST is directly under his responsibility. "DGRST is really my operating cabinet and my means of action."

Determining the science budget, and how it should be spent, is Pierre Aigrain's major task. France currently spends about 1.8% of its gross national product on R&D. Ten years ago, the figure used to be 2.1%; 20 years ago it was well below 1%. In spite of the decrease in this percentage over the past ten years, the volume of research has increased very slightly because of the modest growth in GNP.

On the whole, M. Aigrain believes that the French government is sympathetic to the needs of science and technology. His main problem with parliament, however, is to explain "why, with the budget we have, we should put money on this and that and not necessarily on some of the ideas which are in the news — which may not be the best, or which anyway, cannot be efficient unless we also maintain a sufficient amount of basic research."

He agrees that parliament could be much better informed. A group of MPs, known as Intergroup, used to meet regularly to discuss science policy issues, but with the pressure of seemingly more urgent commitments, this has lapsed and there is now no equivalent of the UK select committee structure wherebvy MPs can gather the opinion of scientists at the grass roots. Something along these lines would be "very useful", however. Scientists "have a duty to try to explain to politicians what the problems of science are, not in party political, but in science policy terms: down to earth terms".

For one reason or another, France has missed out on many of the debates around science policy issues which have aroused public interest in recent years in other industralised countries. In the case of nuclear energy, Pierre Aigrain thinks that the French people recognise that their country, having very few energy resources of its own, is in a particularly difficult position. "There may be many Frenchmen who don't like nuclear energy", he says, "but there are few who doubt that it is necessary".

In the case of genetic engineering, the French public recognised early on, the confusion surrounding the debate in other countries, where concern had centred on the possibility that "some experimentation could be dangerous for the people doing it or the immediate surroundings" and "the vague fear" that genetic engineering could lead to "ethically unacceptable" experiments. "In France I think it was clear from the start that these were two different problems. The problems of the dangers could obviously be handled provided that scientists took the right precautions. People are, I think rightly satisfied that we can handle this problem because we have been handling a much more difficult one for tens of years," – namely the production of large quantities of vaccine from live virus.

About health and safety generally, and concern over carcinogens and toxic substances, M Aigrain says that there are worries in France, but that "up until now, Frenchmen have been a little more rational and a little less emotional. We in France have a big drawback. We tend to have too much of an analytical mind — we are too rationalistic, too Cartesian. In research this is not necessarily an advantage because, in order to be creative, researchers have to be a lot more pragmatic. But it does have the advantage that even the average Frenchman can realise relatively easily that a debate is created on a non-logical basis". □

What the report says about French science

TEN thousand people, of whom 4,500 are qualified scientists, are employed in fundamental physics research in France. The budget for paying their salaries and buying equipment for their work is about 1.7m FF per year. A further 3,500 researchers are employed in physics for engineering.

The DGRST study found French fundamental physics rather healthy, especially in the fields of high energy physics (where France has a reputation for building bubble chambers), nuclear physics, thermonuclear fusion and solid state physics. (The latter is an example of a fundamental branch of research which has good links with industry.) Gravitation, cosmic rays and molecular physics are weak, however.

In applied physics, says the report, the results of research in optics and fluid mechanics are finding their way into industry, but those in solid mechanics and materials sciences are not. The quality of research, for example, in materials sciences is good, but it is unevenly distributed amongst sub-fields and is too academic to be well tuned to the needs of industry. Chemical research done in universities and government laboratories, however, has close links with the chemical industry, but there is need for even greater collaboration. In quantum chemistry, radiochemistry, nuclear chemistry and catalysis (with the exception of catalysis by enzymes) collaboration is good; but it is poor in electrochemistry and chemical engineering. Much of the research is very dispersed and there is a need for some regrouping.

About 4,500 full-time and 6,000 part-time scientists are employed in life sciences research. Areas of which France is particularly proud include cellular and molecular biology, endocrinology, applied immunology and and reproduction and development. Over the past seven years, the programme in the latter has led to a reduction in perinatal death from 23.4 to 16 per thousand. Bacteriology and human nutrition, however, are very weak and neurobiology is currently being 'rejuvenated'.

In energy research, a field with important social and economic implications, the report says that France is doing well in nuclear energy and solar power. It is going ahead in 1982 with plans to start building light water reactors under licence from Westinghouse and with the construction of its demonstration commercial fast breeder reactor, Superphenix. In renewable energy research, it is putting most of its efforts on solar energy and in the long term, thermonuclear fusion. Research on fossil fuels is strong when it comes to prospecting for off-shore for oil (France is well renowned for off-shore technology because of its efforts in this area) but weak when it comes to coal research.

Will China be tainted by western science?

CHINESE science is treading a careful tightrope between its long-term aspiration of a specifically Chinese-orientated scientific outlook and the short-term expediency of importing western ideas wholesale. Recent statements have indicated that although the ideal is still to the forefront of Chinese scientists' minds, it is perhaps being undermined by the very scientific exchanges essential to the modernisation of Chinese science.

The influx of western ideas into the physical sciences pertains to science itself as well as to its social dimension, which includes the administration, funding and general management of science and technology. In the past few months, some ministers and many scientific groups from China have toured scientific institutions in the west, and a large number of western scientific teams are returning the visits. The first impressions apparent in the media were that the Chinese showed great interest in western practice. An example is that after the visit of the State Scientific and Technological Commission delegation to Japan last December, articles appeared discussing the merits of such institutions as the patent system which were introduced into Japan when it became westernised. In fact, however, the Chinese attitude is a cautious one. Commentaries in the party paper on 19 April and 8 May, for instance, stressed that while China is learning advanced science and technology from abroad, it will on no account blindly import social practices; future science policy will be firmly China-based.

On the other hand, in looking towards the future, the Chinese realise that their present system of science management is not perfect. They freely admit, among other things, that their national research efforts are plagued by wasteful duplications as well as damaging omissions — perhaps surprising in view of their central planning. It seems that the misguided motivation of some scientists, together wiht insufficiently democratic coordination between parallel funding offices, are the major factors. Some institutes have recently adopted the practice that individual contribution is judged mainly by the authorship of papers, but such professionalism can sometimes lead to researchers doing only publishable and prestige-promising work. The Chinese believe that the problem of rationalising the organisation of science and the decision-making processes will be solved by the inventiveness of the masses, rather than by imitating another country, no matter how advanced its science. Thus, many of the systems created and evolved in China during the last twelve years are likely to be kept, forming as they do a useful basis for improvement. During this period the achievements of Chinese science have been satisfactory, when compared to the resources available; last year, according to preliminary statistics, more than 12,000 research projects were successfully completed.

This attitude, that a specifically Chinese science can build on a firm past basis, is reiterated in a report on 10 April in the *Guangming Daily* of a meeting of Beijing University professors. The speakers, among them several leading scientists, asserted that as developments from foreign countries are incorporated into the Chinese social system, Chinese science must retain its original character. To us, its outstanding features include an emphasis on combining both traditional and modern knowledge, on linking laboratory investigations with production in the field or factory, on collective rather than individual efforts and rewards, and on the participation of the masses in both the direction and the conduct of research. It is relevant in this context to note that China continues to devote considerable resources to the popularisation of science: it is setting up four additional specialised studios to cater for the rising demand for science and educational films, a demand at present partly satisfied by dubbing films purchased abroad. In the meeting, some professors also argued that political education should be emphasised in the training of young scientists.

For China, at present, the provision of scientific and technological manpower holds the key to the 'fourth modernisation' (*Nature* 19 April, p. 682). A concrete expression of this situation is that instruments such as electron microscopes — if they were foreign imports — are often under-used due to inadequate experimental skill or lack of maintenance.

According to a 1977 report from the Organisation for Economic Co-operation and Development in Paris, there were then some 335,000 scientists and 725,000 technicians working in China (excluding personnel engaged in medical research). At the National Science Conference convened in March 1978, the goal was set that by 1985 an army of 800,000 scientific workers will be formed, double the present number. Chinese universities took in a total of nearly 400,000 new students last year, and to help its educational targets China announced last August that it also plans to send some 10,000 students to the US, the UK, France, Germany and Japan. This year about 500 are going to the States, 200 to England, and 100 undergraduates have already arrived in France.

Although western visitors may import foreign ideas into China, the real danger to the concept of a long-term Chinese-orientated science lies in the return of these many young people after exposure to western scientific culture (although the flow in the opposite direction may also be interesting). This trend will be exacerbated by closer links between students in China and their counterparts abroad.

At present there is a dynamic balance between the ideal of an indigenous Chinese scientific culture and the influence of established western scientific ideas; only in the next five years, as these influences are absorbed, will the future pattern of the world's largest new scientific force become apparent.

T.B. Tang



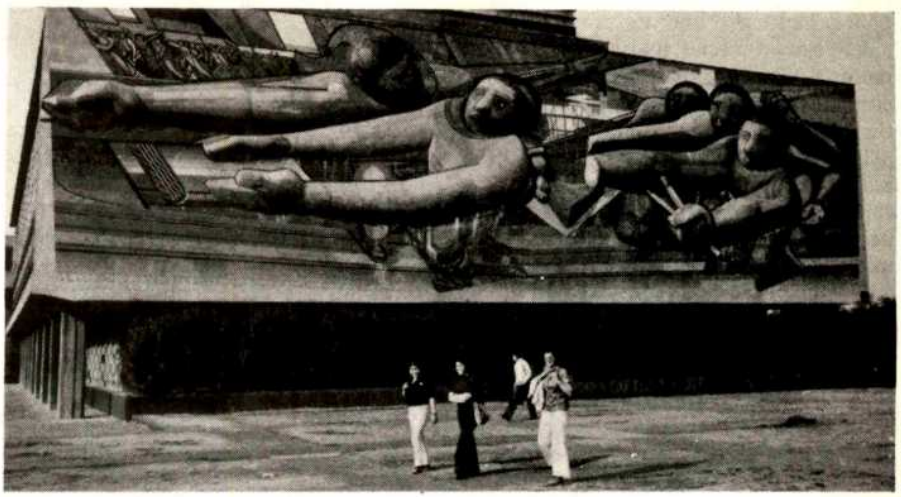
The world's first giant panda born by artificial insemination made its debut at Peking Zoo recently. The panda, named Yuan Jing — meaning "First Crystal" — was born last September weighing only 120 grams. Yuan Jing has aroused interest around the world because of renewed hopes for the famous but uncooperative panda pairs at London Zoo and Washington Zoo. All previous attempts to mate the pairs have failed, including flying in other pandas from the Soviet Union and China. China is the only country so far to have bred pandas successfully in captivity. Baby Yuan Jing started to walk at three months, developed the familiar black and white hair at five and was weaned at seven months.

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Mexico: slow fight against impossible odds

By John Hutton



Universidad Nacional Autonoma de Mexico: increasing demand for education

MEXICO City is one of the largest and fastest growing cities in the world. The colonial heart, built upon the remains of the Aztec capital, is beginning to atrophy as the periphery expands. The volcanic peaks which crown the valley are often hidden in the smog produced by industry and by the ageing vehicles which edge bumper-to-bumper down the arterial thoroughfares designed for a city of a smaller size.

The provision of services cannot cope with the influx of population. There is a chronic housing shortage and, in many parts, scanty supplies of water and electricity are further burdened by illegal tapping. Yet, every day, thousands of people pour into the city. They come to establish themselves in the ranks of power, to enter business, to seek higher education, to find jobs in the factories or to work as household servants.

Mexico City's population of about 13 million is expected to reach 30 million by the turn of the century, and its current rate of growth, the nation's population should exceed that of the USA in fifty years. While 30% of Mexicans enjoy incomes comparable to those in the developed world, more than 50% know the realities of underdevelopment.

Malnutrition is widespread, as is chronic respiratory and gastro-intestinal disease. Life expectancy is about 65 years but infant mortality is estimated at 66 per thousand live births, more than twice that of Cuba and four to five times that of the USA. More than half the population receive no more than a few years schooling and illiteracy stands at 25%. In recent years, the domestic production of foodstuffs has not sufficed and staples, including corn, have had to be imported.

In confronting these problems Mexico can call upon a stable political system, a diversified economy, the vision of its administrators and, in recent years, its vast reserves of petroleum.

In accordance with the spirit of her constitution, Mexico sees the key to development as lying in investment in education and in the construction of an economy with an indigenous base rather than one which relies on imported technologies. In keeping with these plans, investment in research and scientific education has burgeoned in the past ten years. And there now exist pockets of activity which, though not yet mature, are well on the way to manifesting a style which is both in line with the country's aspirations to knowledge and relevant to its material needs.

The body largely responsible for the implementation of this policy has been the 'Consejo Nacional de Ciencia y Technologia' (CONACYT) which was established in 1971 during the presidency of Luis Echeverria. In 1960 Mexico could boast only 385 qualified investigators which, given its rate of demographic growth and the scientific boom in the West, was little advance upon the 30 or so registered in 1929. By a system of grants and scholarships CONACYT brought that number up to 13,300 by 1978. And in the 1978-1982 period a further 17,000 scholarships will be awarded, most of which will be for postgraduate research within the country.

The pattern of the scholarship distribution reflects the priorities of the planners: 17% to studies on the use of energy resources, 13% to agronomy and forestry, 13% to basic research, 11% to nutrition and health, 9% to social development, 7% to fishing, 7% to construction and transport, and 2% to public administration.

This is a far cry from the climate of the 1950s and early 1960s when the glamour of imported technology held sway. In an era often described as 'anti-science' or 'anti-development', overseas-based corporations were given preference in the construction of manufacturing industries; in the field of agriculture the US-inspired 'green revolution' was promulgated; and even in the petroleum industry, nationalised since 1938, much of the geological data concerning the country's resources lay in foreign hands.

Movement away from this ethos gathered impetus in 1961 when the 'Instituto Mexicano del Petroleo' was set up. Apart from direct benefits to the industry, this initiative helped encourage the return of scientists from overseas. By the same token, it provided ammunition for those who wished to draw attention to the dearth of scientists within the country and the need for an adequate training scheme. Meanwhile, in the universities there was a mounting realisation that the nature of the social system had to be changed, a movement which culminated bloodily in the student riots of 1968.

Cries for reform were heeded by the incoming president. In 1970 Echeverria made the unprecedented move of increasing spending on education from 1.8% to 3.1% of the Gross National Product (GNP). At the primary school level the text books were re-written with an emphasis on authentic growth. Science education was begun at the age of six and broadened to include such sensitive issues as human sexuality, a move which required presidential sanction and which met with fierce opposition from conservative sectors of the press and television. The CONACYT scholarship plan was instigated at this time, and spending on research radically increased, from 0.1% of the GNP in 1970 to 0.6% by 1978; it should reach 1.0% by 1982.

During the 1970-1978 period, a policy of support for basic research has evolved. The rationale has been that work of an applied nature would flow more soundly from a solid foundation of scientific principles, and that experience in basic research is essential to the attainment of a 'critical mass' of research facilities and expertise.

The pursuit of such a policy seems to have paid off, as indicated by the emergence of a new type of scientist: one whose work habits are geared to productivity, one who is open to new ideas rather than dedicated to perpetuating dogma, one who demands the critical participation of his students rather than their unswerving loyalty.

It will take time, however, before the new ventures in science make their full impact upon the economic and social development of the country. The successes attained so far have sometimes been marred by the difficulties of realising the economic potential of a discovery. As Dr Arturo Gomez-Pompa, director of the 'Instituto Nacional de Investigacion sobre Recursos Bioticos', put it: "the economic potential of a chemical with interesting

The author visited Mexico on a Nature *travelling fellowship.*

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pharmaceutical properties, extracted from a native plant species, may be lost when the material falls into the hands of a foreign concern which modifies it, patents it and then markets the new compound".

Several ambitious attempts have been made to grapple with this problem, within the framework of a move towards decentralisation. In Tonanzincla a church in the style of Indian baroque, open fields and eucalyptus trees provide an idyllic setting for the 'Instituto Nacional del Astrophysica, Optica y Electronica'. Known in the village as the 'observatory', this once prestigious centre of astrophysical research is now a base for investigation into optics and electronics. Basic research is undertaken by the fifteen or so staff and by the fifty-odd students who are studying for higher degrees.

Alongside the laboratories a pilot factory has been constructed for the manufacture of microscope lenses. Grants are awarded to enable young people from the village to work on the production line.

At the University of Puebla technical expertise in the construction of micro-circuitry is also being developed for research and teaching purposes, in addition to its contribution to the nation's needs. As at Tonanzincla much of the equipment is manufactured at source.

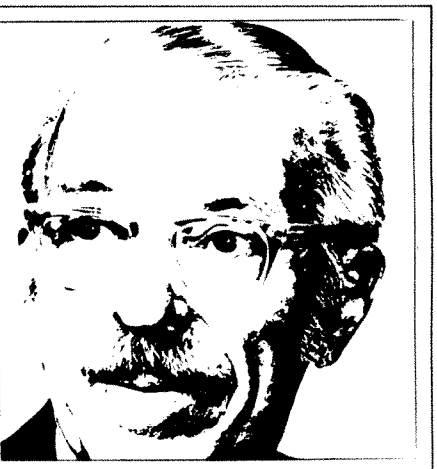
In the State of Durango, the government has set aside a large tract of land representative of the eco-system of the region. This 'biosphere' is designed not only to preserve the genetic diversity of plant and animal species in the region but to provide a laboratory in which experimentation into more rational forms of land use can be made. This project is being conducted in collaboration with the local farmers of the area.

None of these achievements have been gained easily. The constitutional policy of a six year non-renewable presidency brings with it administrative re-shuffling and consequent re-formulation of plans. The scientist, like anyone else, has little guarantee of continuity in his work. Time must be invested in convincing each new administration of the validity of his ideas, either through the cultivation of friends in power or through the publication of his work in a form amenable to the layman. Good though this may be for the purposes of public debate it consumes much of the energy of the successful man.

The scientist must also face the problems of the nation as a whole. He must be able to cope not only with present-day realities but with the contingencies which emerge as the population increases in numbers, literacy, and as more than ever move towards the city. Can the 'Universidad Nacional Autonoma de Mexico', for example, with 150,000 students already, accommodate the increasing demand for higher education without sacrificing teaching standards? Many see it as the race against time.

The scientist, it is hoped, will be instrumental in finding alternatives to mitigate the flood of people reaching the city, people who, each in their vague search for opportunities, constitute a major flight from reality when considered within the nation as a whole. The heroes of Mexican folklore are not Cortes and his men but Quauhtemoc, Father Hidalgo and Emiliano Zapata, men who dared confront the established order against impossible odds. The resourcefulness of the Mexican scientist in achieving what he has in the last ten years shows that such a spirit is still alive. Will it suffice for the coming era? □

All at sea



Thomas H. Jukes

THE muscular American was ahead of me at the cashier's desk in the hotel at Delhi on 3 June. He was arguing forcefully about charges on his bill for more than the actual number of local telephone calls that he said he had made. This auditing impressed me as being unusually precise, but perhaps, I thought, he is a professional accountant. The hotel won a computerized victory, because the local calls were metered to each room.

A few minutes later, I stood beside him in the front entrance to leave for the airport, and I noticed that his luggage was tagged "Agency for International Development". Spontaneously, I spoke to him; I said that I was interested in the public health work of AID, especially in malaria. He said that his was a different field, that of disaster relief. He invited me to ride to the airport, in his officially-provided car, where we would take the same flight, I to Bangalore, but he to an earlier stop, Hyderabad. He had to organize relief for victims of the 11 May cyclone that had devastated a large area in Andhra Pradesh.

I asked him what he had done lately, and he said he had recently been to Yugoslavia to help the survivors of an earthquake. He then launched a discussion of rehabilitation, how, for example, it was better that an Indian farmer receive a live replacement for a dead bullock rather than a gift of food. A variant, I thought, of the old adage about helping a starving man to catch fish.

George Beauchamp was my companion's name, and obviously he was used to taking charge. He deftly piloted me through the churning crowds that pack Indian airports and through the check-points to the departure area. We boarded the air-bus and went to our separate seat assignments after bidding each other goodbye.

As I sat in the plane, I suddenly realized what George Beauchamp must really be like. The surface that he had shown to me was that of a brisk, efficient and business-like public employee, arguing about his bill, and carefully attending to other details of the day. Impulsively, I got out of my seat, and went to find him. "George", I said, "I wish you all future success in your great work for humanity".

He thanked me, and then poured out his indignation at the current treatment of Vietnamese refugees, adrift on a hopeless sea, rejected at all attempts to land. He said that a few years ago he had assured people in Vietnam, in answer to their doubts, that the President of the United States would not let them down. "And now," I replied, "the Americans have forgotten the poem by Emma Lazarus". In answer, he passed me the book he was reading: It was the historical account of the ship that carried doomed and despairing refugees from Hitler from port to port, and finally back to extermination in the gas-chambers of Germany. George pointed to a verse on the front page; there were the wellknown lines I had just quoted to him:

"Give me your tired, your poor,
Your huddled masses, yearning to breathe free,
The wretched refuse of your teeming shore
Send these, the homeless, tempest-tossed to me."

But who will light, once again, the lamp beside the golden door for the despairing waifs from Vietnam? It has been predicted that one of each two will die by drowning. There is a ray of practical idealism in the darkness: a Hong Kong official has pointed out that it may be possible to turn the Vietnamese refugees "into productive labour to support our economic growth. But we must obtain a commitment from other nations to absorb the human wave...".

Will America respond? Numerous among those who make decisions for her to-day are the upwardly-mobile descendants of the "huddled masses" whom Emma Lazarus apostrophized. Have they forgotten the obligations of their heritage? □

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news and views

Peptides in profusion

from John S. Kelly

IN the coming year at least nine major international meetings are being devoted to the peptides present within neural tissue. The first * was a massive affair: for three days, an audience of at least 700 from all over Europe and North America listened to 28 invited speakers. Almost half the speakers devoted their attention to methodology and described how they had used their own particular skills to investigate one or more of these peptides. The others had clearly been instructed to concentrate on one peptide studied with a great variety of techniques, with only one of which they were acknowledged to be expert. By and large these speakers had been selected from the viewpoint that peptides form a new class of neuroactive substances to which certain generalities apply. For instance, peptides may fall into defined groups, each being derived by cleavage from a large precursor molecule. There also seemed to be support for the idea that peptides released from nerve terminals will in general not only mimic the actions of more conventional transmitters such as amino acids, acetylcholine and catecholamines, but that any new unexplained results are suggestive evidence that peptides may mediate their actions on the postsynaptic cell in novel, unconventional ways.

Since much of the work presented has already been published and will in any event be brought together in the proceedings, I shall draw attention here to some of the novel work mentioned only at the meeting and which I feel may prove to constitute the highlights of 1979.

Identification

In the methods section R. Elde (University of Minnesota, Minneapolis) drew attention to difficulties that have delayed the identification of peptide-containing elements at the electron microscopic level. Although he did not discuss methods based on the use of horseradish peroxidase to identify nerve terminals containing immunoreactivity, it is clear that this type of work is still hampered by the compromises needed to preserve the antigenicity of the peptide-containing tissue. Frequently the concentrations of aldehyde fixatives that are tolerated are too low to ensure the integrity of tissue ultrastructure. It therefore seems that although the reaction product in the horseradish peroxidase method is easy to identify at the electron microscopic level, the thin frozen sections which appear ideal for light microscopy are rarely adequate for ultrastructural studies. However, even in these studies, where the reaction product is in the terminals and in apparent association with synaptic vesicles or the presynaptic membrane, this may simply reflect the sites with which aldehydes react most readily. To overcome some of these difficulties Coulter and Elde (*J. Histochem. Cytochem.* in the press) have developed a method based on ultrathin serial sections cut from epoxy-resin-embedded material and the preparation of alternate sections for conventional immunofluorescence and electron microscopy. Although the resolution of the material is highly dependent on the thickness of the sections, single areas of fluorescence 0.2 μm in diameter can be recognised on photomicrographs enlarged to 8,500 times magnification and then identified on the corresponding electron micrographs. Many of the small fluorescent areas can be identified as synaptic vesicles. Coulter and Elde have yet to explore the possibility that post-embedding staining of serial sections will facilitate the localisation of a range of different peptides in adjacent or even the same ultrastructural element.

LHRH release explains enigma

In his review of the neurophysiological status of peptides, J. L. Barker (National Institute of Neurological and Communicative Disease and Stroke) drew attention to the findings of Jans and Kuffler *Proc. natn. Acad. Sci. U.S.A.* **76,** 1501; 1979), which suggested that the release of luteinising-hormone releasing hormone (LHRH) from nerve endings in the frog sympathetic ganglion might explain the enigma of the non-cholinergic, non-aminergic 'late slow e.p.s.p.' first described by Nishi and Koketsu *J. Neurophysiol.* **31,** 109; 1968). This late slow e.p.s.p. lasts for 5 min and is found in both the large B cells and the small C cells. Although substance P, neurotensin, bombesin, somatostatin, thyrotropin-releasing hormone and angiotensin I and II concentrations up to 0.1 mM had no effect, LHRH produced a slow depolarisation of the ganglion incubated in 1 μM dihydro-B-erythroidine and 0.1 μM atropine. The effect was reversible and the depolarisation evoked by 20 μM amounts occluded the nerve-evoked 'late slow response'. Similar depolarisations were evoked by smaller amounts (0.01 μM) of the more potent LHRH analogues (D-Ala^6,) LHRH and ethylamide (D-Trp^6, Pro^9) LHRH. The effect of these analogues was not blocked by the removal of Ca^{2+} from and the addition of Mg^{2+} to the media in concentrations that blocked synaptic transmission through the ganglia. Unpublished work shows both the peptide and nerve-evoked responses to be blocked by LHRH antagonists.

The presence of an LHRH-like substance in the lumbar chain of sympathetic ganglia has also been confirmed by radioimmunoassay using the highly specific antisera developed by Nett and colleagues (*J. clin. endocr. Metab.* **36,** 880; 1973).

Degeneration studies show the LHRH-like material to be confined to the spinal nerves that contain the axons which initiate the late slow e.p.s.p. . LHRH-like material is also present in perfusates of the ganglia made with isotonic KCl and the release seems to be calcium dependent. This discovery of LHRH-mediated potentials in the frog sympathetic ganglion draws attention to the possible role of peptides in transmission in the peripheral nervous system. Substance P and enkephalins are now well established as potent transmitters in the guinea pig myenteric plexus and there is still a number of non-cholinergic, non-adrenergic synaptic potentials to be investigated.

Cholecystokinin

Both J.W. Phillis (College of Medicine, Saskatchewan) and S.H. Snyder (Johns Hopkins University School of Medicine, Baltimore) drew attention to their work which shows fragments of cholecystokinin to have potent actions in the frog spinal cord (Phillis & Kirkpatrick *Can J. Physiol. Pharmac.* in the press) and to be located in specific sites throughout the central nervous system (Innis, Correa, Uhl, Schneider & Snyder *Proc. natn. Acad. Sci. U.S.A.* **76,** 521; 1979). Earlier, Rehfeld and his associates (Larsson & Rehfeld *Brain Res.* **165,** 201; 1979) showed the biologically active octapeptide of cholecystokinin to be located throughout

*A symposium on The Role of Peptides in Neural Function was held on 7–9 May at the National Institutes of Health, Bethesda, Maryland. It was sponsored by the Intramural Program of the National Institute of Neurological and Communicative Disorders and Stroke, and organised by J. L. Barker and T. G. Smith, who will edit the proceedings which will be published by Dekker.

J. S. Kelly is a senior member of the scientific staff of the MRC Neurochemical Pharmacology Unit, Cambridge, appointed Professor of Pharmacology at St. George's Hospital Medical School, London from 1 July 1979.

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the central nervous system. Within the spinal cord its localisation in the substantia gelatinosa is reminiscent of the distribution of substance P in fibres thought to be involved in nocioception. Furthermore, they have demonstrated similar immunoreactivity throughout the autonomic nerves and ganglia of the gut and have a considerable amount of biochemical evidence to suggest that the neurotransmitter released from these nerves is the terminal tetrapeptide common to cholecystokinin and gastrin (Trp-Met-Asp-Phe-NH_2). On the isolated perfused porcine pancreas, Rehfeld and colleagues have shown the tetrapeptide to be a highly potent and specific releaser of insulin, glucagon, somatostatin and pancreatic polypeptide and suggest that the tetrapeptide may be the neurotransmitter of the nerves which innervate the pancreatic islet cells. Dodd and Kelly *(Proc. Physiol. Soc.;* 1979) have confirmed and extended Phillis's suggestion, that the action of cholecystokinin on central neurones is excitatory, by showing that both the octapeptide and tetrapeptide fragments of cholecystokinin excite rat hippocampal neurones.

Retinal peptides

In his review of somatostatin as a neuroactive peptide, J.B. Martin (Harvard Medical School, Boston), drew attention to his work with Rorstad and Brownstein (*Proc. natn. Acad. Sci. U.S.A.* in the press), in which they demonstrate the presence of radioimmunoassayable and bioassayable somatostatin-like material in acid extracts of the retina by using the release of growth hormone from dispersed rat anterior pituitary cells in culture as their assay. Gel-filtration chromatography confirmed that 96% of the material from the retina could be eluted as a single peak identified by the addition of synthetic somatostatin to the system. Increased levels of somatostatin were found in the retina of rats with hereditary degeneration of the photoreceptor cells and in rats in which transection of the optic nerve one year earlier had led not only to complete atrophy of the optic nerves but also to degeneration of the ganglion cells. The somatostatin therefore seems to originate within the retina. This work is complementary to that of Marshak and colleages (Marshak, Yamada, Basinger, Walsh & Stell *Invest. Ophthalmol. vis. Sci., ARVO Abstr. No.25,85; 1979),* who have used similar techniques to demonstrate the presence of somatostatin-like immunoreactivity in extracts of the frog, rat and goldfish retina. They have extended this work on 10 μm cryostatic sections of the goldfish retina by using a conventional immunofluorescence method to show immunoreactivity in two types of amacrine cells and one type of ganglion cell. The processes of the cells ramify in the most distal and most proximal sublaminae of the inner plexiform layer.

A more extensive examination of the localisation of distinctive populations of various peptide-containing amacrine cells in the retina by Brecha, Karten and Laverack (*Proc. natn. Acad. Sci. U.S.A.* in the press) was not, however, mentioned at the meeting. Brecha *et al.* have shown enkephalin-like, substance P-like and neurotensin-like immunoreactivity in the avian retina to be localised in separate subpopulations of amacrine cells with processes that arborise in quite different regions of the inner plexiform layer. The distribution of amacrine cells containing enkephalin-like material is quite regular, with the greatest density in the central region where they are spaced at approximately 40-μm intervals. Enkephalin-like reactivity is also a feature of the amacrine cells of the turtle. Subpopulations of amacrine cells containing substance P-like reactivity are found in the retina of frogs, toads, rats and rabbits.

The presence but not the location of a Met-enkephalin-like material in the avian retina was earlier demonstrated by Humbert, Pradellos, Gros and Dray (*Neurosci. Lett.* **12**, 259; 1979) using highly specific radioimmunoassays in conjunction with high pressure liquid chromatography and gel filtration. Earlier, other workers reported the presence of thyrotropin-releasing hormone-like immunoreactivity in the rat retina (Schaffer *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 579; 1977) and showed the levels of this material to differ in the light and dark. One hopes that these new studies which show subpopulations of amacrine cells to contain at least five different peptides will go some way to elucidating the function of the 15 different types of amacrine cell which Cajal identified in the retina.

The discovery of a possible LHRH pathway in frog sympathetic ganglia, cholecystokinin pathways in the nerves to the islet cells of the pancreas and possibly the spinal cord and the presence of various peptides in the amacrine cells of the retina surely must lead to the conclusion that the peptides in the brain behave no differently from the other substances already thought to be established as neurotransmitters in the central nervous system. □

Atmospheric arsenic

from Peter Brimblecombe

ARSENIC is toxic. This much at least seems to have been known for a long time. Its environmental chemistry is intriguing, because not only does it occur in various oxidation states, but it is converted to volatile compounds by methylation in reducing sediments in much the same way as mercury. The toxicity and mobility of arsenic have made it the subject of many studies in specific ecosystems, but Walsh, Duce and Fasching have discussed the global cycle of the element in a recent issue of the *Journal of Geophysical Research* (**84**, 1710, 1719; 1979). Their two papers on tropospheric arsenic reflect an increased concern that anthropogenic emissions are significantly altering the distribution of trace elements in the environment.

Man's influence on the levels of airborne arsenic has been a matter of concern since the seventeenth century when John Evelyn wrote hopefully in his diary that the charring of London's coal would remove its 'arsenic malignity'. Salmon and his group at Harwell in a more recent study (*Sci. Total Env.* **9**, 161; 1978) have also implicated coal smoke in the elevation of the arsenic concentrations in the air over rural England. They have examined the long term trends in atmospheric trace elements at Chilton in Oxfordshire, through the use of airborne dust samples collected on filters between 1954 and 1974. The arsenic levels commenced at about 8 ng m^{-3} in the late 1950s and declined to slightly less than 5 ng m^{-3} by the early 1970s. These closely follow the concentrations of smoke in the atmosphere, both in the gradual decrease, and the seasonal pattern of high levels in winter and low values in the summer.

There are good reasons for such a correlation. Arsenic is present in coal at levels of about 25 p.p.m. and almost all of this is released during combustion. Of the toxic elements lead, thallium, cadmium and selenium, only lead is found at levels as high as that of arsenic in fly ash. Like these other low boiling point elements, arsenic concentrates preferentially on the smaller sized particles by vapour transport within the furnace. Goldberg has extended this notion to ambient temperatures, arguing that the relatively high amounts of these elements in the atmosphere might well be explained by evaporation from rocks (*Geochem. J.* **12**, 75; 1978) although in the absence of amplification mechanisms this contribution is probably insignificant.

The concentration of arsenic in rural air over England appears to be a few ng m^{-3}, while concentrations determined in remote areas are somewhat lower. (Table 1). The enrichment factors relative to crustal elements range between 23 and 1,940 with a geometric mean of 313. This is largely due to the fact that the arsenic is found associated with sub-micron particles in which the crustal reference

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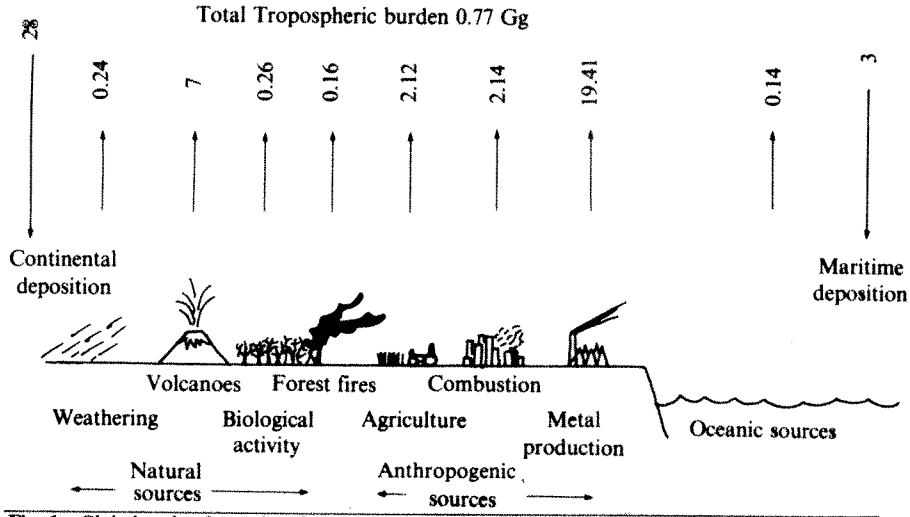


Fig. 1 Global cycle of arsenic (after Walsh *et al.*) Emission and deposition as Gg yr^{-1} (10^9 g yr^{-1}).

elements are depleted. The association with the small particle fraction hints at a vapour phase origin.

Walsh and his colleagues were at pains to distinguish between particulate and vapour phase arsenic (that is, < 0.05 μm). With the exception of some unusual events vapour phase arsenic accounted for only a few per cent of the atmospheric concentrations. This is to be expected as compounds with vapour pressures less than 10^{-11} atm are predominantly associated with particulates; the aerobically stable arsenic trioxide has a vapour pressure of only 6×10^{-12} atm. although they note that the ratio of particulate to vapour phase was inversely related to air temperature. In spite of the low concentrations of arsenic in the vapour phase it is important to remember that such low concentrations may reflect a species with a relatively short lifetime, but one which could be of considerable importance in distributing arsenic among the smallest aerosol particles.

The estimation of emission rates on a global scale is difficult because even where measurements are available the particle size is usually unknown. Natural sources are often even more difficult to evaluate because of the extremely low levels of arsenic involved. The largest natural source would appear to be volcanic (Fig. 1), but it is obvious that this could be subject to wide variations. Wind blown dust and particles from forest fires may both make contributions to atmospheric arsenic. The sea is an emitter and here volatilisation of the small amounts of naturally occurring methyl arsenic compounds may be significant. Biological sources account for about 3%, but even subtle changes in the assumptions about the release of particulates from plants and the methylation of arsenic compounds by bacteria could significantly change the importance of this contribution.

The construction of a global budget for arsenic emphasises the relatively large anthropogenic inputs to the troposphere. The principal use of arsenic is in the manufacture of agrochemicals; arsenic compounds have had a long history of use as insecticides, but during the past 20 years there has been a change from the use of inorganic to organic arsenicals, which can be applied at a much lower rate. There has also been a marked reduction in its use in insecticides, so that it has become largely restricted to herbicidal compounds. Good evidence for these changes are to be seen in the consumption of arsenic in the United States which halved between 1950 and 1970.

The largest man made contribution to the atmosphere arises from releases incurred in the manufacture of metals, as arsenic is frequently associated with ores. The largest emissions occur in the production of copper. Combustion of fuels is an important source, but coal is really the only fuel of significance, with the incineration of waste making an important contribution. The input from agricultural sources and processes such as cotton ginning is of about the same magnitude as the combustion source. the importance of these anthropogenic sources and the low emission rates from the oceans means that higher levels of arsenic are to be expected in the air over the northern hemisphere. The rates of input seem in reasonable agreement with the measured rates of removal. The tropospheric residence time determined from these rates and the atmospheric arsenic burden suggest a residence time of about 9 days, which is about that which would be expected for a tropospheric aerosol. □

Table 1 Concentrations of atmospheric arsenic

	($ng\ m^{-3}$)
Northern Canada	0.3
Sahelian dust plume	1.2
Volcanic plume	2.2 and 5.5
Antarctica	0.019
Bolivian mountains	1.4
Oceanic air	< 1.0

Anomalous H-2 antigens on tumour cells

from Kirsten Fischer Lindahl

FOUR years ago the idea was discussed (*News and Views* **254**, 653; **256**, 7; 1975) that some tumour-specific transplantation antigens (TSTA) were anomalous forms of the major histocompatibility antigens (H-2 in the mouse) found on normal cells of the individual in which the tumour arose. According to this view, the anomalous H-2 antigens would function as fire-alarms, signalling the transformation of a cell to the immune surveillance system.

A recent meeting* offered an overview of the evidence accumulated since then for the presence of anomalous H-2 antigens on tumour cells. The meeting confirmed published evidence that many tumours lose antigens: a heterozygous lymphoma cell line, LDHB, does so spontaneously at a very high rate in culture (B. Holtkamp, University of Cologne). On the other hand, tumours may gain H-2-like antigens. Some mouse tumour cells express H-2-like antigens which in normal spleen cells from the same mouse strain are detectable only in lysates and even then in lower amounts than in the tumour cells. This is the case for the DBA/2 lymphoma, SL2 (P. Robinson, Deutsche Krebsforschungszentrum, Heidelberg) and a retinal cell sarcoma (B. Bonavida (University of California, Los Angeles).

A spontaneous AKR ($H\text{-}2^k$) leukaemia, K36, apparently loses expression of antigens controlled by the $H\text{-}2K^k$ locus, as detected serologically or by T killer cells specific for the $H\text{-}2K^k$ locus. Furthermore, K36 appeared to express two different allelic products of the H-2D locus: the expected $H\text{-}2D^k$ antigen and new $H\text{-}2D^d$-like specificities. W. Schmidt (London Hospital Medical College) showed preliminary peptide maps of the $H\text{-}2D^d$-like antigen precipitated from the K36 tumour cells. These maps were very complex, presumably because of coprecipitated viral antigens; but they seemed to contain all the peaks found in a peptide map of $H\text{-}2D^d$ from normal cells.

The most persuasive evidence for anomalous specificities on tumour cells came from F. Garrido (Facultad de Medicina, Cordoba) and V. Schirrmacher (Deutsche Krebsforschungszentrum, Heidelberg) who have studied MCG4, a methylcholanthrene-induced BALB/c ($H\text{-}2^d$) tumour which has been converted to growth as ascites. The expected $H\text{-}2^d$ antigens were expressed only weakly on the tumour cells. Normal BALB/c mice

*A meeting on 'H-2 Antigens: Genetic Control and Expression on Normal, Tumour and Virally Infected Cells' was held at the London Hospital Medical College on 30 April-3 May. The meeting was sponsored by the European Molecular Biology Organisation and the Cancer Research Campaign of Great Britain.

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responded to the tumour cells with formation of cytotoxic T cells and of antibodies; the latter reacted with cells of almost all mouse strains (except H-2^d strains). Although the MCG4 cells did not express the private serological specificities of H-2^k, they did react with four different monoclonal BALB/c anti-H-$2K^k$ antibodies. Furthermore T killer cells which specifically attacked MCG4 cells also recognised H-$2K^k$ and H-$2D^k$. MCG4 may also carry some H-2^b-like specificities.

Susceptibility of the tumour cells to BALB/c killer cells raised against foreign H-2 antigens does not prove the presence of a particular gene product, because such killer cells are known to be very cross-reactive (more evidence on this point was presented by C. Neauport-Sautes, IRSC, Villejuif). More dramatic was the demonstration that Sendai virus-infected MCG4 tumour cells, but not virus-infected BALB/c lymphoblasts are susceptible to H-$2K^k$-restricted T killer cells from B10.A(4R) mice specific for Sendai virus. Because H-2 restricted killer cells are generally extremely specific, this was taken as evidence that MCG4 expressed the restriction specificity of H-$2K^k$. It must, however, be noted that two cases were presented at the meeting where precisely H-$2K^k$ restricted T killer cells could recognise other restriction elements: Elizabeth Simpson (Clinical Research Centre, Harrow), showed that male-specific killers also reacted with H-$2D^k$ male target cells, and W. Haas (Basel Institute for Immunology) has shown that even cloned killer cells specific for the hapten SP, killed hapten-coupled cells carrying H-$2D^k$, H-$2D^s$ or H-2^r antigens.

Are the new H-2-like specificities transplantation antigens? This seems to be the case for MCG4 which grows better in (BALB/c × H-2^k)F_1 mice than in BALB/c, the strain of origin. Immunisation of BALB/c mice with BALB.K (H-2^k) cells increases their resistance to C-1, another BALB/c tumour found by G. Parmiani and G. Invernizzi (Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan) to express H-$2K^k$ serological specificities. That this tumour was rejected equally efficiently by several (BALB/c × H-2^k)F_1 hybrid mice and by BALB/c may mean that the tumour cells have other TSTA in addition to the H-$2K^k$-like ones. H-2-like TSTA is one possible explanation why M. Bortin (Mount Sinai Medical Center, Milwaukee) could induce resistance in AKR mice to syngeneic leukaemia by immunisation with pooled allogeneic cells, carrying many different foreign H-2 antigens. On the other hand, E. Lennox, (MRC Laboratory of Molecular biology, Cambridge) could biochemically separate the TSTA of a couple of methylcholanthrene-induced tumours from their H-2 antigens by their different affinities for lectins.

In spite of the many reports of new H-2-like antigens on tumour cells, other laboratories study tumours without finding such evidence, and the devil was not short of advocates at the meeting. C-1 is the only tumour so far which has been shown to have the same isoenzyme markers as the presumed strain of origin. But neither this test, nor an analysis of frozen samples of the very mouse from which the tumour was taken would exclude a mix-up with another cell line from an H-2 congenic strain during the many passages in mice or in culture.

Special caution is needed in the interpretation of serological data. Are the antigens detected on tumour cells with anti-H-2 sera also structurally H-2-like, and are the antigens detected by syngeneic antitumour sera on normal mouse cells from other strains H-2-like, not only in strain distribution, but also in tissue distribution and ontogeny? P. Ivanyi (Central Laboratory of the Netherlands Blood Transfusion Service, Amsterdam) presented a cautionary example of an anti-H-2^b serum raised in a virus-free B10.A(H-2^a) line against C57BL/10 cells from a line carrying a milk-transmitted virus. Although these antibodies were completely removed by absorption with virus-free C57BL/10 cells, and thus were H-2^b specific, they also reacted with syngeneic B10.A cells from a line carrying the same virus. This antigen, which was dependent on the milk-transmitted virus, did not appear on spleen cells until day 17 after birth.

In no case has it yet been demonstrated that the new H-2-like specificities are actually encoded by chromosome 17 (as are the known H-2 specificities), but assuming this is so, how do they arise? It has been proposed that "the serologically detected allelic differences [of H-2] are actually the products of different, closely linked genes, and the genetic polymorphism is for the control of whichever of the genes is expressed" (Bodmer *Transplant. Proc.* **5**, 1471; 1973). The day now seems close when this hypothesis will be tested directly by isolation of the genes of the H-2 complex and sequencing of the DNA. According to this model, new H-2-like specificities on tumour cells could arise by derepression of the corresponding genes. The current argument against derepression (and incidentally against a mix-up of cell lines) is that the anomalous antigens had only some, not all, of the specificities of known H-2 haplotypes: an H-2^d tumour might cross-react with H-2^k at the T cell level but still lack the private serological specificities of H-2^k.

Mutation seems a likely cause of new specificities. In normal mice mutations can occur in H-2K and H-2D fairly frequently (4×10^{-5} per locus per gamete). Such mutants have been detected by their ability to cause skin graft rejection. Studies *in vitro* on cytotoxic T cell reactions between mutant and wild type cells (K. Melief, Netherlands Cancer Institute, Amsterdam) showed that such mutations give rise to multiple new specificities, some of which cross-react with known foreign H-2 antigens (Nabholz *et al. Immunogenetics*, **1**, 457; 1975). J. Martin (National Cancer Institute, Bethesda) showed that a transplacentally-induced lung tumour from a C3HfeB/HeN mouse — this strain apparently carries an H-$2K^k$ mutation — expresses wild type H-$2K^k$ antigens, as if it were a revertant, and grows progressively in normal C3H/HeN mice.

Considering the strong reactions elicited by mutant cells, the report by S. Nathenson (Albert Einstein College of Medicine, New York) on the amino acid sequence analysis of eight H-$2K^b$ mutants was indeed surprising. Seven of these had only a single amino acid substitution, and one had two, five residues apart. Furthermore, the changes were all conservative, such as methionine to leucine. Nathenson presented a model in which the H-2K molecule can be likened to a bow knot, the N-terminal end sticking out from the cell surface, the C-terminal end traversing the cell membrane, and two domains held by cystine bridges forming the loops. All the mutations were clustered around the two cysteines of the first domains, suggesting that this is the site of prime importance for T-cell recognition (some of the mutations even lead to changed restriction specificity for virus-specific killer cells). It is not known whether the many amino acid differences between alleles at the N-terminal end play a part serologically.

The reason for the strong T cell reactivity to H-2 mutants may be that T cells during their development are selected for their ability to see minor alterations of self. Presumably somatic mutants that express altered H-2 antigens arise all the time and are normally eliminated by the immune system. It seems structurally almost unavoidable that altered self H-2 antigens would share specificity with one or more foreign H-2 antigens, and priming against such altered self determinants might account for the high frequency of alloreactive T cells in a normal mouse. □

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Glueballs without gullibility

from Probir Roy

A glueball is a newly proposed form of neutral, unstable, quarkless hadronic matter and as such is currently engaging the attention of high energy particle physicists. Hadrons, the strongly interacting elementary particles, are assumed to be composed of pointlike (that is structureless) permanently confined constituents, the quarks. Apart from its spin of half, and fractional charges $\frac{2}{3}$ and $-\frac{1}{3}$

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in units of the proton charge, each quark carries three kinds of a quantum number called colour. Colour is necessary to satisfy the antisymmetrical Fermi–Dirac statistics property of quarks in describing baryons as three-quark composites, the three quarks being in a colour-antisymmetrical state. Colour is also carried in eight different kinds by massless boson quanta (of spin one) called gluons. These are responsible for keeping the quarks 'glued' together in a hadron and are themselves also confined. One special property of colour forces mediated by gluons is the self-coupling of three gluons carrying different colours. As a result, the colour forces become weaker and weaker at shorter and shorter distances. This phenomenon is poetically described as 'asymptotic freedom' and seems to have been observed experimentally (see *News and Views* **277**, 349; 1979). These forces are believed to become stronger at greater distances. The literary acronym for this behaviour is 'infrared slavery' and this is what is supposed to be responsible for colour confinement. According to this view all coloured objects (such as quarks and gluons) should be confined and only colourless hadrons should be observable.

Given the self-coupling of gluons, one can expect to see as real, unstable particles of zero or integral spin, colourless composites made out of gluons alone. These are the hypothetical (at present) glueballs. A glueball may be visualised as a composite of two or three gluons, in analogy to the quark–antiquark picture of a meson or the three-quark picture of a baryon. Several glueballs are likely to exist at different masses. It has been argued (*Nucl. Phys.* **B 130**, 328; 1977) that, owing to the asymptotic freedom of the colour forces, the decay of the lowest mass glueball into the usual hadrons ought to be somewhat inhibited. Consequently, it is expected to be more stable than a typical meson of the same mass. It should have a lifetime of the order of 10^{-21} rather than of the order of 10^{-23} as is usual for mesons. Higher mass glueballs will of course be generally more unstable.

The existence of glueballs was first proposed by Fritzsch and Gell-Mann in 1972. However, they remained an idle curiosity until an intriguing suggestion by Freund and Nambu (*Phys. Rev. Lett.* **34**, 1645; 1975) linked them to an important feature of high energy hadron–hadron collisions. The total cross-section for such collisions depends very weakly on the energy – a behaviour which had for many years defied any real understanding in terms of the otherwise successful Regge picture of exchanges of Regge trajectories on which hadrons lie. Freund and Nambu could provide a credible explanation if the dominant exchanges are those of new Regge trajectories on which real glueballs lie. They required glueballs to exist with spins two, one and zero with masses around 1.5 GeV.

The very existence of glueballs – let alone their masses – is still a matter of considerable theoretical controversy. It was proved by Coleman (*Comm. Math. Phys.* **55**, 113; 1977) that, if the colour forces are described by the laws of classical physics, glueballs cannot exist. In a classical world a glueball would show up as a concentrated colourless gluon-made packet of finite energy which does not spread. Coleman has shown however that all classical packet-like solutions of this theory radiate away their energy very fast. Of course, the nonexistence of classical glueballs says nothing about the existence of quantum glueballs. However, it suggests that a glueball would be a purely quantum occurrence. The question of the existence of a glueball in a quantum field theory is a difficult one but has nonetheless received some attention. Arguments have been given (though not universally accepted) for the existence of such objects in a quantum theory of colour forces in lower dimensions, but none has yet been found for the $(3+1)$ dimensional space-time in which we live.

Instead of pursuing such a general approach, model-builders have considered the glueball question in specific dynamical models. The bag model, which has been developed particularly by the MIT group, is one such scheme. In this, each hadron is considered as a bag in which quarks and gluons are kept confined by chosen boundary conditions. The bag generates volume pressure and/or surface tension to produce confinement. Gluon wavefunctions have been treated by analogy to the transverse electric and magnetic modes of electromagnetic fields confined within a spherical cavity. This approach leads to the prediction of glueballs with spins zero and two and with masses slightly less than 1 GeV.

Another set of models has been pursued by lattice theorists, led by Wilson. They quantise space-time and consider the theory of colour forces on a four-dimensional Euclidean (that is, $x_1x_2x_3x_4 = it$) lattice with nearly vanishing lattice spacings. Quarks and antiquarks, instead of being continuous fields, are attached to lattice sites and gluons identified with the links between such sites. A quarkless glueball then is a closed box of links with no loose ends. Such objects have been shown to exist with spins zero, one and two and their masses calculated by Padé approximant methods to be around 1.5 GeV.

Glueballs have been looked for experimentally although they are generally very difficult to produce in hadronic collisions. The gluons by and large play a passive role in these reactions. However, they do play a pivotal part in the decay of heavy quark–antiquark bound states ('quarkonia') produced by electron-positron annihilation at high energies: for

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Incest re-assessed

IN his advisory article on incest (*News and Views* **279**, 192; 1979), May reinforces his theoretical calculations on the deleterious effects of parent–offspring and brother–sister matings by reference to a Czechoslovakian study reported by E.O. Wilson. As presented, the study provides overwhelming evidence to support May's hypothesis; however a closer examination of the original data must raise a number of doubts as to the validity of the control group employed.

In her study (*Hum. Hered.* **21**, 108; 1971) Seemanova reported that the mean and the modal maternal ages of the non-incestuous control group at childbirth were 24.9 and 21 years while those of the father–daughter matings were 18.9 and 16 years and the brother–sister matings were 19.9 and 14 years. Since the Czech data referred to births from 1933 to 1970, the low modal ages of the mothers in the two incestuous groupings are particularly significant when viewed against the secular decline in the age of menarche during that period (Tanner *Growth at Adolescence* Blackwell, Oxford, 1962) and the high incidence of chromosomal anomalies and congenital malformations associated with pregnancies close to menarche (Carr *Adv. Hum. Genet.* **2**,201; 1971).

A second area of doubt concerns the imbalance between the biological fitness of the parents in the incestuous and the non-incestuous matings. Of the 141 incestuous mothers, 20 were mentally retarded (of whom two additionally were deaf-mutes, two had congenital syphilis and two were epileptics), a further two were deaf-mutes and three were schizophrenic. Among the 46 control mothers, a subgroup of the incestuous mothers, only two were mentally subnormal (one additionally being a deaf-mute) and two others were deaf-mutes. Similarly, of the 138 fathers in the incestuous matings 8 were mentally subnormal, 13 were chronic alcoholics, two had syphilis and four had committed suicide. In the non-incestuous grouping of 52 fathers, none were mentally subnormal, there were two chronic alcoholics and one case of polydactyly.

When considered in conjunction with the specific adverse social factors accompanying incestuous pregnancies, the conclusions on incest drawn on the basis of this particular study appear rather less convincing.

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example, the J/ψ at 3.1 GeV and the upsilon at 9.4 GeV. In these bound states the heavy quark–antiquark pair can decay hadronically only by annihilating into three gluons in much the same way that positronium disintegrates into three photons. The gluons of course have to evolve into colourless hadrons later. The glueball may lurk among such hadrons. One interesting decay mode is when J/ψ goes into an energetic photon plus hadrons. Here the heavy quark–antiquark pair in the J/ψ annihilate into two gluons and a photon. The two gluons could resonate into a glueball in which case the photon will be highly monochromatic. Experiments done by the PLUTO group at the German storage ring DORIS have yielded good quality data for precisely this situation. They rule out the existence of any glueball up to a mass of 1.2 GeV, thereby demolishing the lighter than 1 GeV glueball of the MIT bag model.

Turning to the much heavier upsilon, the three gluons from its decay are expected to generate jet-like hadronic fragments. Normal electron-positron annihilation into hadrons by way of a quark–antiquark pair) above 6 GeV produces two symmetrical jets opposite each other. This jet structure survives the final 'colour-washing' that creates only colourless hadrons out of the quark and the antiquark. As each of the gluons from upsilon decay will on average carry an energy of nearly 3 GeV, gluon jets can be expected there. The three gluons should emerge symmetrically with 120° angles between one another, and there is some preliminary evidence for such events from PLUTO (*CERN Courier* **19**, 108; 1979). Another interesting possible configuration is one in which an energetic gluon dashes off to one side leaving two less energetic collinear ones opposite. This will lead to two jets opposite each other that are asymmetrical. According to a recent suggestion (Roy & Walsh *Phys. Lett.* **78B**, 62; 1978), the energetic gluon can now easily induce the creation of a gluon pair from the vacuum and capture one of its members to form a glueball. The latter should show up as a prominent, not very unstable resonance in one arm of the two jets and should disappear off the upsilon mass. Quantitative estimates, albeit hand-waving, have been made and look extremely favourable for the production and detection of glueballs in this manner, if they exist at all. □

Polymers for blood replacement

from Paul Calvert

SYNTHETIC blood volume expanders are sometimes used in cases of extensive bleeding or burns where much plasma has been lost, or in cases of shock, where contraction of the capillaries increases blood pressure so that water is lost to the tissues, the blood thickens and circulation ceases. Restoration of normal volume can be achieved by natural plasma, solutions of plasma proteins or by solutions of polymers such as dextran. Where blood or plasma are readily available the main reason for using a synthetic expander is to avoid the risk of hepatitis. On the other hand, in countries where blood supplies and storage and separation facilities are more limited, synthetic substitutes should be more important. So, recently published Chinese work on a volume expander derived from corn starch is of interest as an application of science to the needs of a developing country.

Blood has such diverse functions that it is difficult to imagine a completely satisfactory substitute. The role of a volume expander is simply to maintain the correct water balance between blood and the tissues and to allow continued transport of nutrients and gases. The water balance is determined by the osmotic pressure between blood and tissues, and the hydrostatic pressure difference. Lost volume can be restored by addition of an isotonic electrolyte solution such as Ringer's but this does not give the correct osmotic pressure balance as the electrolytes pass readily across membranes into the tissues. It is necessary also to restore the colloidal osmotic (oncotic) pressure normally provided by serum albumin which cannot cross the membrane and so balances the osmotic pressure of the macromolecular cell contents. In practice it does not seem to be agreed whether polymeric (or 'colloidal') solutions work better than salt solutions but they are certainly better in principle (see Pennell, in *Bibl. Haemat.* **29**, 883; 1968).

Solutions of a range of water-soluble polymers have been tried as volume expanders. The chief requirements are that the polymer is of sufficiently high molecular weight to prevent its loss through the kidneys, that it resists degradation and causes no harmful reactions. Dextran is chiefly used but hydroxyethyl starch has had successful clinical trials and polyvinyl pyrrolidone has been tested (Ricketts, *Brit. J. Anaesth.* **45**,958; 1973). Dextran has been used for about 30 years and its side effects are well studied (Gruber & Messmer, *Prog. Surg.* **15**, 49; 1977); Grönwall *et al.*, *Bibl. Haemat.* **29**, 874; 1968). High molecular weight dextrans cause clumping of red cells (rouleaux formation) though, interestingly from the colloid science viewpoint, molecules of low molecular weight lower even the natural degree of clumping. Reduced clumping leads to lower blood viscosity, promotes blood flow and exchange of nutrients with tissues and so is beneficial in shock cases. High molecular weight dextran has anticoagulant activity and it can be used for this purpose after surgery. Low molecular weight dextran is antigenic but there is no evidence for this with clinically-used dextran, although allergic reactions occasionally occur. Various dextrans in the molecular weight range of 40,000—110,000 are used depending on the balance of effect desired.

The Chinese work is from the Institute of Organic Chemistry in Shanghai, one of the three big polymer research groups in China. They describe the production of sodium carboxymethyl amylose by carboxymethylation of amylose from corn starch (*Acta Chimica Sinica* 36, 49 (1978). The molecular weight of the product was characterised by ultracentrifugation, solution viscometry, fractional precipitation and osmotic pressure. Using ^{14}C-labelled carboxymethylation followed by hydrolysis and paper chromatography the reaction was shown to occur primarily on the 2-oxygen. The degradation rate of the polymer in blood decreases with increasing carboxymethylation. A 5% solution of the polymer (molecular weight 60,000, 0.4 carboxyl methyl groups per saccharide) in 0.9% saline is now being tested in animals. It maintains blood volume for 6 h, is 90% lost in 24 h and is said to lack the immunological reactions of dextran. A possible snag, however, is that similar charged polymers are reported to enhance clumping (see Rickets *op.cit.*)

This work is interesting in the Chinese context for a number of reasons. It is a piece of careful, thorough polymer science. It describes the development of a new material from an available resource to replace the complex fermentation process needed for dextran. The work is original, whereas much of polymer science in China has been devoted to reproducing Western materials from patents. Also the work was done between 1961 and 1964 and has waited 15 years to be published. It shows that a relatively modest project can produce something adapted to local needs.

Most Western interest in this area seems to be in the more dramatic problem of an artificial blood substitute capable of carrying oxygen, particularly for organ storage. Geyer (*Drug Design* **VII** (ed. Ariens) Academic Press, 1976), has reviewed these with particular regard to emulsions of perfluorochemicals. With these he was able to replace completely the blood of rats so that they survived in 100% oxygen for 10 days until their red cells regenerated and they could be returned to air. Interestingly, these bloodless rats could also survive in 10% carbon monoxide. Cell-free haemoglobin solutions have also been used for blood replacement but they are degraded and must be continually transfused. There is obviously potential here for an oxygen-chelating polymer but a suitable molecule does not yet seem to be available □

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review article

Hormonal regulation of peptide receptors and target cell responses

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Regulation of plasma membrane receptors for peptide hormones by the prevailing ligand concentration often causes altered target cell function. Receptor number is determined by hormone-induced changes in membrane conformation, irreversible ligand binding, and processing of ligand–receptor complexes during hormone action.

THE ability of target cells to respond to changes in ambient ligand concentration by regulating the number and/or affinity of their surface receptors is now so widely documented in hormone-regulated tissues that the absence of receptor regulation is now considered an unusual finding. Self-regulation of membrane receptors, originally observed with surface antigens, has been demonstrated in cells exposed to ligands including neurotransmitters, peptides, protein and glycoprotein hormones, and surface-modulating factors such as lectins and immunoglobulins[1]. Desensitisation of cellular responses by hormonal ligands has been observed in certain target cells, and in some cases has been correlated with loss of receptor sites—'down-regulation'. The mechanism of receptor regulation by homologous ligands probably depends on the ability of peptide hormone receptors to behave as mobile entities within the plasma membrane. The mobility of hormone receptors is evident during their interactions with membrane effector proteins such as adenylate cyclase, and in ligand-induced patching and capping of receptors[2]. Investigations of the fate of receptors during ligand-induced regulation have drawn attention to the ability of peptide hormones to enter their target cells, in contrast to the previous view that internalisation was a minor or negligible aspect of protein hormone action[3].

The effects of hormone stimulation on target cells include a complex series of events that not only evoke the characteristic cellular response, but also regulate the components of the activation pathway and modify the response to subsequent hormone stimulation. This article reviews recent work on the regulation of hormone receptors, with emphasis on the mechanisms and consequences of receptor regulation and desensitisation in target cells for insulin, catecholamines, angiotensin II and gonadotropins.

General aspects of receptor regulation

The concept of intrinsic membrane proteins diffusing laterally within the lipid bilayer[4] led to the notion that the mobility of receptors within the membrane was responsible for features of peptide hormone action such as the activation of common membrane responses by multiple independent ligands[5], desensitisation, negative cooperativity, and the dependence of receptor affinity on the presence of membrane effector systems[6]. One prediction of the hypothesis of mobile receptors is that the receptors should be physically independent of adenylate cyclase in systems where the response is effected via adenylate cyclase. That prediction is borne out by the resolution of the two activities in detergent-solubilised membranes from cells bearing receptors for glucagon[7], luteinising hormone (LH)[8], or catecholamines[9], by the coupling of receptors from one cell type with the heterologous adenylate cyclase of another cell type when the two are fused[10,11], and by the functional transfer of solubilised ovarian LH receptors to isolated adrenal cells[12]. This evidence suggests that there is a universal mechanism of coupling between receptors and adenylate cyclase but characterisation of solubilised receptors[13–17] has not yet revealed the molecular basis of the supposed coupling.

Edelman[2] has suggested that cell growth, movement and recognition are coordinated by a supramolecular complex of cell-surface receptors and submembranous fibrillar structures, by which changes (surface modulation) in receptor conformation, mobility and distribution lead to changes in the associated cytoplasmic components. These structural interactions are probably responsible for the aggregation (clustering, patching and capping) of receptors that is induced by cross-linking agents such as divalent antibodies and multivalent lectins.

The clustering of receptors into surface aggregates is an important prelude to internalisation of hormone–receptor complexes, as discussed below but may also be important in the acute phase of hormone action. Thus, bivalent antibodies to the insulin receptor can both bind to receptors and exert insulin-like actions in isolated adipocytes[18]. In contrast, monovalent Fab' antibody fragments, although binding to the receptor, do not evoke a biological response. Addition of anti-$F(ab')_2$ antiserum cross-linked the Fab' receptor complexes and restored the insulin-like action of the antibody; also, anti-insulin antibodies enhanced the biological activity of low concentrations of insulin. These effects were independent of microfilaments and microtubules, and indicated that receptor cross-linking or aggregation is important in the insulin-like effects of anti-receptor antibodies and perhaps in the actions of insulin itself. This proposal is consistent with findings that insulin receptors are mobile[19] and can form clusters[20] in the cell membrane, and with the ability of lectins[21] to exert insulin-like effects.

Many peptide hormone receptors are regulated by the homologous hormone, and sometimes by other hormones. Increased hormone concentrations usually cause a decrease in the respective receptors, as demonstrated originally by Roth and co-workers for the insulin receptor of liver[22,23] and cultured lymphocytes[24]. Such down-regulation of insulin receptors occurs in human obesity[25] and in genetic or induced obesity in rodents[26], and probably reflects the action of excess circulating

insulin to decrease insulin receptors. Such receptor loss seems to be a general consequence of the raised blood insulin levels associated with insulin-resistant states. The converse effect, of increased insulin receptors in states of reduced insulin secretion, has been observed in the liver of diabetic hamsters and rats[27,28]. Other receptors down-regulated by the homologous hormones include those for growth hormone, thyrotropin-releasing hormone, catecholamines, gonadotropins and glucagon[29-33]. Certain peptide receptors, such as those for prolactin[34] and angiotensin II[35], may increase in number after exposure to elevated hormone concentrations, but the more common response is loss of receptors from the cell surface. Some receptors, notably those for insulin, may exhibit negative cooperativity on exposure to increased ligand concentrations. This represents an additional form of receptor regulation, in which partial occupancy leads to decreased affinity of the residual sites, with acute reduction of target cell sensitivity in the presence of increased insulin concentrations[36]. Hormone-induced receptor loss has sometimes been found to be dependent on protein synthesis[30,36] and sometimes not[29,31], but the return of peptide receptors seems to require new protein formation[29]. The details of the processes leading to receptor depletion and replenishment are still not clear.

An important factor in receptor regulation is the incompletely reversible nature of the complex formed after the initial phase of hormone binding to the receptor. Analysis of hormone–receptor interactions has traditionally involved methods based on the assumption that the complex is reversible, but kinetic studies have frequently shown the hormone–receptor complex to have an initial rapid phase of dissociation, followed by a prolonged phase of slow and incomplete reversal[37]. This suggests that the initial interaction with hormone leads to a conformational change in the receptor that results in tighter binding of the ligand. One view is that the higher-affinity binding state is brought about by interaction of occupied receptors with adenylate cyclase[38], but similar changes are evident in receptors considered not to operate through adenylate cyclase, such as insulin[39] and angiotensin II[40]. In any case, prolonged occupancy by undissociated ligand is always a potential cause of functional receptor loss, and should be evaluated during studies of receptor regulation. For this purpose, uptake and release of labelled ligand and direct assay of occupied and free sites should be performed, since multiple washing steps and unchanged basal adenylate activity do not exclude residual occupancy by the agonist ligand. As well as causing apparent receptor loss by masking free binding sites, continued occupancy by ligand favours the probability that hormone–receptor complexes will be degraded or otherwise processed and destroyed. This could involve occlusion within the cell membrane or shedding from the cell surface, but accumulating evidence favours a process of endocytosis and subsequent lysosomal breakdown of the internalised hormone–receptor complexes.

Effects of receptor regulation on cell responses

Implicit in the interpretation of receptor regulation has been the expectation that end-organ responsiveness will be altered by changes in receptor concentration. If no other changes occurred, a reduction in membrane receptors would be expected to decrease target-cell sensitivity in tissues containing abundant 'spare' receptors, and to decrease the magnitude of the target-cell response when receptor concentration became the limiting factor in the ability to elicit a hormonal response. The general function of receptor regulation as a mechanism to decrease target-cell sensitivity in the face of high hormone concentrations could have certain biological advantages. An obvious example is blood glucose homeostasis, in which decreased insulin receptors and reduced sensitivity of peripheral tissues to insulin would blunt the effect of hyperinsulinaemia on circulating glucose concentration. Receptor regulation could also minimise or buffer the cardiovascular responses to excessive levels of angiotensin II and catecholamines. This could account for the marked resistance to angiotensin II that occurs in many clinical states of increased renin secretion. However, the biological function of receptor regulation is less evident for other stimulators, such as growth hormone and gonadotropins, which exert more long-term biological effects on their target cells.

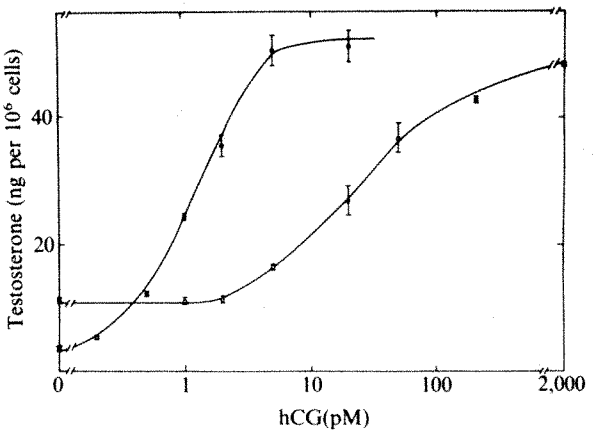


Fig. 1 Decreased sensitivity of testosterone responses of desensitised Leydig cells to gonadotropin stimulation *in vitro*. Cells were prepared from testes of adult male cats treated 48 h earlier with 10 μg hCG by subcutaneous injection (O), or from untreated controls (●). The loss of LH receptors causes a marked increase in the ED_{50} for hCG-stimulated testosterone production.

The effects of receptor regulation on cellular responses to hormones have been studied in several tissues, particularly target cells in which hormone action is mediated by the adenylate cyclase–protein kinase system. Intact cells are particularly useful for such studies, because hormone binding and cyclic AMP production can be correlated with the ultimate cell response[41]. Also, the cyclic AMP produced during hormonal stimulation of isolated cells frequently accumulates in the medium, providing a sensitive index of receptor activation. An important distinction to be made in the study of such hormone-treated tissues is the difference between acute desensitisation (usually of adenylate cyclase) without receptor loss, and the development of true receptor loss, which is a delayed and more slowly reversible consequence of hormone–receptor interaction.

Analysis of the level at which regulation of surface receptors causes altered metabolic responses depends on the ability to measure changes in the hormone-activated pathway that is triggered by receptor binding. For several peptide hormones, such as insulin, growth hormone and prolactin, the immediate mechanism of action on target cells is still unknown. Thus, although insulin indirectly modulates several membrane enzymes, including adenylate cyclase, phosphodiesterase and ATP-ase, the relationship of these effects to its actions on membrane transport systems and anabolic responses remains unclear[42]. Where early events in hormone action have not been identified, the effect of receptor regulation on cell responses can be examined by measuring changes in transport systems and biosynthetic processes. This approach has been used to analyse the effects of insulin receptor regulation in adipocytes or hepatocytes, on glucose or amino acid transport and lipid synthesis[43,44].

The consequences of receptor regulation on target cell function have been extensively studied in steroidogenic cells of the testis and ovary, which respond to trophic hormones with specific steroid secretion via an adenylate cyclase-dependent mechanism. The Leydig cell of the testis and the luteal cell of the ovary possess high-affinity LH receptors that are readily measured with ^{125}I-labelled human chorionic gonadotropin (hCG) and are coupled to steroidogenesis through the adenylate cyclase–cyclic AMP–protein kinase pathway[41,45]. In these cells it is possible to analyse several levels of response, from receptor

occupancy through cyclic AMP production and kinase activation, to stimulation of pregnenolone production and subsequent biosynthetic steps leading to progesterone and testosterone production. Analysis of these processes in enzyme-dispersed cells during hormone stimulation permits evaluation of the changes induced by gonadotropin levels similar to those regulating cell responses *in vivo*. When cells are prepared after desensitising doses of gonadotropin, marked changes in LH receptor content and metabolic responses to hormone stimulation are evident *in vitro*. As described below, such desensitised cells provide evidence for several consequences of receptor regulation induced by hormone treatment in the intact target tissue.

Regulation of β-adrenergic receptors and adenylate cyclase

The actions of catecholamines on receptors and adenylate cyclase have been analysed in avian and amphibian erythrocytes, and in cultured cells bearing β-adrenergic receptors. The ultimate cellular actions of catecholamines in most of these test systems are not yet known, although an association with ion transport has been described in the avian erythrocyte[46].

Measurement of β-adrenergic receptors has been performed by binding assays with labelled antagonists, either ^{125}I-hydroxybenzylpindolol[47] or ^{3}H-dihydroalprenolol (DHA)[48]. In frog erythrocytes, binding studies with DHA have shown available β-adrenergic receptors and adenylate cyclase responses to be reduced by previous exposure to catecholamines[31,48]. In contrast, the β-receptors in turkey erythrocytes do not exhibit altered binding properties on exposure to adrenergic stimuli (G. D. Aurbach, personal communication). The desensitising action of catecholamines in frog cells can be blocked or reversed by the β-adrenergic antagonist propranolol, which does not itself cause desensitisation. These findings indicate that desensitisation or tolerance to catecholamines is caused by a reduction in the number of functional β-adrenergic receptors. It is likely that chronic occupancy by agonist rather than an active process of receptor loss is responsible for the decreased receptor number, as the ligand-induced changes in receptor number and adenylate cyclase were not altered by treatment with cycloheximide to inhibit protein synthesis[49]. Desensitisation was also observed in erythrocyte membranes exposed to β-adrenergic agonists *in vitro*, but was not produced by antagonists such as propranolol, and could be reversed by guanyl nucleotides[50]. These findings suggest that receptor binding of agonist is followed by coupling of the complex to adenylate cyclase, a process accompanied by conformational changes that initially activate the enzyme and subsequently produce the refractory state known as desensitisation. A requirement for such coupling would account for the inability of antagonists to induce desensitisation, and of guanyl nucleotides to reverse desensitisation by enhancing dissociation of the receptor-bound agonist.

Subsequent studies on the interaction of erythrocyte receptors with a labelled β-adrenergic agonist, ^{3}H-hydroxybenzylisoprenaline (HBI), revealed that dissociation of bound agonist is much slower than that of antagonists such as ^{3}H-DHA, and is significantly accelerated by guanyl nucleotides[51]. The persistence of agonist binding could be responsible for desensitisation of β-adrenergic receptors in cell membranes exposed to agonists *in vitro*, and for the apparent loss of receptors measured with labelled antagonists. Formation of the high-affinity complex between β-receptor agonists and adenylate cyclase-coupled receptors is dependent on magnesium, which increases receptor binding affinity for agonists but not for antagonists[52]. This finding, and the absence of a magnesium effect in solubilised receptors, implies that coupling of receptors to adenylate cyclase is involved in the formation of the high-affinity agonist binding state. Confirmation of this comes from the finding that mutant lymphoma cells deficient in adenylate cyclase did not lose receptors on exposure to isoprenaline[53]. In contrast, wild-type cells and mutants deficient in protein kinase, showed loss of β-receptors and adenylate cyclase responses after incubation with isoprenaline, although the kinase mutants lacked the induction of phosphodiesterase seen in normal cells. Thus, activation of adenylate cyclase, but not changes in protein kinase and phosphodiesterase, appears to mediate the decrease in β-receptors and cyclic AMP responses of cells exposed to catecholamines. However, solubilised turkey erythrocyte membranes showed increased affinity for agonists, but not for antagonists[54], so that the formation of a high-affinity agonist binding complex may not depend only on coupling to adenylate cyclase.

Recently, comparison of agonist and antagonist binding assays has shown discrepancies in receptor numbers measured after β-adrenergic desensitisation of frog erythrocytes with isoprenaline in *vitro*[55]. Whereas the loss of available sites measured with the labelled agonist ^{3}H-HBI was correlated with loss of adenylate cyclase responses, binding of the labelled antagonist ^{3}H-DHA was significantly less impaired. In a related study, desensitisation of frog cells by exposure to the radiolabelled agonist led to a loss of antagonist binding sites that exceeded the measured occupancy by residual agonist[56]. These findings raise the possibility that prolonged high-affinity binding of agonist does not entirely account for the loss of β-adrenergic receptors measured by binding studies with labelled alprenolol. Whether this indicates that antagonist sites are regulated independently of agonist sites has yet to be determined. However, it is clear that occupancy of β-adrenergic receptors by agonist ligands is of higher affinity than anticipated from antagonist binding studies, and that *in vitro* desensitisation of adenylate cyclase is probably associated with sustained receptor occupancy.

A physiological role for regulation of β-adrenergic receptors was suggested by the observation that sympathetic control of *N*-acetyltransferase and melatonin synthesis in the rat pineal gland was modulated by marked changes in β-adrenergic receptor number and adenylate cyclase responsiveness during the diurnal light–dark cycle[57]. Pineal glands of light-exposed rats contain more β-receptors and adenylate cyclase than glands from animals kept in the dark, and treatment with isoprenaline causes marked loss of both receptors and enzyme responsiveness to β-agonists. These changes seem to be involved in the varying sensitivity of the pineal gland during light–dark cycles, by modulating the capacity to form cyclic AMP in response to β-adrenergic stimulation. The desensitisation of pineal receptors by catecholamine, as in the frog erythrocyte[48], was not affected by cycloheximide, again suggesting that inactivation and reactivation, rather than receptor turnover, were responsible for the changing receptor concentrations. Modulation of β-adrenergic receptor binding of agonist ligands has also been noted in the myocardium, where guanyl nucleotides reduce binding affinity for agonist but not antagonist ligands[58]. In this tissue, muscarinic cholinergic agents antagonised the GTP-induced reduction in β-receptor affinity for isoprenaline, and attenuated the combined effect of GTP and isoprenaline on myocardial adenylate cyclase. These findings suggest that cholinergic agonists can act through the muscarinic receptors to regulate both β-receptors and adenylate cyclase by modulating the effects of GTP. This action is consistent with the known ability of the cholinergic system to attenuate the cyclic AMP-mediated effects of sympathetic stimulation.

Regulation of gonadotropin receptors and gonadal steroidogenesis

In the Leydig cell of the testis and the luteal cell of the ovary. LH binds to plasma membrane receptors with high specificity and affinity ($K_d = 0.1$ nM). Hormone binding is followed by activation of adenylate cyclase and increased formation of cyclic AMP, which stimulates production of testosterone in Leydig cells and progesterone in luteal cells[59]. Administration of ovine LH or hCG to rats is followed by prolonged loss of LH receptors in gonadal homogenates[60] and membrane preparations[61], as well as in purified Leydig[62] and luteal cells[63]. The decrease of receptor number is both time- and dose-dependent[60–64], and is

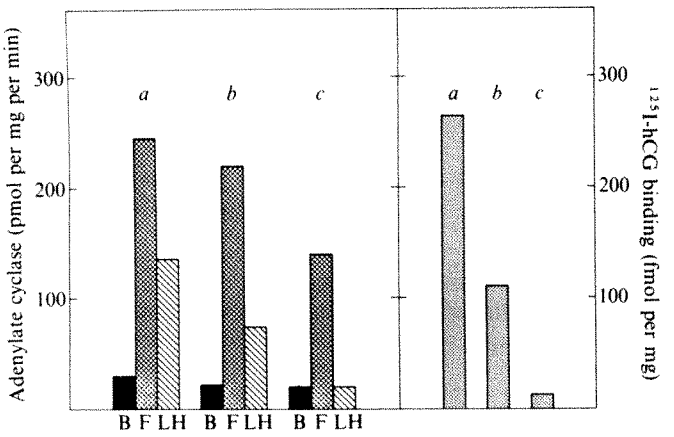


Fig. 2 Concomitant loss of LH receptors (right) and of adenylate cyclase responses to LH (left) in ovarian homogenates from rats treated 24 h earlier with 0.1 μg (*b*) and 20 μg (*c*) of hCG. The control levels of binding and adenylate cyclase (*a*) are shown at the left of each panel. For adenylate cyclase activity, basal (B), fluoride-stimulated (F) and gonadotropin-stimulated (LH) values are shown.

accompanied by prominent changes in the cyclic AMP and steroid responses of the desensitised cells[63,65]. The expected effect on Leydig cell sensitivity to gonadotropins after receptor loss (an increase in the ED_{50} for steroid production) is observed after subcutaneous treatment with hCG, as shown in Fig. 1. However, more marked receptor loss, particularly after intravenous treatment with hCG, is accompanied by a decreased maximum steroid response to subsequent hormone stimulation.

Of particular significance in studies of receptor regulation is the demonstration that the initial loss of LH binding capacity is due to occupancy of LH receptor sites by the desensitising dose of the hormone used to treat the animal[61,65]. This has been shown by administration of ^{125}I-hCG, and directly by elution and radioimmunoassay of receptor-bound hCG after treatment with the unlabelled hormone. The initial occupancy stage, which lasts for up to 24 h, is followed by prolonged loss of LH receptors which is maximum after 1 to 2 d and lasts for up to 8 d in both testis[65] and ovary[66]. After treatment with a low dose of hCG (200 ng), maximum receptor loss of about 60% is evident at 4 d. At this time, the decrease in binding cannot be due to occupancy, and represents depletion of LH receptors from the plasma membrane. The extent of true receptor loss is much greater than the initial occupancy by administered hormone[61,65], as also noted after growth hormone binding to cultured lymphocytes[29]. These findings suggest that the initial occupancy induces an active or cooperative process in the cell membrane that augments the degree of receptor loss. In both testis and ovary, the period of net receptor loss is followed by return of the receptor number to normal over the next 6 to 10 d. The reappearance of gonadotropin receptors probably depends on synthesis of new receptor protein rather than recycling or reactivation of the lost receptor sites.

Receptor regulation in the testis and ovary has also been demonstrated following elevation of endogenous LH levels by administration of gonadotropin-releasing hormone (GnRH or LHRH) and its analogues. These studies also revealed marked and reversible loss of prolactin receptors, and regression of testis function[67–69]. Administration of GnRH to pregnant rats caused a fall in progesterone secretion and termination of pregnancy[70], probably due to functional luteolysis following loss of ovarian receptors for prolactin and LH, and desensitisation of the corpus luteum of pregnancy[71]. In addition, a direct effect of GnRH on the ovary may contribute to these regressive changes.

The disappearance of functional LH receptors, whether due to initial occupancy or subsequent receptor loss, is accompanied by decreased ability of LH to stimulate adenylate cyclase activity and cyclic AMP formation in the ovary[61] and testis[65]. At the time of true receptor loss, the receptor number correlates with the degree of adenylate cyclase stimulation by LH in ovarian particles (Fig. 2). Subsequently, the recovery of receptors is accompanied by restoration of LH-stimulation of adenylate cyclase. Modulation of receptor number and adenylate cyclase stimulation has been shown with low doses of hCG, equivalent to the LH levels that occur during the normal reproductive cycle in the rat. Changes in adenylate cyclase responsiveness to LH after both induced and spontaneous ovulation[72,73] suggest that receptor regulation and its consequences play a part in the regulation of ovarian function. Apart from their relevance to receptor regulation, these studies provide evidence that the binding sites measured with ^{125}I-hCG represent the functional LH receptors that are coupled to adenylate cyclase and the steroid activation mechanism.

The loss of adenylate cyclase responsiveness to gonadotropins during receptor occupancy shows that the gonadal enzyme, like that of the frog erythrocyte, becomes refractory after the initial hormone–receptor interaction. At this time, free LH receptors are still present but cannot undergo functional coupling to the catalytic unit of the enzyme. Transient loss of the fluoride effect on adenylate cyclase also occurs during desensitisation by hCG, with recovery in 24 to 36 h[74], corresponding closely with the occupancy phase of the LH receptors. Adrenaline can also stimulate adenylate cyclase in the ovarian luteal cell, and during hCG-induced desensitisation, when no loss of β-adrenergic receptors could be detected, there was decreased enzyme response to adrenaline[75]. These findings suggest that hormone interaction with the LH receptor is followed by a transient refractory state in which the system cannot be stimulated by occupancy of free LH receptors or β-adrenergic receptors, or by fluoride. This refractory state is reversed within 24 h, as shown by the return of the fluoride and adrenaline responses. In contrast, the loss of LH receptors results in a prolonged loss of receptor-mediated LH activation of adenylate cyclase for several days.

Changes in Leydig and luteal cell LH receptors and adenylate cyclase activation have marked effects on the responses of the intact cell to gonadotropins[62,63,76]. The production of cyclic AMP in desensitised cells is generally well correlated with changes in receptor number in both cell types, and steroid production is also reduced in proportion to receptor loss. In the ovary, treatment with 2 μg of hCG, enough to produce > 90% reduction in the luteal LH receptors, caused almost complete loss of cyclic AMP and progesterone responses in isolated cells stimulated with gonadotropin *in vitro*. A lower dose of hCG (100 ng) caused a 50% fall in the receptor number and produced a similar decrease in both cell responses. In the Leydig cells of the testis, there was again a close correlation between decreased receptor number and loss of the steroid response to hormone stimulation. However, loss of steroid production was not fully accounted for by loss of LH receptors and the consequent failure of cyclic AMP generation, because addition of dibutyryl cyclic AMP or cholera toxin to over-ride the block in cyclic AMP synthesis failed to stimulate steroid production[62]. In the ovary, the loss of steroid response to hCG in partially desensitised cells was also seen with cholera toxin and dibutyryl cyclic AMP, and in fully desensitised cells these two stimuli had no effect on progesterone production[63].

The nature of the additional block beyond the level of cyclic AMP formation was characterised in the Leydig cells from hCG-treated rats. Depending on the desensitising dose of hCG, decreased conversion of steroid precursors to androgens was evident at specific points in the biosynthetic pathway[77]. Although high doses of hCG caused a decrease in the formation of pregnenolone from cholesterol, lower doses of hCG did not impair the side-chain cleavage step while still causing a marked fall in androgen production. Detailed analysis of the testosterone biosynthetic pathway indicated that the 17, 20 desmolase step becomes rate-limiting in the desensitised state, with accumulation of 17-hydroxylated precursors. Also, reductions in 3β-hydroxysteroid dehydrogenase and 17-hydroxylase activi-

ties were suggested by the increased levels of pregnenolone and progesterone formed in desensitised cells. The mechanism by which gonadotropin treatment causes these reductions in enzyme activity has not been elucidated, but may depend on local inhibitory feedback by oestrogen produced during the initial stimulation by the desensitising dose of hCG. It is also likely that maintenance of the steroid biosynthetic pathway requires the 'trophic' influence of LH, and that receptor loss impairs this long-term action of gonadotropins as well as their acute effect on steroid biosynthesis and secretion.

Regulation of angiotensin II receptors and adrenal function

The renin–angiotensin system plays a major part in the control of aldosterone secretion during changes in electrolyte intake and sodium balance. This action is exerted through the binding of angiotensin II to specific receptors in the adrenal zona glomerulosa cells, with stimulation of aldosterone production via a non-cyclic AMP-dependent mechanism. In addition to this acute action on steroid secretion, the renin–angiotensin system exerts an important trophic action on the glomerulosa zone, as seen in prolonged sodium deficiency. The adrenal angiotensin II receptors are predominantly in the glomerulosa layer, and exhibit high-affinity binding of agonist and antagonist derivatives of angiotensin II, with association constants in proportion to their biological activities.

Angiotensin II receptors are regulated by the prevailing concentration of the peptide ligand[35], and also by changes in electrolyte intake[78]. The latter action is at least partly exerted through changes in blood angiotensin II concentration. Decreased sodium intake and increased potassium intake, which both markedly elevate aldosterone secretion, also cause increased angiotensin II receptor content in the glomerulosa cell[78]. Conversely, high sodium or low potassium intake, which both reduce aldosterone secretion, cause a decrease in adrenal receptors for angiotensin II. The sodium-induced changes are largely attributable to the altered renin secretion and angiotensin II concentrations that accompany variations in sodium intake. Thus, blood angiotensin II levels are increased in sodium deficiency and decreased in sodium loading, and both adrenal angiotensin II receptors and aldosterone secretion are commensurately altered by changing sodium intake. Changing potassium intake has the opposite action to sodium on angiotensin II levels, but the same effect on glomerulosa cell receptors and aldosterone production, by an independent direct effect of potassium on the zona glomerulosa.

During sodium deficiency, the adrenal becomes more sensitive to angiotensin II, whereas vascular smooth muscle becomes less sensitive and pressor responses to the octapeptide are blunted. These changes partly depend on altered receptor properties, as even short periods (36 h) of sodium deficiency caused increased binding of angiotensin II by the rat adrenal *in vivo* and *in vitro*, whereas sodium loading had the opposite effect[79]. Enhancement of binding by sodium restriction was due to a rapid increase in receptor affinity, followed within a few days by increased receptor content. Conversely, sodium loading resulted in a fall in the number of angiotensin II binding sites per adrenal glomerulosa cell. In each case, the aldosterone responses of isolated glomerulosa cells were commensurate with the changes in angiotensin receptors. Thus, cells exhibited increased sensitivity to angiotensin II when receptor affinity was raised, increased maximum aldosterone secretion when receptor content became elevated, and decreased responses when receptor content was diminished. These changes in receptors and steroidogenic activity of the glomerulosa cells contribute to the regulation of sensitivity and maximum responsiveness of the adrenal to angiotensin II during altered sodium balance. The presence of a converse effect in smooth muscle receptors during low sodium intake was consistent with the reduced sensitivity of vascular smooth muscle to angiotensin II during sodium deficiency. The contrasting effects of low sodium intake on adrenal and uterine smooth muscle receptors are shown in Fig. 3, the reciprocal changes in receptors being parallel to the altered sensitivity of each tissue to angiotensin II.

The trophic actions of angiotensin II in the glomerulosa cell include effects on angiotensin II receptors and enzymes in the steroid biosynthetic pathway, as well as on glomerulosa cell differentiation. Infusion of angiotensin II enhances adrenal sensitivity to the peptide in man and rat, and increases the angiotensin II receptor content of the rat adrenal, with an accompanying rise in aldosterone secretory capacity[35]. Although injection of large doses of angiotensin II can decrease the adrenal and uterine receptors in nephrectomised rats[80,81], treatment of intact rats with smaller amounts of angiotensin II has the converse effect, increasing angiotensin II receptors and aldosterone responses in the adrenal glomerulosa cell[35].

The ability of angiotensin II to cause positive regulation of its own receptor sites in the adrenal suggests that most or all of the effects of sodium deficiency on aldosterone secretion could be mediated by changes in angiotensin II secretion and adrenal sensitivity to angiotensin. This controversial concept has recently been supported by the results of direct assay of adrenal receptors and glomerulosa cell responses[79]. Further evidence has been provided by the use of an inhibitor of converting enzyme to prevent angiotensin II formation during sodium restriction, which would normally cause increased adrenal receptors and aldosterone production[82]. Blockade of angiotensin II generation during sodium restriction completely abolished the increased receptor binding and aldosterone responses that occur in sodium deficiency, indicating the importance of angiotensin II in mediating the adrenal response to altered sodium balance. This finding emphasises the central role of the renin–angiotensin system in the regulation of aldosterone secretion during short-term physiological changes in sodium intake.

Fate of the hormone–receptor complex

Most studies of peptide hormone action have concentrated on the acute responses that follow the binding of hormone to its cell-surface receptor. Much less is known about the equally important long-term actions on cell growth and differentiation, or about the termination of hormonal stimuli. These events may be related to the fate of the hormone–receptor complex. As noted above, there is evidence that the initial interaction is followed by formation of a higher-affinity binding state that causes the complex to remain associated for prolonged periods. That, together with increasing evidence for the presence of hormones and binding sites within target cells, has led to the

Table 1 Formation and degradation of the hormone–receptor (H–R) complex

Formation and actions of the H–R complex

A H–R becomes coupled to ACy or other effectors to form H–R–ACy
B H–R–ACy initiates acute response to hormone
C H–R–ACy complex becomes refractory (desensitisation)
D H–R becomes uncoupled from ACy

Fate of H–R complex

A H released, R re-utilised with turnover independent of occupancy (traditional)

or

B H–R released from cell surface (shedding)

or

C H–R internalised
1. Loss of occupied surface receptors (down-regulation)
2. Concomitant endocytosis of adjacent unoccupied receptors
3. Lysosomal degradation of H, possibly of H–R
4. Mediates intracellular trophic action

D H metabolised or inactivated, R remains in inactive (non-binding) form

The most clearly demonstrated mechanism of receptor regulation is by internalisation of the H–R complex. The other pathways could operate in specific tissues and their relative contributions probably vary with the functional state of the cell. ACy, adenylate cyclase.

view that internalisation is a frequent consequence of hormone–receptor interaction. Interest in this process was stimulated by demonstration of the uptake and turnover of cholinergic receptors[83] and low-density lipoprotein–receptor complexes[84]. Subsequently, increasing emphasis has been placed on the importance of internalisation in the processing of hormone–receptor complexes in endocrine target tissues. The mechanism of the internalisation process for peptide hormones probably has many features in common with the processes of surface modulation in lymphocytes[2] and the coated pit system responsible for uptake of macromolecules such as LDL[85]. The basis of the increase in target cell receptors induced by peptides such as angiotensin II and prolactin requires further study but is likely to be related initially to changes in membrane conformation and later to increased receptor synthesis.

Several studies have implicated cytoplasmic contractile elements in receptor mobility and regulation. In lymphoid cells, ligand–receptor interaction is followed by patch formation, aggregation into a few larger patches or a single cap, and finally by internalisation or shedding of the cross-linked receptors. Examination of surface receptor capping by fluorescence staining of the receptor-bound ligand and intracellular actin or myosin showed that patching of surface antigens in splenic T lymphocytes (and lectin receptors in HeLa cells) was accompanied by collection of actin or myosin beneath the aggregated receptors[86]. In the presence of sodium azide, the ligands induced a patchy redistribution of receptors and a concomitant rearrangement of membrane-associated actin on the cytoplasmic surface of the cell membrane. In the absence of azide, the patched receptors became capped, with accumulation of the underlying actin and myosin beneath the cap. These observations, and previous findings on Ig receptor capping in B lymphocytes[87], led to the proposal that a general mechanism of capping depends on the collection together of patches by actin and myosin, analogous to the sliding filament mechanism of muscle contraction[86]. This mechanism also provides a plausible link between capping and subsequent endocytosis, by the concerted action of actin and myosin to initiate invagination of the capped region of the cell membrane. Cellular contractile elements have been also implicated during capping of lectin receptors in cultured ovarian granulosa cells[88], in which microfilaments were active in the redistribution of surface receptors during capping, and microtubules had an orientating effect on the direction of flow of receptor complexes.

Although receptor patching and capping commonly occur in cells exposed to multivalent ligands such as immunoglobulins and lectins, the progression from patching to capping may not apply when receptors are occupied by univalent ligands such as hormones and transmitters. However, the processes leading to aggregation and internalisation of multivalent ligands may be relevant to the turnover and fate of peptide hormone receptors, particularly during receptor regulation in response to increased concentration of hormonal ligands. In recent studies, described below, redistribution of receptors for hormones and other univalent ligands has been found to precede internalisation of receptor aggregates and subsequent degradation of the complexes.

The association of hormonal internalisation with ligand-induced receptor loss was clearly documented for epidermal growth factor (EGF) in human fibroblasts[89]. The cell-bound mitogen was rapidly degraded in a manner consistent with internalisation and lysosomal breakdown, and surface receptors for EGF simultaneously disappeared from the cells. Loss of EGF receptors after peptide binding was also demonstrated by affinity labelling, which showed that rapid proteolytic processing of receptor–hormone complexes accompanied the decrease in membrane receptor sites[90]. Also, both receptor processing and DNA synthesis were stimulated half-maximally when 10% of the receptors were occupied by the mitogen. However, in cultured ovarian cells the luteinisation of granulosa cells was associated with increased EGF receptors and retention of the ability to internalise bound EGF, but with loss of responsiveness to the mitogenic action of EGF[91]. At least in these cells, there was no correlation between mitogenic activity and the capacity for EGF binding and internalisation.

Direct visualisation of the internalisation of fluorescent analogues of EGF and insulin has been achieved by image intensification during fluoresence microscopy[92]. The hormone–receptor complexes were initially distributed uniformly on the cell membrane, then rapidly became aggregated into patches that were internalised to form endocytic vesicles within the cytoplasm. These events could explain both the negative cooperativity of insulin receptors and the down-regulation of receptors and hormone degradation by target cells. Binding and internalisation of EGF has also been observed by conventional fluorescence microscopy in epithelioid carcinoma cells with 25 to 30 times more EGF receptors than in fibroblasts[93]. The bound EGF was again rapidly internalised as fluorescent endocytic vesicles, which later formed a prominent perinuclear ring. Although patch formation did not occur in the epithelioid cells, the occurrence of micro-clusters of receptors was demonstrated by electron microscopy after binding of ferritin-EGF to the cell surface (S. Cohen, personal communication).

These observations on internalisation of EGF are probably applicable to other peptide ligands, although the probability of differences between *in vitro* and *in vivo* regulation should be noted, as should the degree of variability in the rate and extent of receptor processing in various cell types. This is illustrated by the fate of receptor-bound insulin after uptake by adipocytes, lymphocytes and hepatocytes. The specific binding of ^{125}I-insulin to adipocytes became less reversible with time, as if compartmentalised and less accessible to dissociating agents[94]. Also, the bound tracer was rapidly converted to iodotyrosyl fragments, suggesting that internalisation and processing is responsible for the degradation of much of the insulin bound to receptor sites. In contrast to the cultured lymphocyte, where translocation was minor, insulin bound to hepatocytes was progressively internalised and underwent lysosomal degradation[95]. This process must have occurred by endocytosis of the hormone–receptor complex, and would explain ligand-induced receptor loss. However, down-regulation of insulin receptors is also prominent in cultured lymphocytes, where the intracellular translocation of hormone is small in relation to the final degree of receptor loss. Given the time required for insulin-induced receptor loss in cultured lymphocytes, the rate of internalisation could be adequate to account for the receptor loss observed after several hours in the cultured cells.

Further evidence for hormone internalisation was provided by studies on the uptake and metabolism of gonadotropins in cells of the testis and ovary. The presence of gonadotropin binding sites in the cytoplasm of ovarian cells has been demon-

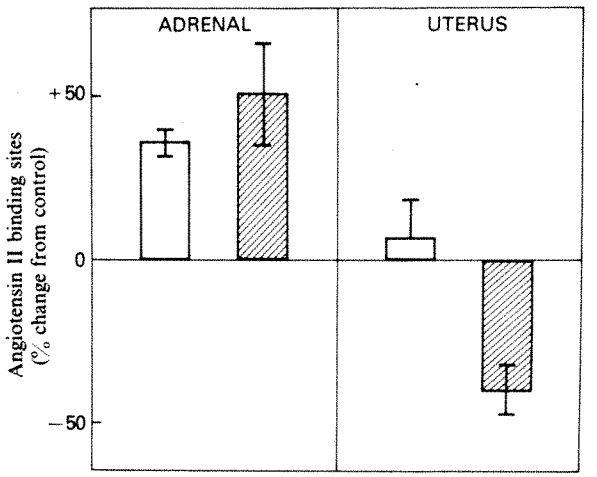


Fig. 3 The reciprocal effects of low sodium intake on angiotensin II receptors of adrenal glomerulosa cells and uterine smooth muscle. Open column, 36-h low sodium: cross-hatched column, 7-d low sodium.

strated by immunocytochemistry and autoradiography. After *in vivo* treatment with hCG, localisation of the hormone was demonstrated in membrane, cytoplasmic and perinuclear sites, much of the hormone being within the cytoplasm[3]. More direct evidence has come from studies of the ovine corpus luteum, where significant amounts of ^{125}I-hCG were taken up within endocytic vesicles and incorporated into dense bodies, presumably lysosomes[96]. Lysosomal degradation of gonadotropins occurs in Leydig tumour cells, in which the binding and metabolism of ^{125}I-hCG was not affected by agents which alter microfilaments and microtubules[97]. In these cells, steroidogenesis is not dependent on hormone degradation, consistent with the rapidity with which binding to receptors is followed by the acute metabolic effects of the hormone. The loss of gonadotropin receptors induced by hCG in the ovary is accompanied by progressive internalisation of receptor-bound ^{125}I-hCG, and at least a proportion of the translocated radioactivity can be identified as the hormone–receptor complex after solubilisation and density gradient centrifugation[98]. Such findings have indicated that internalisation is a common sequel to hormone–receptor interaction, and is likely to be responsible for the down-regulation of surface receptors that frequently follows exposure to elevated concentrations of the homologous hormone.

Conclusions

The effects of receptor regulation on target cell function are both marked and widespread in peptide-modulated endocrine tissues. The initial phase of receptor occupancy and target cell activation is frequently followed by a period of diminished responsiveness that accompanies the presence of residual hormone on receptor sites. This corresponds to the short-term phenomenon described variously as desensitisation, tachyphylaxis or refractoriness, and is sometimes caused by temporary inactivation of adenylate cyclase. In the case of non-cyclic AMP-mediated hormones, other membrane effector systems are presumably inactivated during receptor occupancy. A general scheme of the sequence of events following hormone–receptor interaction is shown in Table 1, which presents the known and hypothetical events that follow the initial event of activation by binding of the homologous hormone to the specific membrane receptors.

In certain tissues, such as the amphibian erythrocyte, refractoriness may depend largely on such transient inactivation of receptors and adenylate cyclase, with subsequent reactivation of these membrane systems when the agonist–receptor complex dissociates. In other cells, depending on the affinity with which the ligand is bound and the rate and extent of receptor and membrane turnover, occupancy is followed by a true loss of receptor sites due to processing and internalisation of the hormone–receptor complex. The degree to which different ligands are able to decrease or increase their specific receptors must also depend on the relative rates of receptor processing and resynthesis in the respective target cells. Thus, minor loss of receptors may not be evident if the rate of receptor synthesis or activation is markedly increased by the initial hormonal stimulus, a mechanism that could account for the increased receptor number in certain cells exposed to high hormone levels. In the more general case, where marked loss of receptor sites follows hormone binding, it is likely that peptide hormone receptors are not necessarily vacated and re-utilised, but may be occupied only once and then degraded or processed.

The fate of processed hormone–receptor complexes, and perhaps some of the adjacent free receptors, probably depends on internalisation and lysosomal degradation. The initial event involves incorporation of both hormone and receptors into endocytic vesicles probably through a coated pit mechanism, with subsequent rapid metabolism of the hormone. Whether recycling of membrane components occurs during receptor regeneration is unclear, but this seems unlikely given the prolonged time needed for recovery of receptors in most tissues. The biological role of hormone–receptor internalisation and degradation remains uncertain, other than as a mechanism for terminating hormone action initiated by tightly bound ligands. These processes may also protect target cells from prolonged or excessive stimulation, and could exert long-term effects on target cell growth and differentiation.

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articles

Accretion temperature of the Tieschitz, H3, chondritic meteorite

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The Tieschitz, H3, chondritic meteorite apparently formed as part of a parent-body by the accretion of both molten and solid materials with a mean temperature of 800 ± 100 °C, followed by slow cooling to lower temperatures. Our data argue against the currently accepted model of equilibration with a cooling gas of solar composition.

KNOWLEDGE of the conditions of planetary formation in the Solar System is largely obtained from meteorite studies. Of particular interest are the unequilibrated chondrites, rare meteorites in which disequilibrium mineral assemblages have survived because they were unaffected by the period of thermal metamorphism which produced crystallisation in most chondrites. Tieschitz fell in Czechoslovakia in 1878. It belongs to the high iron, H, group of chondrites, has prominent chondrules many of which contain glass, and its olivines and pyroxenes have wide ranges of composition. Tieschitz is, therefore, an unequilibrated chondrite *par excellence*[1,2]. All chondrites contain small amounts of the volatile metals Tl, Bi and In, whose presence is explained either by the accretion of various proportions of high temperature and low temperature fractions, followed by closed system metamorphism[3–5], or else by total accretion at low temperature followed by open system metamorphism[6,7]. In either case, models are based on the condensation of volatiles from a gas of solar composition. Recently, Ikrammudin *et al.*[8] heated aliquots of powdered Tieschitz for a week at different temperatures and under a low pressure of hydrogen. Ten elements were determined in each aliquot and elemental loss from unheated material was calculated for each temperature. It was concluded that, in the experimental conditions, open system metamorphism of Tieschitz starting material could not account for trace elemental abundances in more metamorphosed chondrites.

We have carried out some 600 analyses of Tieschitz silicate minerals, glasses or fine-grained intergrowths, sulphide and metal. One of us (J.R.A.) has used high-voltage electron microscopy to study textures and identify minerals in the fine-grained matrix. Mobility during accretion of nepheline-normative liquids allows us to use data from experimental petrology to constrain accretion temperatures. Finally, we discuss the implication that, because of the presence of troilite, the Tieschitz assemblage was not in equilibrium with a gas of solar composition[9,10].

Results

The meteorite comprises millimetre fragments and spherical chondrules of silicate, all of which have opaque rims, together with smaller, near spherical bodies of metal or metal plus troilite. Outside the opaque rims, between fragments and chondrules, there may be a channel of variable width filled by transparent material; this has been termed by Christophe-Michel-Levy[11] "white matrix", in contrast to the "black matrix" of the opaque rims. A few of the fragments clearly represent pieces broken from solid, chemically fractionated, pre-existing rock. For example, one fragment is composed of a granular intergrowth of pigeonite, olivine (Fo_{86}), and crystalline plagioclase (An_{78-68}); this rock is a norite. However, other angular fragments have minerals of variable composition set in devitrifying glass and were apparently broken from solid chondrules or pre-existing rock.

Most chondrules exhibit quench textures. Olivine and/or pyroxene crystals are associated either with clear glass or with fine-grained turbid material which is interpreted as devitrified glass. Some chondrules contain blobs of metal or sulphide or both. Most olivines and pyroxenes are zoned. In all cases zoning is normal, olivines tending towards iron enrichment in rims and pyroxenes tending towards iron and/or calcium enrichment. In

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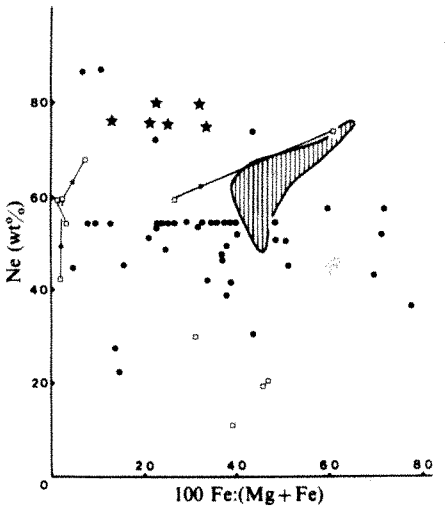


Fig. 1 'Nepheline' (wt %) versus 100 Fe:(Fe+Mg) (atomic), in Tieschitz glasses and fine-grained matrices. 'Nepheline' is the figure obtained from casting the bulk analysis in the SiO_2, nepheline, kalsilite residual system (see Fig. 2). Shaded area represents the white matrix. ●, Chondrule glasses and matrices. ★, Lilac glass from dumb-bell-shaped chondrule. □, Inclusions in olivine; tie lines link those from the same host crystal. A row of solid circles has normative feldspar close to albite, which projects at 54% Ne.

composition, olivine cores in 30 chondrules or rock fragments range from pure forsterite to Fo_{60}. Seven isolated olivines from the matrix between chondrules are either Fo_{80} or about Fo_{60}; in addition one matrix olivine is Fo_{90}.

Glasses or turbid mesostasis in 30 chondrules or chondrule fragments were analysed. Compositions vary from chondrule to chondrule, and, to some extent, within chondrules. The atomic 100 Fe: (Fe + Mg) ratio ranges from 4 to 80 (Fig. 1) and the lime content (wt) from about 1% to over 14%. Analyses were performed using an energy dispersive system on an electron microprobe. Usually, the energy spectrum was integrated over 80 s on a single point, but in some cases a defocused beam was used and in others the spectrum was integrated for four 20-s counts each on a different spot. There was no evidence of loss of alkalis, and glasses apparently did not decompose under electron bombardment; totals were always between 96 and 102%. Figure 2 shows the analyses cast in the silica–nepheline ($NaAlSiO_4$)–kalsilite ($KAlSiO_4$) ternary diagram (wt), after assigning SiO_2 to the various normative mafic and calcium-bearing minerals. All interstitial materials within chondrules plot close to, or on, the SiO_2-nepheline join, because of their lack of K_2O. However, in some chondrules, phenocrysts of olivine contain inclusions, some of which have over 1.5% K_2O. In one pair of inclusions and one triplet, each from a single olivine, a decrease in K_2O is accompanied by an increase in the iron-to-magnesium ratio. This negative correlation of the K_2O and the iron-to-magnesium ratio is contrary to the normal consequences of igneous fractionation. Hence we believe that, in some chondrules, loss of potassium by volatilisation took place. Early, potassium-rich liquids were preserved by their complete enclosure in olivine. On the ternary plot, about half of the chondrule matrices project at the albite composition; in this respect many Tieschitz chondrules resemble those of Bjurböle[12].

A small proportion of the chondrules have non-spherical shapes which were determined, after accretion, by the impingement of adjacent objects on them. At least some of these mis-shapen chondrules were undoubtedly still partially molten during aggregation. One encountered in the present study is roughly dumb-bell-shaped (Fig. 3) and has skeletal crystals of olivine set in turbid, lilac-coloured glass. The olivines are close to pure forsterite, but the glass shows some iron enrichment; this latter is highly nepheline-normative and contains up to about 0.5 wt % K_2O; analyses gave totals from 98 to 102%. Texturally it seems evident that this material was plastic when incorporated into the meteorite parent. Subsequent compaction by other, more solid objects caused it to adjust by flow to fit the irregular space available. A second mis-shapen chondrule (Fig. 4) is micro-crystalline. It too, was clearly plastic or molten when accreted. Subsequent deformation caused the dark rim to rupture as part of the contents squeezed out. The micro-crystalline material is potassium-poor, with variable undersaturation in silica. The 100 Fe:(Fe+Mg) atomic ratio also varies from 45 to 70. Some decomposition occurred under the electron beam and totals were rather low, with a maximum of 97.5; we suspect that the fine-grained material may be slightly hydrated, as the surface is highly polished.

White matrix occupies some narrow, interstitial areas outside the dark rims of chondrules and clasts[11]. In thin section, between crossed polars, it consists of a fine-grained intergrowth of crystals of low birefringence; electron diffraction indicates that nepheline is common and some plagioclase is present. Only broken grains were found in the electron microscope study, but some large (>10 μm) fragile nepheline fragments were probably generated close to their present location. A polished surface in reflected light confirms that white matrix is composed of angular to rounded fragments, the largest observed being close to 20 μm across. The bulk compositions of compact white matrix (Fig. 2) indicate the same nepheline plus plagioclase mineralogy. Textural evidence (Fig. 5) shows that cohesive masses of this white matrix were mobile during the accretion of the chondrite. Analysis totals for white matrix are low (80–97%; compare ref. 11), because it does not become highly polished (due to its fractured nature?), and decomposes in the electron beam. Nevertheless, compared with chondrules, our data (analyses of 25 locations) establish a striking consistency of composition, throughout a 2.5-cm^2 thin section (Figs 1, 2). White matrix usually has 8–10% Na_2O, about 2% CaO and 100 Fe:(Fe+Mg) of 40–60.

Discussion

It seems that some constituents of Tieschitz were molten or plastic after aggregation. Experimental petrology may be used to constrain the conditions in which flow could have taken place. We can then outline some of the consequences imposed by these conditions for the cooling history of the meteorite.

There are two temperature minima on the silica–nepheline join, separated by the albite ($NaAlSi_3O_8$) composition (Fig. 2). On cooling, liquids with compositions between albite and silica crystallise one or other phase, depending on initial liquid composition. This drives the composition of the residual liquid towards the minimum at which both phases crystallise together at 1,062 °C (for the dry system) until completely solidified[13]. For

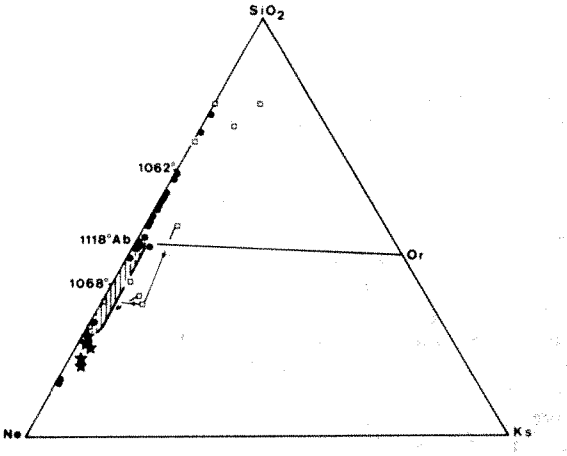


Fig. 2 Residual system SiO_2-nepheline ($NaAlSiO_4$)–kalsilite ($KAlSiO_4$) (wt). Symbols as in Fig. 1. The melting point of albite, and minima on the SiO_2–nepheline join are in °C.

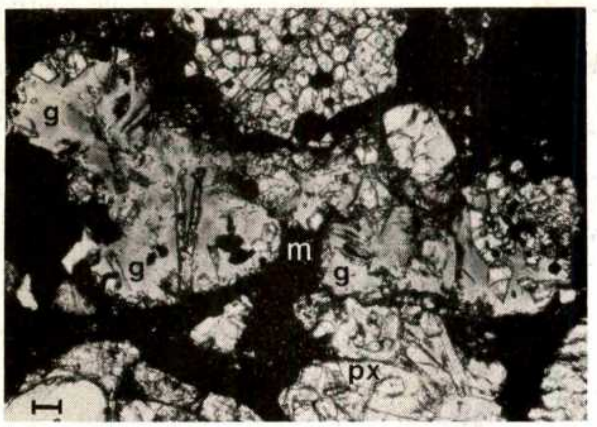


Fig. 3 Dumb-bell-shaped chondrule. m, Metal grain in dark matrix; g, lilac-coloured glass, partly turbid; px, porphyritic pyroxene chondrule. Although some brittle fracturing occurred above and to the right of 'm', the two long, fragile olivine crystals between 'g' and 'm' (lower left of centre) are undeformed and grew *in situ* from the melt after the shape of the chondrule had been determined. Scale bar, 0.05 mm.

liquids with composition between albite and nepheline, crystallisation of one of these phases drives the liquid towards a minimum at 1,068 °C. Because white matrix compositions straddle this second minimum, they probably represent solidified, fractionated liquids. Between the two minima is a thermal barrier which cannot be crossed by a crystallising liquid at low pressure. We interpret the white matrix as being representative of different liquids which tended towards the 1,068 °C minimum by crystal-liquid fractionation. On the other hand, many chondrule liquids are oversaturated with silica and so tended to fractionate towards the 1,062 °C minimum. But these liquids are more viscous than the undersaturated ones and hence were not mobile during accretion.

We envisage that white matrix liquids were available in small quantity distributed among the aggregating mass of chondrules, clasts, metal, sulphide and mineral fragments. These liquids show a genetic relationship with nepheline-normative chondrules. Initially, white matrix must have been sufficiently mobile for it to have flowed for distances of a few mm along interchondrule channels up to 50 μm wide. The question then arises in what conditions could the white matrix have been mobile? One measured composition (SiO_2 56.2% (wt); TiO_2 0.50; Al_2O_3 18.3; FeO 4.75; MgO 3.32; CaO 3.86; Na_2O 8.9; K_2O 0.40; sum 96.23), with 1 wt % H_2O, has a calculated minimum viscosity of 10^4 P at 900 °C (H. Pinkerton and L. Wilson, personal communication, and refs 14, 15). Water is present in Tieschitz[1]; 1% water in white matrix lowers the viscosity by a factor of 2–5, and slightly lowers the liquidius temperature. The calculated viscosity is comparable to that of rhyolite liquids during eruption; their viscosities are so high that it is surprisng that they can be erupted at all. A liquid of 10^4 P requires a pressure gradient of about 50 kbar cm^{-1} to cause it to flow for 5 mm along a channel 25–50 μm wide. The pressure required is greatly in excess of the measured compressive strengths of meteorites[16], hence injection of white matrix as a liquid at 900 °C or less, is impossible. The calculated yield strength of liquid white matrix at 900 °C is 10^8 c.g.s. units[15]. Emplacement as a solidifying liquid, by comminution, requires a pressure gradient of 20 kbar cm^{-1} which is still unacceptably high. Crystallisation would exacerbate the situation. The Tieschitz meteorite is, in fact, noted for the absence of significant deformation effects[11]. We must therefore assume that white matrix was emplaced in a fluid state which requires a temperature above 900 °C. Now, a 100-μm thick film of silicate liquid, in a vacuum, would solidify from 1,100 °C in microseconds. So, if Tieschitz accreted mainly as chondrules, clasts and metal at 170 °C (~455 K, for the two-component model championed by Anders and co-workers[17]), interspersed with a few per cent of molten chondrules and white matrix liquid, the melts would have solidified immediately.

Our only solution to this problem seems to be that hot, liquid, white matrix and plastic chondrules were accreted with chondrules, clasts, metal and sulphide at a cooler, though still high, ambient temperature. We now try to constrain this temperature. Chondrules show the effects of rapid quenching and we observe gross compositional heterogeneities between olivine and pyroxene crystals. The main, low-calcium pyroxene is the twinned monoclinic variety. There is also pigeonite exsolved on a very fine scale (~10 nm) in crystals with a higher lime content. All these features indicate rapid cooling to around 800 °C, or less. In addition, low-calcium pyroxenes with a variety of iron contents have remained unattacked by engulfing white matrix which, being nepheline-normative, must have been strongly out of equilibrium with this pyroxene. Either the chondrules and debris came together at about 800 °C, with residual alkaline liquids at higher temperature and still crystallising matrix olivine (Fo_{60-80}), or accretion took place with all the components at temperatures above 800 °C, during rapid cooling. In either case we propose that the effective accretion temperature was 800 °C, this being the highest temperature that can plausibly be invoked for the end of rapid cooling of the mafic silicates.

An upper limit to the accretion temperature is fixed at 988 °C by the minimum in the Fe–FeS system[18]. The addition of nickel lowers the minimum, but precise data are not available. If the ambient temperature during accretion of Tieschitz had exceeded 900 °C, then metal plus sulphide should have been mobilised[19], and squeezed between the chondrules. As this is not observed, we can set the maximum temperature during accretion at 900 °C. Because the Fe–FeS eutectic temperature is well below the solidus on the nepheline–albite join, we can eliminate metamorphic reheating as a cause of white matrix mobility. Had the Tieschitz assemblage aggregated cold, subsequent heating would have caused the flow of metal plus sulphide liquids when the eutectic was reached at above 900 °C. A further increase of over 100 °C would have been required to produce melting in the white matrix. We have observed no textural evidence of metamorphism except for compaction in some chondritic clasts. In them the same unequilibrated silicates occur as in the meteorite as a whole. A lower limit to the accretion temperature may be established as follows: metal in Tieschitz occurs in a number of textural associations. Nickel distribution in the metal grains was measured using a con-

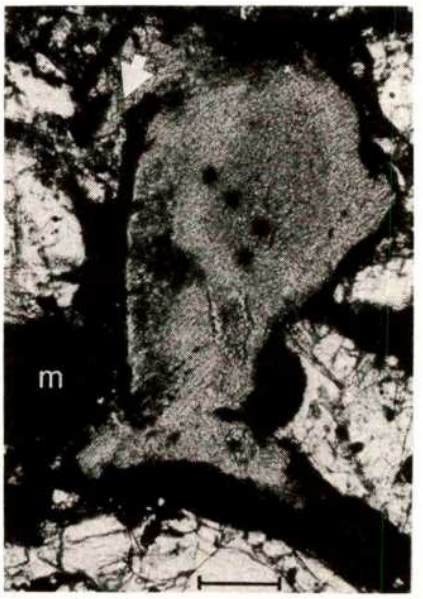


Fig. 4 Mis-shapen chondrule. m, Metal. Compression from left and right caused the dark matrix surrounding the chondrule to rupture, forcing the contents out (see arrow). The four dark spots are 'probe' burns. Scale bar, 0.05 mm.

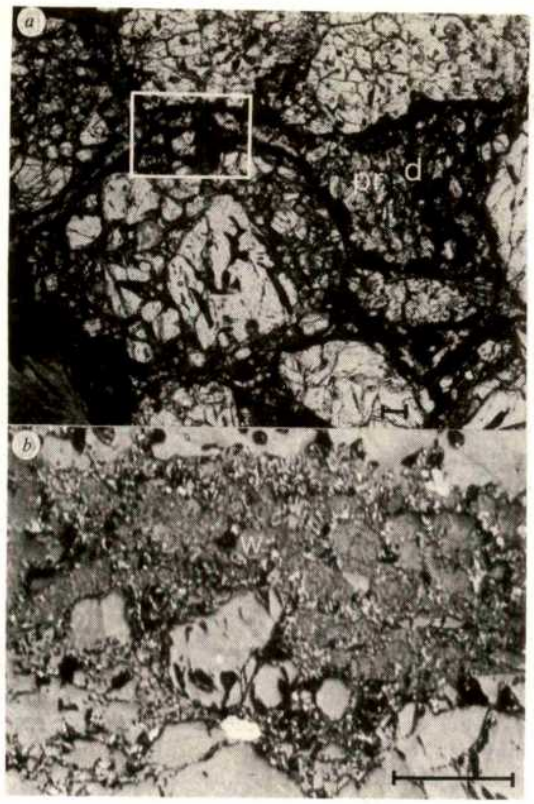


Fig. 5 White matrix: *a*, transmitted light; *b*, reflected light. The field of *b* is the rectangular outline in *a*. In the top left of *a* white matrix fills channels between three chondrules outlined by dark matrix. To the right, one channel with white matrix runs into a pyroxene-rich area pr, which is delimited on the right by dark matrix *d*. *b* Illustrates the block-like nature of white matrix, one piece of which is marked (w). Metal is brilliant white. Vermicular, light lines are cracks outlined by adhering carbon coat which was cleaned from most of the surface. Scale bars, 0.05 mm.

ventional, wavelength-dispersive microprobe technique. Both kamacite and taenite grains show a relationship between size and central nickel content, indicating that all metal cooled through about 500 °C *in situ*, thus confirming the conclusion of Wood[20]. The cooling cycle must have begun near 700 °C, the temperature at which bulk Tieschitz metal (11.8% Ni, ref. 21) would enter the $(\alpha+\gamma)$ field, if the effect of undercooling is neglected. Thus the lower limit to accretion temperature is fixed at 700 °C. We can therefore conclude that Tieschitz effectively accreted at (800 ± 100) °C.

Christophe-Michel-Levy argued that white matrix represents part of the contents of chondrules which had been leached out and redeposited by a fluid phase[11]. However, it seems impossible to mobilise preferentially a silica undersaturated component, white matrix, without attacking low-calcium pyroxene. (For example, in the terrestrial, aqueous environment low-calcium pyroxene tends to be very unstable.) Moreover, the presence of water could not have been responsible for the deformation of the chondrule in Fig. 4, because the effect of water in reducing the viscosity of the chondrule liquid would have been much less than the effect of falling temperature in increasing it.

Accretion of an unequilibrated ordinary chondrite at 800 °C has several implications. This temperature is higher than that of the reaction of H_2S gas with metal to form troilite plus hydrogen from a gas of solar composition[9,10]. We must then assume that any gas in equilibrium with the accreting components of Tieschitz was depleted in hydrogen relative to a gas of solar composition[10]. Obviously, this can be overcome by *ad hoc* assumptions such as rapid formation of chondrules and metal plus sulphide during, for example, an impact event—but this must be a non-equilibrium reaction. In contrast, the story in the Tieschitz metal supports equilibration and slow cooling over temperatures around 500 °C, which is still above the temperature at which troilite can form by reaction from a gas of solar composition. Following Herndon and Suess[10], our findings indicate that the Tieschitz assemblage was probably associated with a gas depleted in hydrogen by a factor of 100 or more relative to solar composition.

These implications may be taken further. In the two-component model, or variations of it, the temperature of accretion of chondrites has been defined by the estimated temperature at which a low temperature, volatile-rich component ceased to equilibrate with a cooling gas of solar composition. Larimer's estimate for Tieschitz is ≤445 K (170 °C)[17], it depends, among other things, on gas composition. A temperature of 170 °C may be reconciled with our estimate of 800 °C for the accretion temperature if it is assumed that the highly porous Tieschitz[11,22] continued to equilibrate with gas during slow cooling. During this stage of the meteorite's history the partition of Ni between kamacite and taenite occurred. In addition the meteorite received its complement of the volatile metals Tl, Bi, In and Pb by condensation from the coexisting gas. Such a mechanism has been suggested for the L3 chondrite, Khohar, on the basis of Bi distribution between metal grains[23]. Where did the volatile metals go? They probably condensed into any suitable substrate, such as dark matrix around chondrules and clasts. Dark rims (=matrix) to chondrules and clasts were, in some instances at least, picked up when chondrules were liquid. We cannot, therefore, assume that the volatiles are present in the dark rim material, or that a low temperature carrier of volatiles existed and was available during accretion of solids. There is some evidence that in ordinary chondrites Pb is not highly concentrated in the sulphide[23,24], as would be expected for equilibrium condensation from a gas with free access to sulphide grains. Furthermore, the existence of troilite in a high temperature mineral assemblage indicates that thermodynamic modelling of condensation must be extended to embrace non-solar compositions of gas. Also, a purely hydrogen atmosphere[8] is probably inappropriate for experimental heating of chondrites.

Finally, the presence of dark rims around norite clasts indicates that at least one period of fractionation preceded the accretion of Tieschitz. Perhaps ordinary chondrites are not really primitive objects, or maybe depletion in hydrogen occurred due to kinetic effects in the atmosphere of a growing planet which had received material from the break-up of an already differentiated body. This argument has already been put forward[25].

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Morphology and intracortical projections of functionally characterised neurones in the cat visual cortex

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The neuronal structure and connectivity underlying receptive field organisation of cells in the cat visual cortex have been investigated. Intracellular recordings were made using a micropipette filled with a histochemical marker, which was injected into the cells after their receptive fields had been characterised. This allowed visualisation of the dendritic and axonal arborisations of functionally identified neurones

CONSIDERABLE insight into the stages of visual information processing has been obtained from analysis of single cell responses to patterned light stimulation. This approach revealed that cells in the visual cortex respond optimally to specific stimuli, such as line segments of given orientation and length[1,2], and that neurones differ in their specificity. Cortical neurones also show great morphological diversity[3-7], suggesting that there may be a correlation between the function of a cell and its structure. Another feature of cortical organisation is that cells are segregated into layers, and in each layer the cells have distinct size, shape and packing density. This lamination has long been thought to be important in cortical function[8-10]. The layers differ further in the receptive field properties of the cells within them[2,11-13], in the inputs that they receive[14-17] and in the sites to which their cells project[18-22]. This information was derived from extracellular recording and anotomical tracing, but now intracellular marking techniques, first used in the visual cortex by Kelly and Van Essen[23], can extend these findings by showing the detailed intracortical wiring of cells with known response properties.

In this study we have been able to trace the intracortical projections of functionally identified neurones in different layers by intracellular injection of the enzyme horseradish peroxidase (HRP)[24,25]. Comparison of the receptive field properties of marked cells with those of cells in the layers to which they project provides evidence for the manner in which receptive fields are constructed.

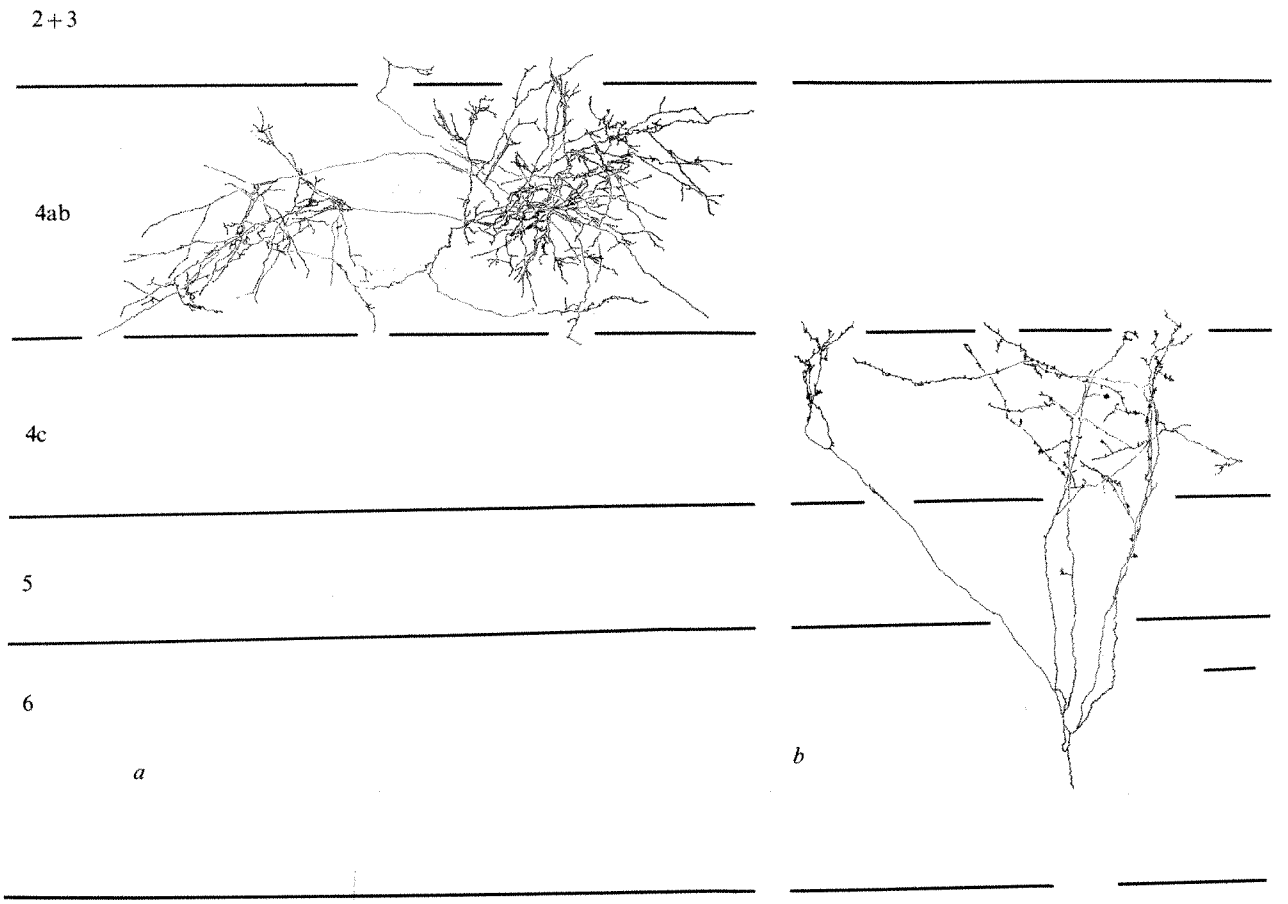


Fig. 1 Two afferents from the lateral geniculate nucleus, injected within the cortex. Was an off-centre Y-cell (centre size 1.5°, located 5° from the area centralis), and ramified entirely within layer 4ab. Was an off-centre X-cell (centre size 1°, located 3° from the area centralis), which ramified entirely within layer 4c. Scale bar, 100 μm.

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Table 1 Numbers and laminar positions of injected cells

Layer	No. of cells
2+3	16
4ab	4
4c	2
5	19
6	11
Afferents	9
Total %	61

Functional characterisation and labelling of visual cortical neurones

Recordings were made in cats maintained on sodium thiopental anaesthesia, paralysed with succinylcholine and artificially respirated. The animals' electrocardiogram, electroencephalogram, temperature and expired CO_2 concentration were monitored. A small hole was drilled in the skull above the visual cortex, and the dura and pia were opened. Half-micrometre bevelled-tip electrodes were filled with a solution of 4% HRP (Boehringer–Mannheim, grade I) in 0.2 M KAc, *p*H 7.6. After penetration of the brain surface with the electrode, the hole in the skull was filled with agar to reduce pulsations. The electrodes were advanced through the brain with a stepping microdrive (Transvertex) until a cell or process was penetrated, as indicated by a sudden change in the resting potential, the appearance of large action potentials and often synaptic activity. The receptive field properties of the cells were determined using either a hand-held projector or an optical bench.

The cells were injected with HRP either by pulses of pressure ranging from 0.5 to 5 atm or by pulses of positive current ranging from 1 to 2 nA in a 4 ms-on/4 ms-off duty cycle. At the end of the experiment each animal was perfused with a short buffer rinse followed by a long perfusion with 2% glutaraldehyde in the same buffer. After the brain was blocked, 150-μm coronal sections were cut on a Vibratome (Oxford Instruments), and then treated with a combination of the diaminobutyric acid reaction at acid *p*H[26] and cobalt intensification[27]. The injected cells were reconstructed from serial sections by using a microscope equipped with a drawing tube. Subsequently, the sections were counterstained with cresyl violet, and the laminar position of cells and their processes were determined using the criteria of Otsuka and Hassler[28]. Table 1 lists the cells in each layer that we have injected to date.

Cortical afferents

Different morphological and physiological classes of retinal ganglion and geniculate cells are known to exist[29–32]. Chief among these are the X- and Y-cells. These are defined by the linearity of spatial summation within their receptive fields[29], and also have been found to differ in their firing properties and axon conduction velocities[30,33,34]. The dorsal geniculate laminae, which contain a mixture of X- and Y-cells[35], project to layers 4 and 6 in the visual cortex[16,17].

In this study we were able to penetrate and inject individual axons coming from the lateral geniculate nucleus either before or after they enter the cortex. We used the criteria of spatial summation to classify X- and Y-afferents[29,36]. A reconstruction of each type is shown in Fig. 1. The Y-afferent (Fig. 1*a*) had off-centre/on-surround organisation, showed nonlinear summation, had a relatively large field centre (1.5°) and gave a transient response to a stationary flashing spot of light. It ended in layer 4ab in a rich arbor distributed in two patches separated by a terminal-free gap. The patches presumably correspond to ocular dominance columns driven by one eye, and the intervening gap to the column driven by the opposite eye[15,37] Some of the injected Y-afferents also sent collaterals to the upper half of layer 6.

In contrast to the afferents showing nonlinear summation, the X-afferent arborised entirely within layer 4c (Fig. 1*b*). No afferents were found to arborise in both sublayers of layer 4. This demonstrates, in agreement with Ferster and Levay[38], that the dorsal geniculate layers, which contain a mixture of at least two principal cell types, keep the input from the two types segregated on their arrival in the cortex.

Layer 4 cells

Cells in layer 4 lie in the terminal field of the geniculate afferents, and consequently represent the first level of processing in the cortex. Extracellular recordings show that the overwhelming majority of the cells in layer 4 have simple receptive fields[1,11,23], as defined by Hubel and T.N.W.[37]. In addition to their specificity for stimulus orientation, simple cells can show a reduction in response to slits longer than the receptive field[11,39,40]. This property, known as end-inhibition, makes cells optimally responsive to orientated lines of a defined length. Figure 2*a* shows an example of a simple cell in layer 4ab. It was a spiny stellate cell; the axon branched several times soon after leaving the soma, with a number of collaterals innervating in layer 4ab and then giving off a rich terminal arborisation in layer 2+3. The axon proceeded out of the cortex into the white matter, sending a few collaterals into the lower layers in its downward course. This general projection pattern was seen for all injected 4ab spiny cells and has also been observed with Golgi stains[3–6]. The horizontal extent of the axonal arborisation was much larger than that of the dendritic arborisation. This divergence could produce a further mixing in the input from the two eyes onto layer 2+3 cells, and could account for the higher proportion of binocularly driven cells found in that layer than in layer 4 (refs 1, 11).

Only two cells have been injected in layer 4c, one a small spiny stellate cell and the other a smooth stellate cell (Fig. 2*b*). They had very similar receptive field properties; both had simple receptive fields with on-centres, which were much smaller than those of the layer 4ab simple cells. The smooth stellate cell's axon ramified extensively throughout layer 4. The axon of the spiny stellate cell gave off many collaterals, some remaining within layer 4c and others extending to layer 4ab. Although the cell's axon was restricted to layer 4 (which is consistent with Golgi findings[5]), the existence of complex cells with very small receptive fields in layer 2+3 (refs 1, 11) leads us to expect that there should be other layer 4c cells with projections to that layer.

Layer 2+3 cells

The predominant intracortical projection from layer 4 seems to be to layer 2+3, which then represents the second level of cortical processing. Layer 2+3 almost exclusively contains complex cells[1,11]. They are orientation selective, but differ from simple cells in having uniform receptive fields not divisible into separate on- and off-subregions and in responding continuously as a slit of light is moved across their fields[1,11,41]. Like simple cells, they can be directional and end-inhibited. For complex cells, specificity for orientation and length is maintained, but compared with simple cells they have gained some freedom in the precise position of the stimulus along the movement axis[1].

A complex cell with a small receptive field (1°×1°), showing no end-inhibition, is shown in Fig. 3. It was a pyramidal cell located in the upper part of layer 2+3. The basal dendrites ramified close to the soma and the apical dendrite extended into the lower part of layer 1. Its axon branched richly in its layer of origin, both in a region adjacent to the cell's dendritic field and in a region rather more distant, perhaps to a column of cells with the same orientation preference. The axon collaterals also extended into layer 1. The descending axon projected extensively within layer 5; this was a common feature of pyramidal cells in this layer, in agreement with degeneration[42–44] and Golgi[4,5,45] studies. The axon proceeded out of the cortex, presumably to innervate another cortical area or areas[21].

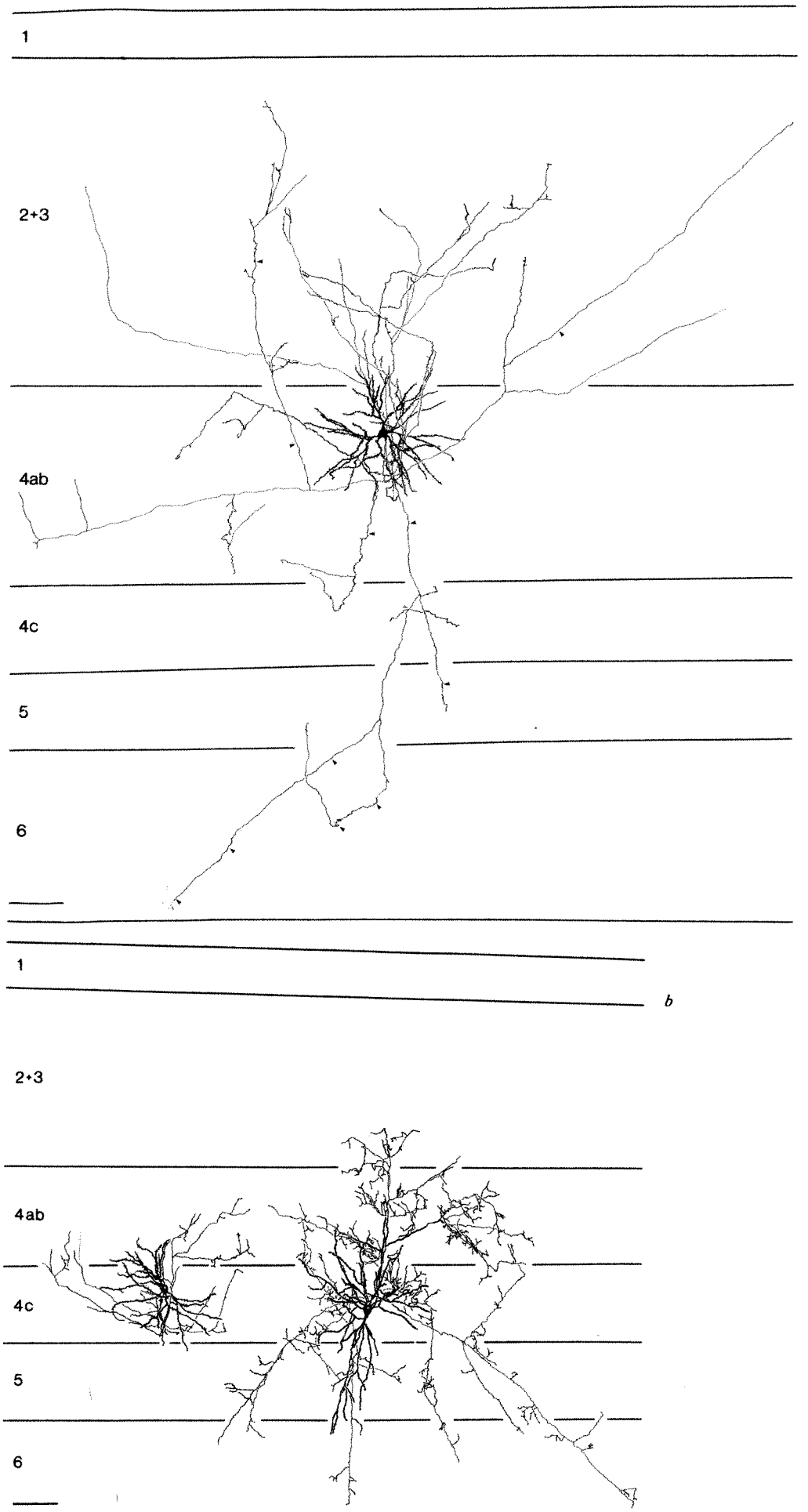


Fig. 2 *a*, A spiny stellate cell in layer 4ab having a simple receptive field with an on-centre and off-flanks, and showing no end-inhibition. The field size was $3° \times 4°$, and it was located 10° from the area centralis. The arrows indicate the positions of nodes of Ranvier. *b*, A spiny stellate cell (left) and a smooth stellate cell (right) in layer 4c. Both had simple receptive fields with on-centre and off-flanks, the spiny stellate cell showing no end-inhibition and the smooth stellate cell showing 50% end-inhibition. The size of the central on-portion of the field for both cells was $1° \times \frac{1}{2}°$, and both were located 2° from the area centralis. Because the plane of section was not perpendicular to the layers, this reconstruction makes it seem as if several sets of collaterals of the smooth stellate cell extend into layers 5 and 6, whereas in fact they remain within 4c. Scale bar, 100 μm.

Layer 5 cells

Cells in layer 5 have complex receptive fields like those in layer 2+3, but differ most notably in their large field sizes. Previous studies[11,20] have shown that within layer 5 two cell types can be distinguished: one that shows summation for increased slit length (the standard complex cell) and one that responds optimally to a small moving slit of light placed anywhere within its relatively large field, showing no summation for increased slit length (the special complex cell). Consequently, the special complex cell maintains specificity for orientation, length and direction of stimulus movement, but gains some freedom in the precise position of the stimulus along the orientation axis. Like simple and standard complex cells, the special complex cell can exhibit end-inhibition[11].

Figure 4 shows a standard complex cell in layer 5. A surprising finding was that the cell's axon sent an extensive projection to layer 6, passing 6–8 mm down the medial bank, still remaining within area 17. The cell's receptive field was $2\frac{3}{4}° \times 1\frac{3}{4}°$, and its axon spanned an area of cortex representing up to 15° of visual field. Thus, it reaches areas that deal with parts of the field of view far outside the cell's receptive field. This projection has not been seen with Golgi stains, possibly because the axon becomes myelinated soon after leaving the cell body, and would therefore not impregnate with the Golgi technique. The distribution of the axonal field as viewed from the cortical surface was long and narrow. One might expect that the overall axis of distribution of the axon would be related to the orientation axis of the cell's receptive field. The axonal distribution and orientation of this cell were consistent with this view. The apical dendrite extended up into layer 1, branching repeatedly there, and several of its processes passed just underneath the pia. The cell had a dense dendritic arbor near its cell body, with a number of processes leaving the base of the apical dendrite at the layer 4/5 border, and a set of basal dendrites extending down into the upper part of layer 6.

We have also injected special complex cells in layer 5. Their axons seemed not to project as richly to layer 6 as those of the standard complex cells, and set a large trunk out of the cortex, presumably to innervate the superior colliculus[20,21].

Layer 6 cells

Layer 6 is of special interest as it receives a direct projection from the lateral geniculate nucleus[11] and contains a mixture of simple and complex cells[1,11,23]. Its cells have unique receptive field properties, often requiring long slits for activation and showing summation with slit length up to very large values (16°)[11]. Figure 5 shows an example of a layer 6 simple cell. The basal dendrite of the cell branched in the upper part of the layer; this is interesting as the collaterals of geniculate axons in layer 6 end in the upper part of the layer (see ref. 38 and also above). Its axon projected mainly to layer 4ab, in the vicinity of its apical dendrite, which branched extensively and ended in 4ab. Other cells, both pyramidal and smooth stellate, had axonal fields that lay almost exclusively within layer 6.

Although the simple cells of layer 6 were restricted to the upper half of the layer, we found complex cells throughout the layer. The apical dendrites of these cells branched at different levels within the cortex, which is reminiscent of the pattern observed in the monkey by Lund and Boothe[45]. Like the cell in Fig. 5, they often sent collaterals primarily to layer 4, and these were restricted to either 4ab or 4c. The injected smooth stellate cell had a complex receptive field.

Intracortical pathways and receptive field construction

Although we have not yet injected all the morphological cell types found in the cortex[4], some patterns of projection have



Fig. 3 A pyramidal cell in the upper part of layer 2+3, with a complex receptive field showing no end-inhibition. The field size was 1°×1°, and it was located 4° from the area centralis. Scale bar, 100 μm.

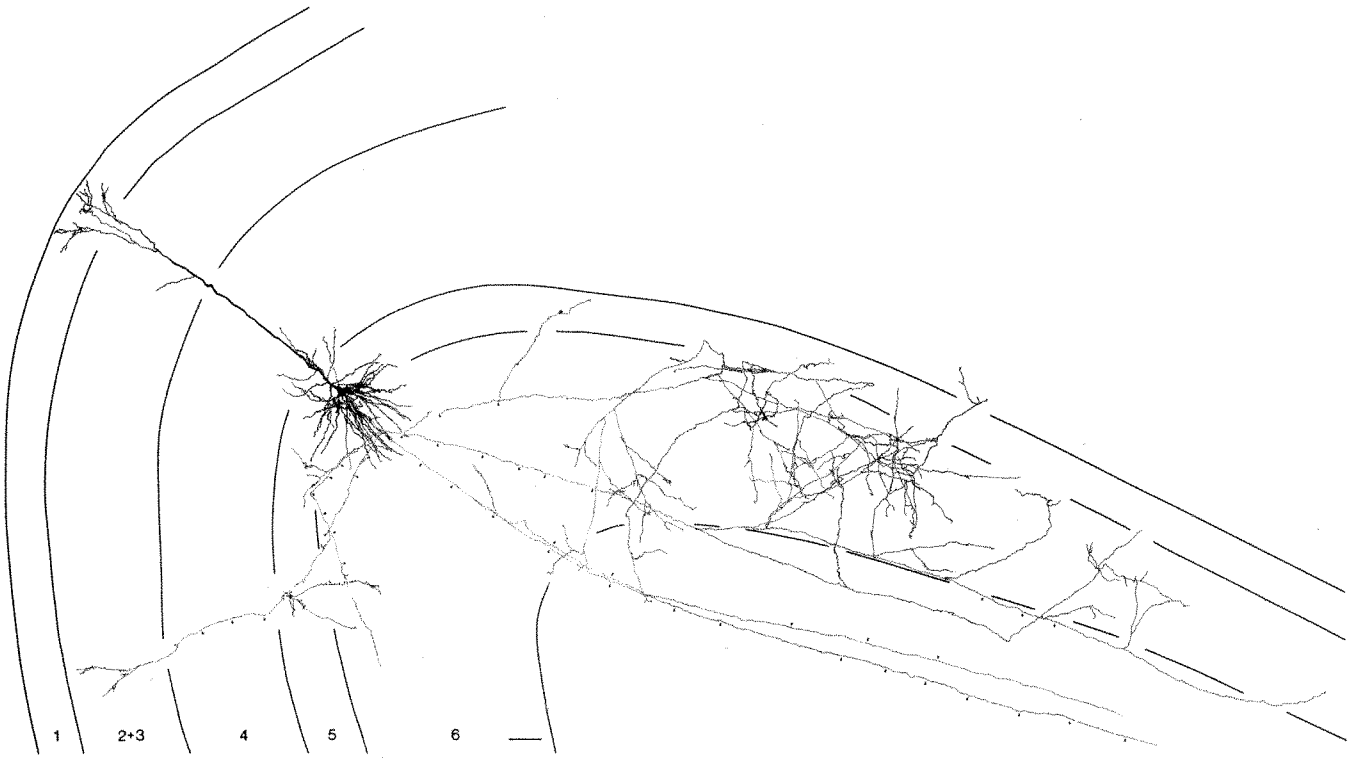


Fig. 4 A pyramidal cell in layer 5, with a standard complex receptive field showing no end-inhibition. The field size was $2\frac{3}{4}° \times 1\frac{3}{4}°$, and it was located on the area centralis. The branches of the apical dendrite within layer 1 all run quite close to the pia, but this is not apparent due to the plane of the section relative to the cortical layers. Scale bar, 100 μm.

emerged from our present catalogue of injected cells. The predominant stages of information processing in the cortex seem to be as follows: the major geniculate input to the cortex arrives in layer 4, with the X-input and the Y-input arriving separately in layers 4c and 4ab. Both inputs also project to the upper half of layer 6. From layer 4 the sequence of processing continues to the upper layers, from the upper layers to layer 5, and from layer 5 to layer 6. Layer 6 then sends output back into layer 4. The cortex is tapped for output to other regions at several stages: cortical areas from layer 2 + 3, superior colliculus from layer 5, and lateral geniculate nucleus from layer 6. Also, the spread seen in the horizontal axonal projections (Fig. 4) extends far beyond that expected from previous studies using the Golgi technique[3–6].

This pattern enabled us to form hypotheses as to the manner in which receptive fields are constructed within the cortex. As originally suggested by Hubel and T.N.W.[46], simple receptive fields are constructed from the fields of lateral geniculate neurones, as simple cells are the predominant class in layer 4, the major geniculate afferent zone. The correspondence between simple receptive field type and geniculate input is also seen for the cells that lie in the upper part of layer 6. Although they share the same general receptive field type, cells in layer 4c have smaller fields than those in 4ab. This difference is consistent with the two features of the geniculate input to layer 4—the X-afferents have smaller receptive fields than the Y-afferents, and the 4ab afferents have a much wider terminal arborisation than the 4c afferents[38]. Although the receptive fields of the injected 4c cells were quite similar, spiny cells are thought to be excitatory and smooth cells inhibitory. These presumptions are based on both their synaptic morphology and transmitter neurochemistry[47–49].

The superficial layer complex cells receive their input from the layer 4 simple cells. As cells in layer 4ab project to layer 2 + 3, and as, except for the bottom of layer 3, the geniculate does not project to this layer, it is likely that the receptive fields of cells in the upper layers are generated primarily by input from layer 4.

The layer 5 complex cells may form their fields from the concatenation of the fields of the superficial complex cells, as suggested by the extensive projection from the layer 2 + 3 cells into layer 5. The lateral spread of this projection can account for the increase in receptive field size from the superficial layers to layer 5. Because their apical dendrites pass through geniculate afferent zones, the layer 5 complex cells may also receive geniculate input. Evidence from serial electron microscopic reconstructions suggests, however, that they do not[50]. We do not know what are the differences in the inputs to standard and special complex cells which account for their differences in receptive field properties.

The substantial horizontal traverse of the layer 5 axon in layer 6 is an intriguing feature, as the layer 6 cells have very long receptive fields, showing summation for increased slit length up to very large values, some reaching 16° or more[11]. We have seen no other inputs to layer 6 or intrinsic connections within layer 6 that can account for this receptive field property; the input to layer 6 from the lateral geniculate nucleus is, if anything, more restricted than the geniculate input to layer 4 (refs 17, 38). Also, none of the cells within layer 6 that we have injected thus far have axons that ramify within the layer over nearly as large an area as do the axons from the layer 5 cells. This hypothesis requires us to account for elongation in both simple and complex fields in layer 6 despite the fact that the input from layer 5 is exclusively complex. The simple fields may be produced by an interaction between the geniculate input to the upper half of layer 6 and the input from layer 5.

The rich projection from layer 6 to layer 4 suggests that some properties manifested by layer 4 cells may not depend solely on convergence of geniculate input onto layer 4 cells or on connections made by those cells within the layer. It could be that, due to their intimate involvement with layer 4 through dendritic arborisation and axonal projection, the layer 6 cells have a role in producing orientation specificity, preference for direction of stimulus movement and/or end-inhibition.

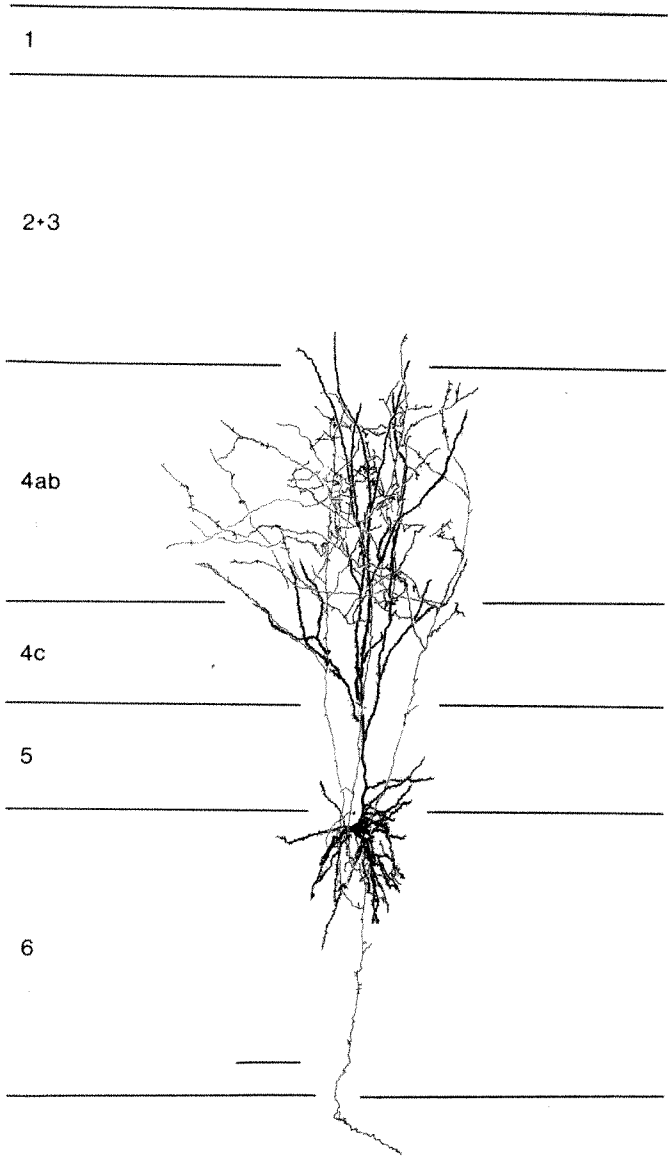


Fig. 5 A pyramidal cell in layer 6, with a simple receptive field showing the lack of end-inhibition characteristic of cells in this layer. The field size was $1\frac{1}{2}° \times 2°$, centred 4° from the area centralis. It did not show obvious summation to long slits and may represent a population of layer 6 cells with shorter fields. Scale bar, 100 μm.

Dendritic morphology and receptive field construction

Several issues are raised in considering correlations between morphology and function. As seen from our injections in layer 5, for example, large neurones have large receptive fields. This is presumably due to their large dendritic fields, which enable the cells to collect input over a wide area. Another determining factor in receptive field size is the extent of axonal ramification of the inputs to a given cell. This is seen for the layer 6 cells; although their dendritic fields are relatively small, they receive input from layer 5 over a very large area, due to the wide spread of the layer 5 cell axons. The layer 5 fields may derive their large area from both mechanisms: they have very wide dendritic arbors, and the layer 2+3 cells have widely arborising axons in layer 5.

A correlation between simple receptive field type and stellate morphology was found by Kelly and Van Essen[23], but, as they indicated, this was not a strict correlation. We have injected simple cells in layer 5a and 6 that were clearly pyramidal in morphology and one complex cell in layer 6 that was stellate. Thus, the simple/complex receptive field classification seems not to be the pertinent feature in relating functional properties to the stellate/pyramidal categories. The determinant factor as to whether a pyramidal cell is simple or complex seems to be the position of its basal dendrites relative to the geniculate input. The function of apical dendrites remains unknown; they may ramify at different levels within the cortex, but so far this does not seem to correlate with any receptive field differences. It is possible that, as seen for some cells in layer 6, the role of the apical dendrite is to participate in the formation of recurrent loops within the cortex.

In addition to the dichotomy between stellate and pyramidal cells, there is, even for a given layer, considerable variability within each of these morphological classes. At this early stage in our study we do not know how this diversity is reflected in the receptive field and/or firing properties of cells. There are, on the other hand, certain receptive field features, such as orientation specificity and preference for direction of stimulus movement, which may correspond to particular aspects of dendritic arborisation. Another functional difference between cells is their inhibitory or excitatory postsynaptic effects, which depend on the transmitters they use. As mentioned above, this may be reflected in morphological differences and not in differences in receptive field properties. Pharmacological evidence suggests that inhibition is important in enhancing certain receptive field features, such as orientation specificity[51]. The relationship between function and dendritic morphology remains a challenge. We expect that further analysis of and additions to our catalogue of injected cells will reveal clues to this relationship.

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letters

The 78.4-day period of Cygnus XR-1

CYGNUS XR-1/HDE226868 is a multiple star system in which the secondary is the best astronomical candidate for a black hole. The existence of a third body in the system could affect the mass derived for the secondary from the analysis of spectroscopic radial velocity curves[1]. Hence, the presence of a periodicity in the system other than that of the 5.6-d binary orbit has a major bearing on the identification of the secondary with a black hole, especially if such a periodicity can be related to the orbital period of a third body. The existence of a 78.4 (or 39.2)-d periodicity in the optical and low-energy X-ray wavelength region of the emission from this system has been reported by Kemp *et al.*[2,3]. The existence of a 39.2-d modulation in the optical and low-energy X-ray luminosities has been questioned by Walker *et al.*[4]. We report here a search for a 78.4-d modulation in the high-energy X-ray flux observed with OSO 8 and in the U-band optical polarisation previously observed[5], with negative results. We suggest, however, that if such modulation does exist, it is more likely to be related to the rotation of the free modes of oscillation of the primary than to the existence of a third body in the system.

Analysis of the power spectrum of the U-band polarisation of HDE226868 on 225 nights between 1974 and 1977 led to the original detection by Kemp *et al.*[2] of a 39.2-d modulation in the magnitude of polarisation with peak-to-peak amplitude of 0.25% (or 0.0054 mag). A modulation with a 78.4-d period was detected by Kemp *et al.*[3] in the power spectrum analysis of the U-band photometry of the system taken during 43 nights in 1974 and 84 nights in 1977. The peak-to-peak modulation was approximately 0.015 mag. A reanalysis of the UV filter polarimetry at the 78.4-d period produced a double-peaked modulation; the 0.0054 mag modulation in the polarisation was equivalent to a peak-to-peak amplitude of approximately 8% of the mean polarisation. In a similar analysis of the power spectrum of the X-ray flux in the energy range 3–6 keV from Cyg XR-1 observed between October 1974 and December 1977 with the all-sky monitor on Ariel 5, Kemp *et al.*[3] detected a 78.4-d modulation with peak-to-peak amplitude of approximately 15% of the mean flux. The modulation seems to be in phase with the U-band photometric modulation. This periodic modulation was detected only when the X-ray source was in an intensity 'low state'.

Cyg XR-1 was observed with the high energy X-ray spectrometer[6] on OSO 8 during periods of 10–20 d in each of the years between 1975 and 1977 (ref. 7). The source exhibited a transition from the intensity low state to the intensity high state during the observation in 1975 (ref. 8). Consequently, only the data obtained in 1976 and 1977, when the source was continuously in a low state, were analysed for a 78.4-d modulation. The counting rates from the source between 23 and 153 keV, binned in intervals of one-tenth the 5.5995-d binary period, were folded modulo the 78.4-d period with epoch of zero phase as given by Kemp *et al.*[3] (Fig. 1*a*). The weighted mean counting rate over intervals of 0.1 in phase are shown in Fig. 1*b* for direct comparison with the 3 to 6 keV X-ray data of Kemp *et al.* (Fig. 1*c*). The modulation observed by Kemp *et al.* at lower energies is not immediately apparent in our data. The variations evident in Fig. 1*b* have a peak-to-peak range of 19% of the mean flux between 23 and 153 keV. If any 78.4 (or 39.2)-d modulation exists in the high-energy X-ray flux from Cyg XR-1, it must be at an amplitude significantly lower than this. Because of the irregular spacing of the data and the fact that all observing runs were short compared with the 78.4-d period, no more stringent limits could be placed on the existence of a 78.4-d modulation by the use of power spectrum analysis.

Observations of the polarisation of HDE226868 in the U-band were obtained over 23 nights in 1973, 1974 and 1975 (ref. 5). The individual observations were intended to investigate short time-scale phenomena: each U-band polarisation measurement was completed in 10 min. The observations were folded modulo the 78.4-d period with the same epoch of zero

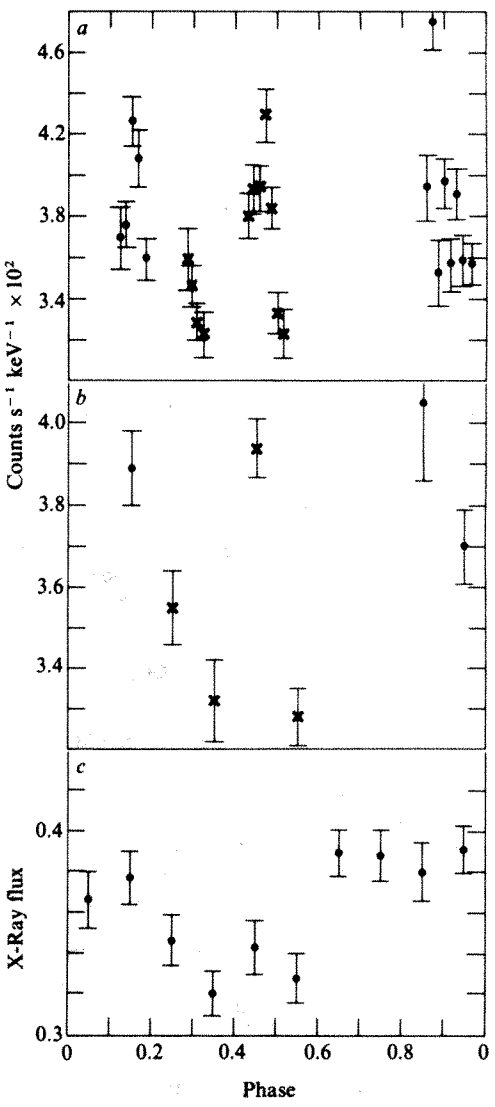


Fig. 1 X-ray counting rates observed from Cyg XR-1 plotted modulo the 78.4-d period of Kemp *et al.*[3]. (*a*), Individual observations of the 23–153 keV counting rates taken in 1976 (×) and 1977 (●). (*b*), Weighted mean counting rate of the observations in (*a*) over intervals of 0.1 in phase. (*c*), The 3–6 keV flux observed by Kemp *et al.*[3].

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phase as above. The weighted mean polarisation in intervals of 0.1 in phase shows variability with a peak-to-peak amplitude of 34% of the mean polarisation. Variations typically of this magnitude have been ascribed[5] to Rayleigh scattering of light from intercomponent gas streams known to exist in the system. A χ^2 test shows that the data points are equally consistent with a random distribution about either a non-varying polarisation or the type of curve fitted to their data by Kemp *et al.*[3]. The data do place an upper limit on the existence, during our observations, of any variability of the type seen by Kemp *et al.*, but only at an amplitude four times that seen by them. Once again, the irregular spacing and greatly varying statistical weights of the individual observations preclude using a power spectrum analysis to set more stringent limits on any periodic modulation.

Kemp *et al.*[3] propose a physical interpretation of a 78.4-d period in terms of either a ternary system, the precession of an accretion disk, or some kind of pulsation. They discuss the three-body possibility, although they point out that their analysis of spectroscopic radial velocity observations of the visible primary limits any third body in the system with a 78.4-d period to a mass of less than $(0.8 \pm 0.6)\ M_\odot$. We believe that a more likely interpretation of a 78.4-d periodicity in the system, if confirmed, is related to the fundamental rotation properties of the free modes of oscillation of the primary. In the Wolff and Kondo model[9,10] of X-ray binary systems, for example, the non-degenerate star pulsates in a broad array of gravitational modes (g-modes), global in scale and lacking spherical symmetry. Under slow rotation and sufficient non-linear coupling, the g-modes can combine to form a much smaller set of non-linear modes departing from azimuthal symmetry. As the main antinodes of these non-linear modes rotate past each other, the oscillatory power density at these locations is temporarily enhanced. The occurrence of transitions between intensity states of the X-ray source is ascribed by Dolan *et al.*[7] to sudden changes in the amount of mass transferred whenever the main antinodes are aligned with the sub-secondary point on the surface of the primary. In this picture, the 78.4-d period would be the time between effective alignments of certain g-modes.

As noted by Kemp *et al.*[3], excess mass transfer between the binary components modulated with a 78.4 d period could explain all the modulation they see in the X-ray and UV emission. The continued existence of this period over 3 years implies that it may be maintained by some forcing function. If the 78.4-d period is an exact integral multiple of the 5.6-d orbital period of the secondary[2,3], tidal forces occurring every periastron passage of the secondary in its eccentric orbit ($e = 0.06$) could provide such a forcing function (see ref. 11). Further observations are necessary to determine whether the 78.4-d modulation can be confirmed, and whether it can be related to the rotation properties of the primary's free modes of oscillation.

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Simultaneous effects of CO_2 and chlorofluoromethanes on stratospheric ozone

THE amount of ozone in the stratosphere is determined by a dynamic balance between processes from three disciplines: photochemical kinetics, radiative transfer and dynamical meteorology. These processes are interactively coupled, principally through the temperature of the air molecules; because of the low pressure and solar UV light a chain reaction is possible by which the composition of the stratosphere, and particularly its ozone content, may be affected by comparatively small quantities of trace species. Calculations have indicated that oxides of nitrogen from high-flying aircraft and nuclear weapons, nitrous oxide from artificial fertilisers and various chlorine-containing derivatives of methane and ethane (mainly CF_2Cl_2, $CFCl_3$, CCl_4 and CH_3CCl_3) could be large enough to act in this way. Reductions in ozone column density could lead to increases in the amount of solar UV light transmitted to the planetary surface in the wavelength region 290–320 nm, with possible effects on human health and biological activity. We have already shown that projected increases in carbon dioxide amounts in the atmosphere would lead to cooling and thus to ozone enhancement in the stratosphere, particularly at the upper levels. Here we extend these calculations to include simultaneous perturbations of CO_2 and chlorofluoromethanes.

The photochemical-radiative column model is as used previously[1,2], but it incorporates the following modifications. The solar radiative transfer scheme for the computation of photodissociation coefficients has been reformulated to include the effects of multiple scattering, in a manner similar to that of Isaksen *et al.*[3]. The chemical kinetic scheme has been expanded

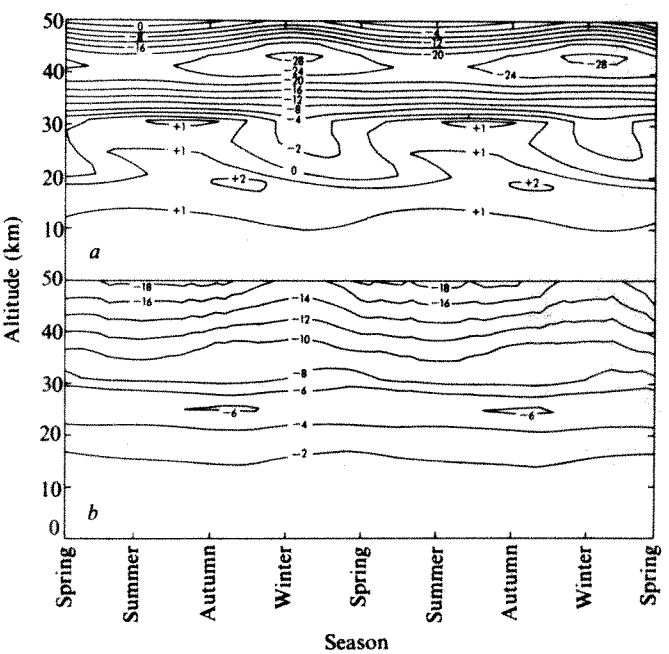


Fig. 1 Integration 5 minus integration 1 from Table 3. *a*, Percentage change in ozone number density; *b*, change in temperature (K).

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Table 1 Photochemical kinetic mechanism and rate coefficients

Reaction	Mechanism	Rate coefficients (cm mol^{-1} s^{-1})
1	$O_2 + h\nu \rightarrow O + O$	Photodissociations have diurnal and seasonal variation included explicitly
2	$O_3 + h\nu \rightarrow O(^1D) + O_2$	
3	$O_3 + h\nu \rightarrow O + O_2$	
4	$N_2O + h\nu \rightarrow O(^1D) + N_2$	
5	$NO + h\nu \rightarrow N + O$	
6	$NO_2 + h\nu \rightarrow NO + O$	
7	$HNO_3 + h\nu \rightarrow OH + NO_2$	
8	$NO_3 + h\nu \rightarrow NO_2 + O$	
9	$N_2O_5 + h\nu \rightarrow NO_2 + NO_3$	
10	$HO_2 + h\nu \rightarrow OH + O$	
11	$H_2O_2 + h\nu \rightarrow OH + OH$	
12	$HCl + h\nu \rightarrow H + Cl$	
13	$CH_3Cl + h\nu \rightarrow CH_3 + Cl$	
14	$ClONO_2 + h\nu \rightarrow NO_2 + ClO$	
15*	$CF_2Cl_2 + h\nu \rightarrow 2Cl$	
16*	$CFCl_3 + h\nu \rightarrow 3Cl$	
17*	$CCl_4 + h\nu \rightarrow 4Cl$	
18	$HNO_4 + h\nu \rightarrow HO_2 + NO_2$	
19	$O + O_2 + M \rightarrow O_3 + M$	$1.1\times10^{-34}\exp(510/T)$
20	$O + O_3 \rightarrow O_2 + O_2$	$1.9\times10^{-11}\exp(-2{,}300/T)$
21	$O(^1D) + M \rightarrow O + M$	$2.0\times10^{-11}\exp(107/T)$
22	$O(^1D) + N_2O \rightarrow NO + NO$	5.5×10^{-11}
23	$O(^1D) + N_2O \rightarrow N_2 + O_2$	5.5×10^{-11}
24	$NO + O_3 \rightarrow NO_2 + O_2$	$9.0\times10^{-13}\exp(-1{,}200/T)$
25	$NO_2 + O \rightarrow NO + O_2$	9.1×10^{-12}
26	$NO_2 + O_3 \rightarrow NO_3 + O_2$	$1.2\times10^{-13}\exp(-2{,}450/T)$
27	$NO + NO_3 \rightarrow NO_2 + NO_2$	8.7×10^{-12}
28	$NO_2 + NO_3 \rightarrow N_2O_5$	See Table 2.
29	$OH + ClNO_3 \rightarrow NO_3 + (HOCl)$	$1.2\times10^{-12}\exp(-333/T)$
30	$N + NO \rightarrow N_2 + O$	$8.2\times10^{-11}\exp(-410/T)$
31	$N + NO_2 \rightarrow N_2O + O$	$2.0\times10^{-11}\exp(-800/T)$
32	$N + O_2 \rightarrow NO + O$	$5.5\times10^{-12}\exp(-3{,}220/T)$
33	$N + O_3 \rightarrow NO + O_2$	$5.0\times10^{-12}\exp(-650/T)$
34	$N_2O_5 + M \rightarrow NO_2 + NO_3$	$k_{28}\times5.7\times10^{14}$ $\exp(-10{,}600/T)/3.8\times10^{-12}$
35	$O(^1D) + H_2O \rightarrow OH + OH$	2.3×10^{-10}
36	$O(^1D) + CH_4 \rightarrow OH + CH_3$	1.4×10^{-10}
37	$OH + O_3 \rightarrow HO_2 + O_2$	$1.5\times10^{-12}\exp(-1{,}000/T)$
38	$O + HO_2 \rightarrow OH + O_2$	3.5×10^{-11}
39	$O + OH \rightarrow O_2 + H$	4.2×10^{-11}
40	$H + O_3 \rightarrow OH + O_2$	2.6×10^{-11}
41	$H + O_2 + M \rightarrow HO_2 + M$	$2.1\times10^{-32}\exp(290/T)$
42	$HO_2 + O_3 \rightarrow OH + O_2 + O_2$	$1.4\times10^{-14}\exp(-580/T)$
43	$OH + HO_2 \rightarrow H_2O + O_2$	5.1×10^{-11}
44	$HO_2 + HO_2 \rightarrow H_2O_2 + O_2$	$6.4\times10^{-13}\exp(500/T)$
45	$OH + H_2O_2 \rightarrow HO_2 + H_2O$	$1.0\times10^{-11}\exp(-750/T)$
46	$OH + CH_4 \rightarrow H_2O + CH_3$	$2.4\times10^{-12}\exp(-1{,}710/T)$
47	$OH + NO_2 \rightarrow HNO_3$	$k_{47} = UV[M]/(U + V[M])$; $U = (68 - 0.22T)10^{-12}$, $V = 3.7\times10^{-32}\exp(1{,}093/T)$
48	$NO + HO_2 \rightarrow NO_2 + OH$	8.1×10^{-12}
49	$OH + HNO_3 \rightarrow H_2O + NO_3$	8.0×10^{-14}
50	$OH + OH \rightarrow H_2O + O$	$1.0\times10^{-11}\exp(-550/T)$
51	$OH + CO \rightarrow (CO_2) + H$	$(1.0 + 4.18\times10^{-20}[M])$ $\times2.1\times10^{-13}\exp(-115/T)$
52	$NO_2 + HO_2 + M \rightarrow HNO_4 + M$	See Table 2
53	$HNO_4 + M \rightarrow HO_2 + NO_2 + M$	See Table 2
54	$Cl + O_3 \rightarrow ClO + O_2$	$2.7\times10^{-11}\exp(-257/T)$
55	$O + ClO \rightarrow Cl + O_2$	$1.07\times10^{-10}\exp(-224/T)$
56	$ClO + NO_2 + M \rightarrow ClONO_2 + M$	See Table 2
57	$ClO + NO \rightarrow NO_2 + Cl$	$8.0\times10^{-12}\exp(250/T)$
58	$O + ClONO_2 \rightarrow ClO + NO_3$	$4.5\times10^{-12}\exp(-840/T)$
59	$Cl + CH_4 \rightarrow HCl + CH_3$	$7.3\times10^{-12}\exp(-1260/T)$
60*	$OH + HCl \rightarrow H_2O + Cl$	$3.1\times10^{-12}\exp(-437/T)$
61	$Cl + HO_2 \rightarrow HCl + O_2$	2.5×10^{-11}
62*	$OH + CH_3Cl \rightarrow H_2O + Cl$	$2.2\times10^{-12}\exp(-1{,}142/T)$
63	$O(^1D) + CH_3Cl \rightarrow ClO + CH_3$	1.8×10^{-10}
64*	$O(^1D) + CF_2Cl_2 \rightarrow ClO + Cl$	2.0×10^{-10}
65*	$O(^1D) + CFCl_3 \rightarrow ClO + 2Cl$	2.3×10^{-10}
66*	$O(^1D) + CCl_4 \rightarrow ClO + 3Cl$	2.5×10^{-10}
67	$O(^1D) + HCl \rightarrow OH + Cl$	1.4×10^{-10}
68	$CH_3 \rightarrow 3HO_2$	Parameterisation; $t_{CH_3} = 100$ s

* In these reactions, effective products are shown. Bracketed product molecules are not carried in the calculation.

Table 2 Rate coefficients for pressure-dependent reactions

Altitude (km)	Reaction 28 (cm^{3}mol^{-1}s^{-1})	Reaction 52 (cm^{3}mol^{-1}s^{-1})	Reaction 53 (s^{-1})	Reaction 56 (cm^{3}mol^{-1}s^{-1})
48	4.8×10^{-14}	7.3×10^{-15}	1.2×10^{-5}	5.5×10^{-15}
46	6.5	1.1×10^{-14}	5.5×10^{-6}	8.4
44	8.7×10^{-13}	1.6	2.9	1.3×10^{-14}
42	1.2	2.3	2.2	1.8
40	1.5	3.4	1.5	2.7
38	2.0	5.0	6.0×10^{-6}	3.9
36	2.4	7.2	3.2	5.8
34	3.0	1.0×10^{-13}	2.0	8.0
32	3.9	1.5	1.4	1.2×10^{-13}
30	4.5	2.0	9.6×10^{-8}	1.6
28	5.2	2.7	8.5	2.2
26	6.0	3.7	7.0	3.0
24	7.0	4.8	5.5	3.9
22	8.3	6.2	4.0	5.2
20	1.1×10^{-12}	7.5	3.5	6.5
18	1.3	9.2	4.0	8.0
16	1.7	1.1×10^{-12}	8.0	9.3
14	2.0	1.3	1.5×10^{-7}	1.1×10^{-12}
12	2.2	1.4	3.2	1.1
10	2.2	1.5	4.4	1.2
8	2.2	1.6	5.5×10^{-6}	1.2
6	2.3	1.6	6.0×10^{-5}	1.2
4	2.5	1.7	8.0×10^{-4}	1.2
2	2.5	1.7	9.0×10^{-3}	1.2
0	2.5	1.8	1.3×10^{-1}	1.3

to that detailed in Tables 1 and 2. The lower boundary conditions are as before except for nitrous oxide, the mixing ratio of which is 3.25×10^{-7}, in line with recent measurements[4]. The mixing ratios of stable chlorine-containing species are listed in Table 3; the new soluble species such as HCl have been added to the list of molecules rained out. A series of five integrations has been made, as shown in Table 3. The design of our radiative transfer scheme does not allow realistic calculation of the effects of CO_2 increases much beyond a factor or two from 350 p.p.m.v. Therefore we have not attempted to simulate the case of simultaneous projected CO_2 (approximately 1,100 p.p.m.v.) and steady state CFM amounts at 1975 release rates, which may occur about the period 2060–2070. Instead, we have computed the effects of steady state CFM release with 600 p.p.m.v. of CO_2. Integration 5 may be viewed as a possibly realistic schedule for simultaneous CO_2 and CFM release, with the former at 600 p.p.m.v. and the latter at half their steady state values. This is projected to represent approximately the period 2020–2030. It is apparent that the net decrease in ozone column density computed for this time is 2.6%. Earlier integrations performed without chlorine chemistry in the model[2] yielded an increase of 5.5% when the CO_2 was allowed to rise from 275 p.p.m.v. to 600 p.p.m.v.; the extra 2.3% compared with integration 2 of Table 3 must thus be attributed to this absence of chlorine from

Table 3 Mixing ratios of stable chlorine-containing species

Integration no.	CO_2 (p.p.m.v.)	CH_3Cl (10^{10} v.m.r.)	CCl_4 (10^{10} v.m.r.)	$CFCl_3$ (10^9 v.m.r.)	CF_2Cl_2 (10^9 v.m.r.)	% Change in ozone column density	Comments
1	275	7.5	1.25*	0	0	—	Reference atmosphere
2	600	7.5	1.25	0	0	+3.2	
3	600	7.5	1.25	0.8	2.3	−6.3	Steady state CFMs 1975 rates
4	275	7.5	1.25	0.8	2.3	−8.6	Steady state CFMs 1975 rates
5	600	7.5	1.25	0.4	1.15	−2.6	Approximately 2020 AD

* There may be both natural and man-made contributions to this species in the atmosphere[5–7].

the model. The remaining integrations demonstrate that the effects of CO_2 and CFMs on ozone column density are not quite linearly additive. Given this, it is apparent that in the simulated period about 2020, the CO_2 at 600 p.p.m.v. substantially alleviates the reductions in stratospheric ozone caused by continued CFM release at 1975 rates. The result of integration 4 yields a mean annual reduction of 8.6% in the ozone column density from steady state CFMs, if no increase in CO_2 over that in the reference atmosphere occurs. This is substantially less than recent estimates of some other column models[8,9], which have given figures of 15–20%. Presumably a fully diurnal model with interactive radiative transfer is more stable towards chlorine perturbation.

The comparatively small reduction in O_3 column density predicted in integration 5 was achieved at the cost of a substantial redistribution as a function of altitude, with a concomitant change in vertical temperature structure, as shown in Fig. 1. The importance of the coupling between ozone, temperature and large scale stratospheric dynamics[10] in this respect should be recalled.

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Geochemical and geophysical evidence on the geothermal potential of Caledonian granites in Britain

TECHNIQUES for the extraction of geothermal energy from hot dry granite are being developed with considerable success, principally at Los Alamos, New Mexico[1–3]. The method involves drilling to depths where the mean temperature exceeds about 160 °C and then using hydraulic fracturing techniques to create an artificial fracture system through which water is circulated and recovered for energy extraction by heat exchangers. At Los Alamos, the required bottom hole temperatures are reached within 3 km of the surface (>50 °C km^{-1}). Although gradients in Britain do not reach this value, a recent economic assessment (J. Garnish, personal communication, based on data in refs 2 and 4) of UK hot rock potential indicates that the Los Alamos technique may become cost effective where the required temperatures occur at <5 km depth; an average minimum thermal gradient of 30–35 °C km^{-1}. The Cornubian granite batholith of Hercynian age and, to a lesser extent the Tertiary granites of northwestern Scotland, have been considered to have potential as hot rock geothermal sources[4,5], but less emphasis has been placed on the Caledonian granites of Britain. Recent studies of heat flow in Britain[6,7], together with an increasing volume of interpreted geochemical and geophysical data, suggest that certain Caledonian granites of Lower Devonian age may have potential as hot rock sources comparable with that of the Cornubian batholith. Few direct measurements of temperature in Caledonian terrain exist although northern England is known to have high heat flow[6,7], and here we review the available data which have a bearing on the geothermal potential of these granites.

Table 1 Neutron activation analyses for important radioelements in the Cairngorm and Foyers granites

Sample no.	K (%)	U (p.p.m.)	Th (p.p.m.)
Cairngorm			
1	4.85	8.7	29
2	4.89	2.8	14
3	5.96	4.2	24
4	4.58	6.9	20
5	4.07	3.2	15
6	3.08	6.3	37
7	4.37	17.2	25
8	1.92	14.1	16
9	5.24	20.6	33
10	3.59	20.3	32
11	4.91	14.2	37
12	3.84	32.9	39
Foyers			
1	1.48	1.5	3.7
2	1.57	1.7	4.9
3	2.19	0.9	2.2
4	3.19	2.0	12.8
5	<0.80	1.2	6.9
6	2.52	1.5	5.9
7	2.16	1.7	3.5
8	2.75	0.9	3.5
9	2.38	1.3	5.1
10	2.73	0.9	2.7
11	2.81	1.9	4.3
12	3.21	1.3	5.8
Lewisian Inliers			
Mean values	<1.00	<1.0	1.5

U was determined by the delayed neutron method; K and Th were determined by instrumental neutron activation.

The first important feature is radiogenic heat production in the zone of interest. This is because of the known empirical relationship between high surface heat flow and high radioactivity in the crust: $q_0 = q^* + A_0 D$ (where q^* is the probable heat flow contribution from deep sources—the background heat flow—q_0 is the surface heat flow, A_0 is the surface heat productivity and D is a function of the thickness of the radioactive layer[7,9,10] (see also ref. 8)). The near-surface thermal gradient across a crustal segment is proportional to the measured heat flow (q_0); clearly, this will depend on both radioactivity (A_0) and the value of background heat flow (q^*). For southern Britain, Oxburgh and Richardson[7] have shown, from limited data, that heat flow and heat productivity are linearly correlated. It follows that in areas where heat flow data are limited it is important to identify zones in which q^* is likely to be most augmented by crustal heat sources. For example, the Cornubian batholith is located in a high heat flow province[4–6] and is characterised by high contents of the major crustal heat-producing radioelements U, Th and K. Shallow drilling in the Carnmenellis granite to 175 km has indicated gradients between 30 and 35 °C km^{-1} (ref. 11). The downward continuation of this gradient depends on certain assumptions about the variation of heat production with depth but there are good indications that the area is interesting from a geothermal energy viewpoint, especially where granite is overlain by low conductivity sediments.

In the Caledonian province, much of the region north of the Scottish Midland Valley is apparently underlain by a thick granulite succession[12] which, from geochemical investigations at outcrop[13–17] and from regional geochemical maps[18], appears to be depleted in heat-producing elements. The cover succession of Moine and Dalradian metasedimentary assemblages contains only low to average crustal abundances of K, U and Th as do the older, pre-Silurian granites, the migmatites and vein complexes,

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and the newer forceful granites[13,14,17,18]. Thus, the northern British Caledonides lack high heat productivity on a regional scale, but this does not exclude the possibility of geothermal hot spots of potential interest which are now tentatively identified.

Regional geochemical maps[18] indicate a high concentration of uranium and potassium over the Cairngorm granite and further studies of the distribution of uranium concentration in British granites[14] have confirmed the relatively high levels in Cairngorm and other Caledonian granites of Lower Devonian age, particularly Etive and Monadhliath from the northern province. To emphasise this point, Table 1 compares neutron activation analyses for K, U and Th in rock samples from Cairngorm, Foyers (a nearby older 'forceful' intrusion) and mean values for some Lewisian inliers. It is clear that Cairngorm has a much higher heat productivity than Foyers and values calculated from Table 1 using appropriate conversion factors[19] are 5.57 $\mu W\ m^{-3}$ and 0.96 $\mu W\ m^{-3}$ respectively.

Lower Devonian granites with high heat productivity are a feature of the entire British Caledonides, including northern England which is a recognised high heat flow province[6,7]: geophysical[22] and heat productivity[13,14,17] data for some of these granites are compared with the Cornubian batholith[20] in Table 2. Additional geochemical data for some of these granites indicate that they have features in common with the Cornubian batholith—high levels of K, Rb, U, Th, Li, Be, low K/Rb ratios, low total Sr, low initial $^{87}Sr/^{86}Sr$ ratios and chondrite normalised rare earth element (REE) patterns characterised by light RE enrichments with marked negative Eu anomalies[13,14,17,21]. Both suites of intrusions also have large negative Bouguer gravity anomalies[21-23] which indicate that they have large volumes and extend to deep crustal levels. Studies of thin sections using the Lexan plastic film technique also indicate that uranium, at least, is located in primary mineral phases such as zircon and apatite[14,24]. Thus, the high levels of radioelements in these granites at outcrop are unlikely to have resulted from mineralisation. Together with the evidence of shallow drilling in south-west England and Weardale[11,25] this suggests that the heat productivities in Table 2 may be regarded as representative of larger volumes extending to depth, although this conclusion needs testing by deep drilling and by considering the geochemical evidence for magmatic fractionation. In the absence of heat flow data to the contrary it seems that the essential characteristics of several newer Caledonian granites are comparable with the Cornubian prospect from a geothermal viewpoint.

The factors identified here so far which favour high geothermal gradients within the top few kilometres of crust are: (1) high background heat flow; (2) high heat productivity, normally associated with granites; (3) large intrusive volumes recorded by substantial negative Bouguer gravity anomalies (see also ref. 26).

Table 2 Radioelement concentrations, heat productivities and Bouguer gravity anomaly amplitudes for selected Lower Devonian granites from the British Caledonides compared with data for the Hercynian Cornubian granite

Intrusion	K (%)	U (p.p.m.)	Th (p.p.m.)	Heat production* ($\mu W\ m^{-3}$)	Amplitude of Bouguer gravity anomaly† (mgal)
Skiddaw	3.86	8.70	18.2	3.91	−19
Weardale	4.29	12.89	12.3	4.63	−37
Shap	4.23	9.68	38.1	5.61	−24
Cairngorm	4.27	12.60	26.8	5.57	−30
Cornubian batholith	4.32‡	11.3‡	19.1‡	4.68	~−50

* Refs 8, 14, 21 and this work.

† Refs 22, 26, 28.

‡ Average from unweathered and unkaolinised samples over entire batholith[23].

From Table 2, we deduce that factors (2) and (3) may be as effective in raising thermal gradients over the Cairngorm and Weardale intrusions as over the Cornubian batholith. Unfortunately, Weardale is the only Caledonian intrusion of those listed in Table 2 to have been drilled. Although the thermal gradient within the granite is only 31 °C km^{-1} (ref. 27), higher values may be encountered in the coalfield region to the east, within sediments underlain by granite. Such an elevated gradient may be postulated from the much lower thermal conductivity of coal-bearing strata which effectively insulate the underlying region. Another factor favouring high thermal gradients in shallow crust arises: (4) a low conductivity sedimentary cover.

It follows that large volume radioactive granites buried beneath suitable sedimentary sequences provide prime targets for hot rock geothermal exploration. In the British Caledonides such granites may be identified from lows in the Bouguer gravity field since, at outcrop, only this type of granite is associated with high radioactivity.

In conclusion, there are several important geothermal targets in the British Caledonides which might usefully receive further attention (see ref. 28). These include the northern England granites, including postulated buried granites in eastern England (for example, at Market Weighton[29]) and the region surrounding the Cairngorm and related intrusions. All but the latter lie in a high heat flow province and the Lower Devonian granites of northern Scotland may prove to be relative hot spots in an otherwise barren geothermal province.

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Pliocene eustasy and the onset of sand barrier formation in Gippsland, Victoria

GIPPSLAND possesses a classical development of coastal lakes formed behind a sand barrier system which achieved its present form during the Quaternary. New evidence is assembled here, showing that a transgressive–regressive–transgressive cycle in the late Miocene–Pliocene ended in an earlier phase of barrier formation and lacustrine deposition. Another period of regression in the late Pliocene completed the cycle. Sufficient remnants of the deposits of the Pliocene lake and sand barrier have survived Pleistocene erosion to establish their former existence.

The Gippsland Lakes (lat 38° S. long 148° E.) are a series of brackish and freshwater lakes (Fig. 1), distributed over an area of about 500 km^2 (refs 1, 2). They overlie a 'continental shelf basin' which has accumulated sediment throughout much of the Tertiary[3] and are separated from the Tasman Sea by a sand-barrier system which, in its present form, is undoubtedly Quaternary in age[4].

This Tertiary sequence contains evidence of a regression in the latter part of the Miocene, first expressed as a facies change from bryozoal to shelly limestone in the upper part of the Bairnsdale Limestone[5,6] (Fig. 2, phase A) and continuing during the deposition of the overlying glauconitic clays, to culminate in the phosphatic nodule bed (phases B–D). During the regression, the sea retreated from the western (Bairnsdale) area, depositing there only a reduced thickness of Tambo River Formation[3]. Marine sediments of younger age are known in outcrop only from the Lakes Entrance–Lake Tyers area and in deep wells south and south-west of Bairnsdale[5]. The early Pliocene transgression is expressed by the Jemmys Point Formation, which was deposited in the initially deeper eastern part of the basin as the sea level rose. Deposition at this time did not extend to the Bairnsdale area and did not resume there until the sea level had risen sufficiently. The Jemmys Point Formation is composed of marine silty clay and near its base it contains a molluscan fauna including the tropical bivalve genus *Pinctada*

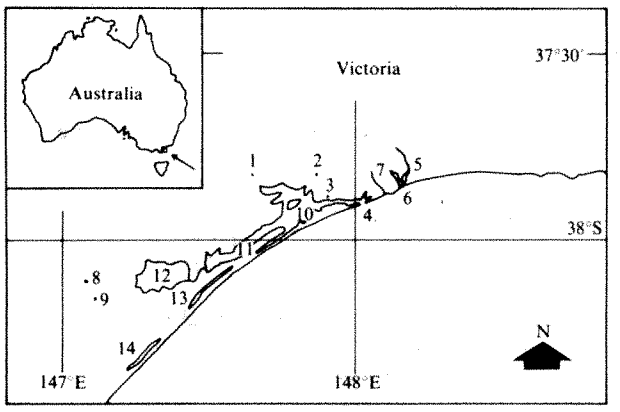


Fig. 1 Locality map of the Gippsland Lakes area. 1, Bairnsdale; 2, Swan Reach; 3, Nungurner; 4, Lakes Entrance; 5, Lake Tyers; 6, Red Bluff; 7, Bunga Creek; 8, Sale; 9, Longford; 10, Lake King; 11, Lake Victoria; 12, Lake Wellington; 13, Lake Reeve; 14, Lake Denison.

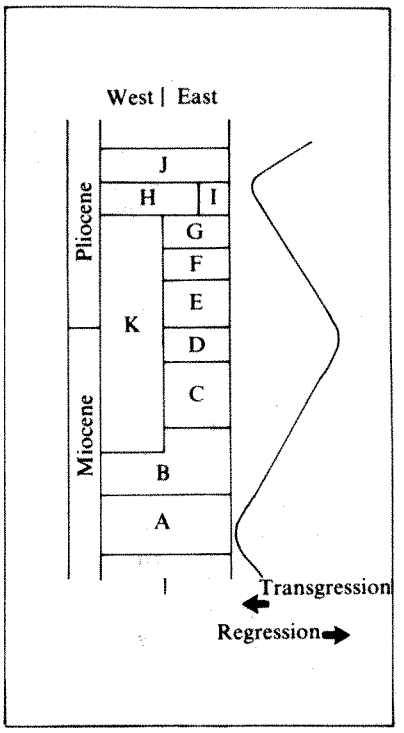


Fig. 2 Stratigraphic column (not to scale) of Miocene–Pliocene formations in Gippsland, correlated with general trends of fluctuations of sea level. Left hand side of column represents western part of area (Bairnsdale district); right hand side represents eastern part (Lakes Entrance–Lake Tyers). A, Bairnsdale Limestone; B, Tambo River Formation; C, Long Point Formation (Carter MS); D, phosphatic nodule bed; E, Jemmys Point Formation; F, Remnant of Pliocene barrier formation within Jemmys Point Formation; G, estuarine beds at top of Jemmys Point Formation; H, Moitun Creek Formation; I, Nyerimalang Formation; J, Haunted Hill Gravel; K, strata missing on unconformity at Bairnsdale.

(the 'pearl shell'), suggesting a warm water environment. It is dated by a lower pelagic foraminiferal zone of *Globorotalia puncticulata*, overlain by a zone of *G. inflata*. In this latter respect, it resembles Pliocene zones in New Zealand[7].

Just below (1–2 m) the top of the Jemmys Point Formation, a bed of cross-bedded sandstone (Fig. 2, Bed F) is intercalated in the otherwise predominantly argillaceous sediment. In places, it possesses sedimentary structures similar to those described from California in a beach formation resting on an unstable substratum[8]. This bed is traceable along the strike for at least 16 km; its continuity and contrasting lithology are highly significant in a depositional facies not noted for continuity of individual beds. This bed is overlain by a further development of the same argillaceous lithology that comprises the bulk of the Jemmys Point Formation, but never more than 2 m thick. However, it has a more estuarine aspect, as evidenced by the abundance of carbonaceous plant debris in the sediment.

Above this level, near the present coast, the depositional facies changes abruptly to littoral sandstones (up to 10 m in thickness), intertwining with lacustrine sediments (Fig. 3). These latter (Moitun Creek Formation) are traceable intermittently for 60 km westwards from Lake Tyers and remnants can be seen as far away as 90 km to the south-west, near Longford[9]. At some places (for example, Red Bluff and Cross's Landing), sections through the littoral sandstone facies show up to 10 m of relatively coarse sandstone and shell fragments, suggesting barrier remnants; elsewhere (such as at the east side of Lake Tyers and Bunga Creek) finer grained sandstones with occasional marine fossils suggest beach or dune developments and both types of sandstone are seen to intertwine with brown siltstones of lacustrine origin. The extent of the Jemmys Point Formation, landwards of the barrier remnants and overlain by the Moitun

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Creek Formation, suggests that the barriers occupied an offshore position at the time of their initial formation.

Noteworthy features of this sequence are that the degrees of overlap suggest that the Miocene transgression extended further inland than the Pliocene transgression, suggesting in turn that the Miocene sea level was relatively higher than that in the Pliocene. During the late Miocene regression, non-marine sediments were not deposited on the emerged sea floor, which was not covered by sediment until the Pliocene transgression, with the unusual event of lacustrine sediments deposited behind the sand barrier being transgressive. However, during the late Pliocene regression, the Haunted Hill Gravel was deposited on the emerged sediment surface composed of Moitun Creek and Nyerimalang Formations. Rapid accumulation of Haunted Hill Gravel is suggested by its poorly sorted character and relatively rapid regression is also indicated.

Recent field work[10] at Lake Tyers has demonstrated a regressive sequence in the mid to late Miocene, from the Bairnsdale Limestone to the phosphatic nodule bed (Figs 2 and 3), a fuller account of which will be given elsewhere. This Miocene regression is widely recognisable[11]. The resumption of deposition on the Gippsland continental shelf in the early Pliocene, following the regression, is also compatible with world-wide seismic data[11] and with the transgressive stratotypes of the Pliocene Stages (Tabianian–Piacenzian) in northern Italy[12], overlain by late Pliocene limestone ('Astian' facies). In the Piacenzian, there is also a lower zone of *Globorotalia puncticulata* and an upper zone of *G. inflata*, though with two intermediate zones[12] which have not yet been recognised in Gippsland. In East Anglia (UK) there are no sediments representing the Miocene transgression, but the phosphatic 'boxstones' of Suffolk occur on an unconformity surface[13]—a stratigraphical position compatible with an interpretation of their formation during a period favourable to world-wide phosphatic deposition in the late Miocene[10]. The overlying Pliocene deposits ('Coralline Crag') have been described as submarine banks[14,15] which in a general sense are not greatly different from sand barriers, suggesting an incidence of coastal sand bar formation in the Pliocene, similar to that in Gippsland. One notes also, transgressive–regressive cycles in the Pliocene of South Australia[16], South Africa[17] and Morocco[18].

The strongly onlapping lacustrine sediments of the Moitun Creek Formation (Figs 2 and 3, Bed H) represent deposition at the time of maximum transgression in the Pliocene, after which another regression occurs. The widespread lacustrine sediments require a barrier to account for their non-marine sedimentary facies and a phase of high sea level to account for their areal extent (see Fig. 3). Non-marine clays, sands and gravels (Haunted Hill Gravel) overlie the sediments of phase H–I and these formed during the late Pliocene regression.

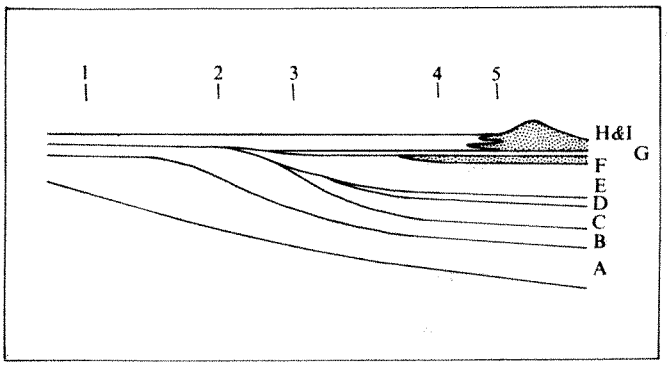


Fig. 3 Reconstruction of the northeastern part of the Gippsland Basin, showing Neogene formations at the end of the Pliocene transgression, before the late Pliocene regression and Pleistocene erosion (not to scale). 1, Bairnsdale; 2, Swan Reach; 3, Nungurner; 4, Lakes Entrance; 5, Lake Tyers. H, Moitun Creek Formation; I, Nyerimalang Formation. Other lettering as in Fig. 2. Stippling indicates sand barrier formations.

During the Pleistocene, coastal erosion destroyed most of the Pliocene barrier and undoubtedly redistributed its sediments during the periods of high sea level and probably also destroyed some of the lacustrine deposits. In the intervening periods of low sea level, river erosion removed large sections of the lake deposits.

It follows from the field evidence that the initial sand barrier formed after a period of rising sea level. Its establishment may have been due to a period of sea level stability but there is no positive evidence either for or against a postulated still stand. It is clear, however, that a further rise of sea level occurred after the initial barrier formation, causing the destruction of the first barrier, whose remnant can be seen (Bed F) but formation of a second barrier (Bed I) and a further rise of sea level allowed the formation of a coastal lake, in which Bed H was deposited.

The presence of Pliocene barrier remnants in Gippsland suggests that, in areas where extensive sand barriers still exist, it would be worthwhile seeking evidence of Pliocene precursors of these barriers, in view of the support they give to the concept of Pliocene eustasy and to the likelihood that they formed at times of still stand during a generally transgressive regime.

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Holocene submergence of southern Long Island, New York

RATES of submergence of the northeastern seaboard of the US during the past several thousand years as determined from studies of coastal marsh deposits show considerable variation. The marshes became established on submerging shorelines and have grown upwards and landwards with the continued relative rise in sea level, thereby often forming thick deposits of peat. A series of radiocarbon dates taken at various levels in the marsh can be used to reconstruct the history of submergence. This letter presents a study of the relative change in sea level in southern Long Island, New York, over the past 8,000 yr, and shows that the changing rates of submergence have been an important factor in the initiation and continued development of the area's saltmarshes.

Samples of basal peat from south-central Long Island were dated by the radiocarbon method and a local curve of submergence was constructed for the past 8,000 yr. Three basal-peat samples from marsh areas behind the modern barriers have been dated in the present study. In addition, two previously unpublished dates of organic-rich silty clays (originally back-barrier deposits) from the modern shoreface became available.

Two shallow basal peats from the back-barrier area were collected by hand coring in the saltmarshes fringing the northern margins of western Great South Bay (Fig. 1). Another sample of

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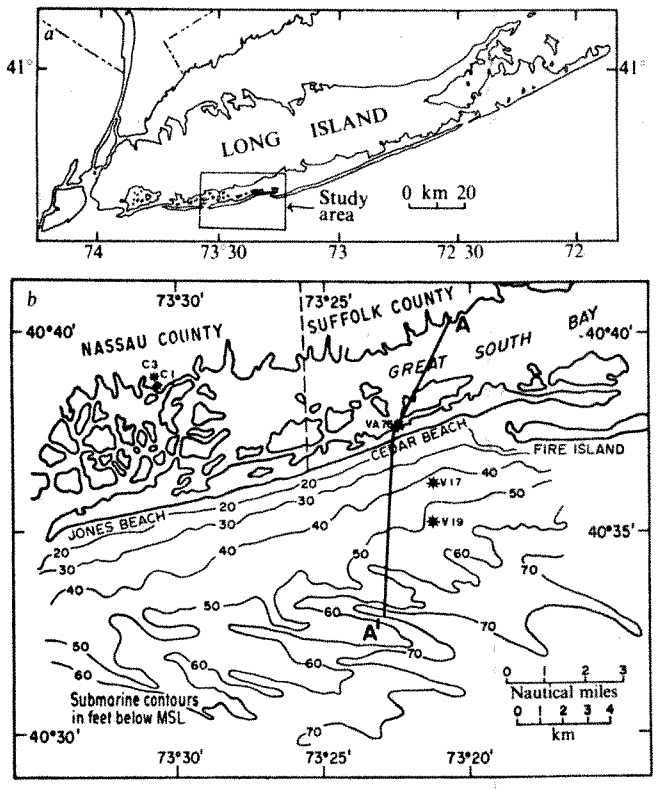


Fig. 1 Location map of the study area showing positions of the borings and cores (*) from which radiocarbon-dated material was taken. Data from the line of borings, A to A', were used to construct the section in Fig. 2. Offshore contours after Williams[4].

basal peat was collected by vibracoring at a depth of −10 m MSL directly behind and beneath the modern barrier island. Locations of these radiocarbon-dated cores are shown in Fig. 1; the dates are plotted on a schematic cross-section of the Holocene stratigraphic sequence as determined from several hundred boreholes and cores in Fig. 2.

Basal peat in tube core 1 was dated at 1,015 ± 100 yr BP (Queens College no QC-315) at approximately −1.1 m MSL. Basal peat in tube core 3 was dated at 300 ± 85 yr BP (QC-316) at approximately −0.3 m MSL. The peat sample taken from vibracore VA-76 at approximately −10.1 m MSL was dated at 5,055 ± 120 yr BP (QC-314).

For each of these dates the samples were of salt- to brackish-water peat, directly overlying Upper Wisconsinan glacial outwash. Therefore, compaction of the underlying outwash sands and gravels is thought to have been minimal.

Organic silty clays collected by vibracoring on the modern shoreface (Fig. 1) were also dated by the radiocarbon method. These former back-barrier sediments were taken from vibracore V-17 at a depth in the core of 1.0 m in 12.6 m of water and from vibracore V-19 at 0.16 m core depth, in 16.4 m of water (Fig. 2). The sediments are lagoonal or saltmarsh deposits now exposed on the shoreface by barrier retreat. These deposits mark the position of a former back-barrier lagoon which existed when the barrier islands were located some 7 km south-east of the present shoreline. The detailed history of the retreat of the barriers of southern Long Island will be discussed elsewhere[1]. These back-barrier sediments were probably deposited at or slightly below sea level. They are over-consolidated, which indicates post-depositional compaction. Radiocarbon dating of these silty clays, performed on total organic material in the samples, gave 7,815 ± 300 yr BP for sample V-17 (1.0 m) and 7,130 ± 380 yr BP for sample V-19 (0.16 m) (dating by Teledyne Isotopes).

These dates from south-central Long Island have been compared with other published dates and curves of Holocene sea level from the Long Island area[2–4], and with the submergence curves which have been constructed for adjacent areas of the Atlantic Coastal Plain[5–8] (Fig. 3).

The submergence curve constructed from the data for southern Long Island represents the combined effects of eustatic sea-level rise, local tectonism, tidal variation, water loading and other variables. Therefore, the curve cannot be extrapolated directly to other areas. However, the submergence curve for south-central Long Island can be used in palaeogeographic reconstructions to locate the position of the palaeoshoreline, and as an indicator of changes in the relative rates of submergence during the past 8,000 yr.

The submergence curve constructed from these data suggests that between 7,000 and 3,000 yr BP the Long Island coast was being submerged at a rate of about 25 cm per 100 yr, and that during the past 3,000 yr, the submergence rate slowed markedly to about 10 cm per 100 yr. Rates of submergence before 7,000 yr BP are uncertain. Sea-level data presented here show considerable scatter before 7,000 yr BP (Fig. 3). It has been suggested that, between 9,000 and 7,500 yr BP, submergence rates in adjacent coastal areas were as high as 50 cm per 100 yr (refs 9, 10).

The submergence curve for Long Island, therefore, shows a marked reduction in submergence rate at about 3,000 yr BP, and the possibility of an earlier reduction in the rate of submergence at around 7,000 yr BP. Studies of relative sea-level rise in other areas of the Atlantic coast have outlined a similar history of changes in the submergence rate[5,11–13]. For example, the submergence rate for the Delaware coast was approximately 47 cm per 100 yr between 10,000 and 7,000 yr BP, slowed to 20 to 30 cm per 100 yr from 7,000 to 4,000 yr BP, and has continued at about 8.0 to 12.5 cm per 100 yr for the past 3,000 to 4,000 yr (refs 14, 15). Note, however, that the smoothly averaged curves presented here could be reinterpreted in terms of fluctuating relative sea level.

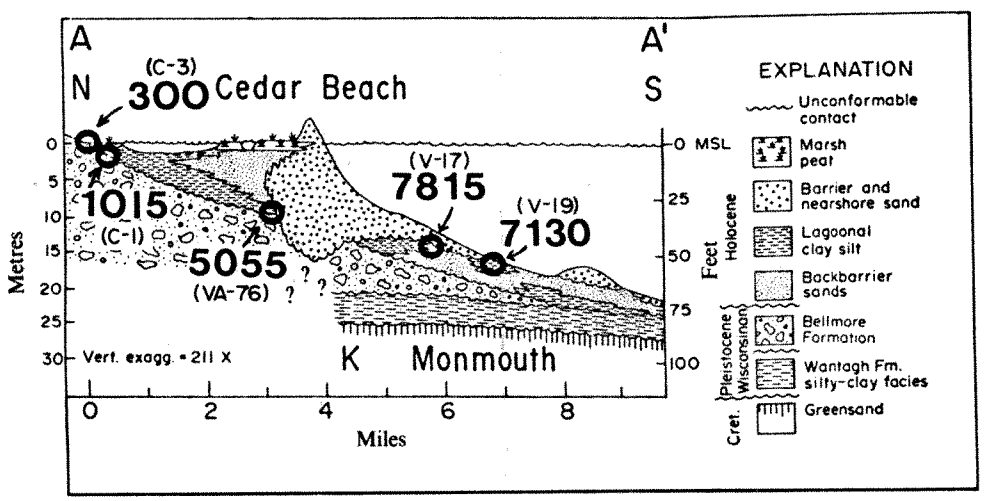


Fig. 2 Radiocarbon-dated samples plotted on schematic, interpretive profile and section A to A' showing the Holocene and Late Pleistocene stratigraphy of south-central Long Island. The positions of samples C-1 (1,015 ± 100 yr BP) and C-3 (300 ± 85 yr BP) are projected from cores to the west of this section.

The method of determining relative sea level by point-plotting the ages of basal-peat samples, and then drawing a best-fit curve will average out any fluctuations in the history of submergence. Postulated curves of fluctuating sea level[16] cannot be adequately tested in this manner. Careful stratigraphic analyses are required to test such an hypothesis.

One possible method is to study the stratigraphy of saltmarshes to determine changes in vegetational patterns and shifts in marsh environments that would accompany variations in sea level. No such consistent variations were found in examination of the cores of marsh sediment recovered in the present study.

It has been suggested that changes in the rate of submergence have been an important factor in the development and maintenance of back-barrier saltmarshes[5,11,17]. The radiocarbon dating of basal peats from southern Long Island sheds light on the mode of formation of the marsh areas. Peat samples from approximate depths of −1.1 and −0.3 m MSL gave radiocarbon ages of 1,015 ± 100 and 300 ± 85 yr BP, respectively (Fig. 2). These samples of brackish to saltmarsh peat presumably mark the approximate positions of sea level at those times. The fringing and mid-bay marsh areas of Great South Bay are therefore rather young. Peat has been probed with steel rods to depths of about −2 m MSL which, according to sea-level curves for adjacent areas, would put the inception of these marsh areas at about 2,000 yr BP (Fig. 3).

This conclusion is supported by the report of a basal peat layer at a depth of −7.5 feet (−2.4 m) MSL in Shinnecock Bay, in eastern Long Island, dated at 2,300 yr BP[18]. This date indicates that the back-barrier marshes in eastern Long Island were established at least 2,300 yr ago.

It seems that a back-barrier environment, with open-lagoonal conditions, existed for some time before the more recent development of the saltmarshes. The colonisation of previously open-lagoon environments by marsh grasses may have been related to a lessening in the submergence rate in the area about 3,000 yr BP. A decrease in the rate of submergence would have allowed sedimentation to build the floor of the lagoon to a level at which the marsh grass *Spartina alterniflora* could grow. As stands of *S. alterniflora* became established and began to trap fine sediment particles, a thin layer of peat would form across former tidal-flat areas. Once the level of the marsh had built to about mean high water, *Spartina patens* could colonise the marsh surface, and marsh building would continue to keep pace with submergence, thus forming the present marshes[5].

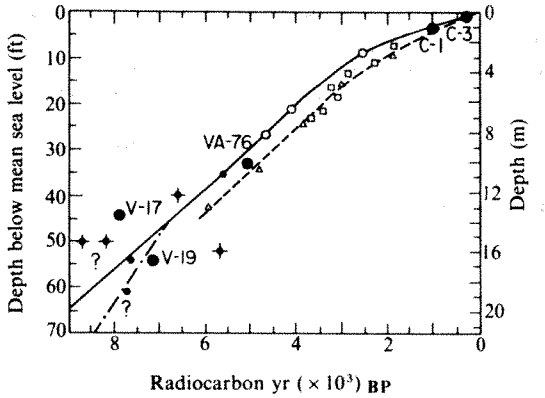


Fig. 3 Submergence curve for southern Long Island during the past 8,000 yr (solid curve). The curve is based on radiocarbon-dated samples reported in this paper (large solid circles) and on previously published dates from northern Long Island[3,4] (crossed solid circles), southern Long Island[2,4] (small solid circles) and Iona Island, New York[7] (open circles). The dashed and dotted curve indicates possible submergence rates of ~50 cm per 100 yr before 7,000 yr BP suggested for adjacent areas[9,14]. The dashed curve is drawn through points of dated samples from New Jersey[6] (triangles) and Cape Cod, Massachusetts[5] (open squares). An assumption of relatively smooth change in sea level has been made in constructing these curves.

In many studies of saltmarshes of the northeastern United States, a relationship has been established between marsh initiation and a marked slowing in the submergence rate between 3,000 to 4,000 yr BP[5,11,13,17]. It seems likely that the change from open lagoon with intertidal-flat areas to saltmarsh was initiated in western Great South Bay by a decrease in the rate of submergence at about 3,000 yr BP. This slowing of the submergence rate allowed sedimentation to build up the floor of the lagoon by about 2,000 yr BP, to a point at which marsh grasses could colonise former tidal-flat areas along the northern margins of the lagoons and also grow on former tidal-delta deposits. For the past 2,000 yr, upward marsh building has for the most part kept pace with the slow submergence.

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Mössbauer spectral studies of the diagenesis of iron in a sulphide-rich sediment core

ONE stage in sedimentary pyrite formation[1–3] is 'FeS' + S → FeS_2, where 'FeS' represents black precursor sulphides such as amorphous iron sulphides and fine-grained mackinawite ($Fe_{1+x}S$). We report here a Mössbauer spectral study of the formation of pyrite, in sulphide-rich sediments, from the reaction of H_2S with hydrated ferric oxides and with ferrous ions in fine-grained chlorite or in amorphous silicates. Approximately 50% of total Fe deposited is converted to pyrite within a few years, with the remainder being converted over several hundred years. Less than 10% of total Fe is present as precursor sulphides. With suitable constraints in computation, Mössbauer methods show considerable potential for measuring rates of reaction of iron compounds in sediments.

Lake St George is a kettle lake situated 60 km north of Toronto, Ontario. It has a surface area of 10 hectares, a maximum depth of 14 m, and is strongly stratified from May until turnover in late October, with the thermocline at ~9 m. The

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surface sediments are rich in organic matter (~25% dry weight) and in sulphide (1.7% total S in the top 6 cm, increasing to ~2.2% below 50 cm, compared with 1.6% Fe). Optical and scanning electron microscopy showed abundant pyrite framboids in all sections of core. X-ray diffraction methods revealed in decreasing order of abundance, quartz, calcite, feldspar, pyrite. Cores were obtained through surface ice in December 1977 using a photoactivated-sphincter lightweight corer[4]. The uniformly grey cores were extruded, sectioned and bottled under nitrogen, to minimise oxidation. The fractions were frozen immediately and freeze-dried 8 h later on return to the laboratory. Mössbauer spectra of the freeze-dried residuals were recorded in January 1978 at University College of Wales, Aberystwyth, and McMaster University, and computer-fitted using Stone's programs[5]. Half widths and peak areas within a quadrupole doublet were constrained to be equal. Low-spin ferrous ions in pyrite and high-spin ferric ions give rise to superimposable doublets[6,7], and additional constraints invoked are described below. The Ambrosia pollen horizon occurs at 15 cm, corresponding to colonial settlement in ~1830; this is consistent with current rates of sedimentation of ~1 mm yr^{-1} calculated from the weight of material collected in sediment traps.

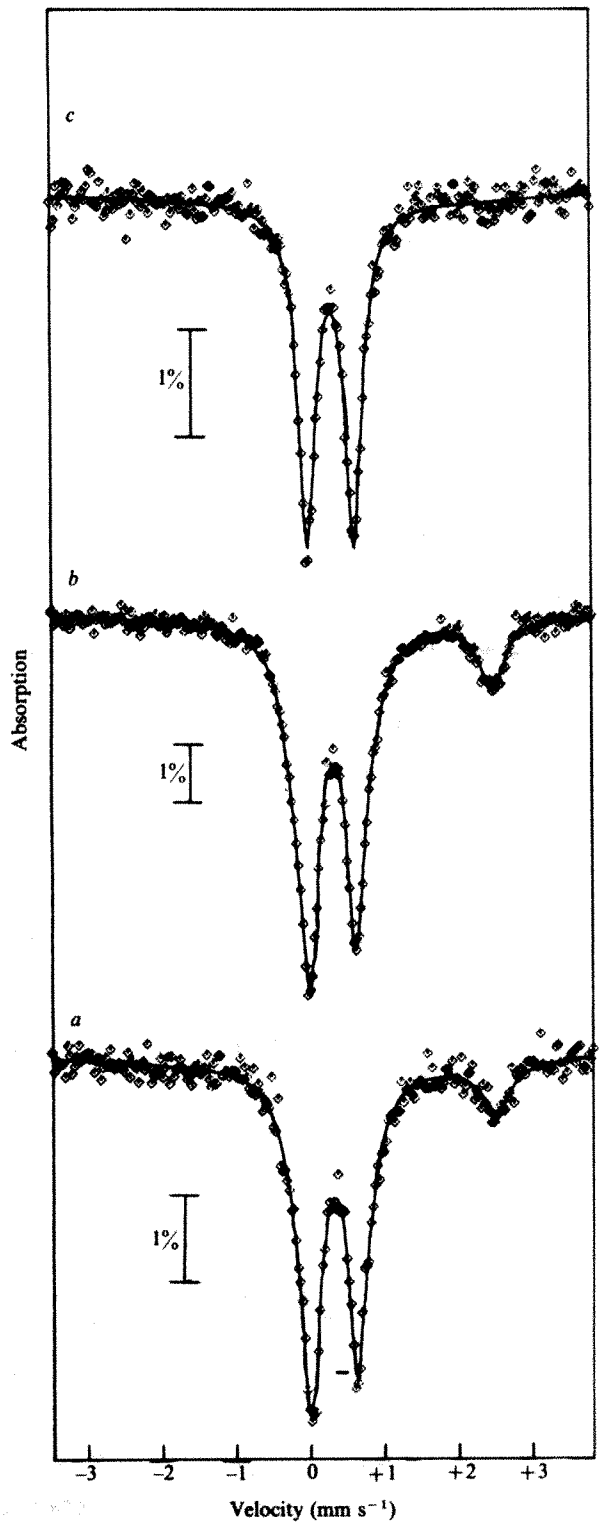


Fig. 1 Room-temperature Mössbauer spectra of freeze-dried Lake St George sediments: *a*, 0–1 cm; *b*, 1–2 cm; and *c*, 86–88 cm fractions. Continuous line represents a computed fit, based on one doublet for the 86–88 cm fraction and on three doublets for the other two. χ^2 values are, respectively, 220 with 239 d.f., 260 with 239 d.f., and 270 with 244 d.f.

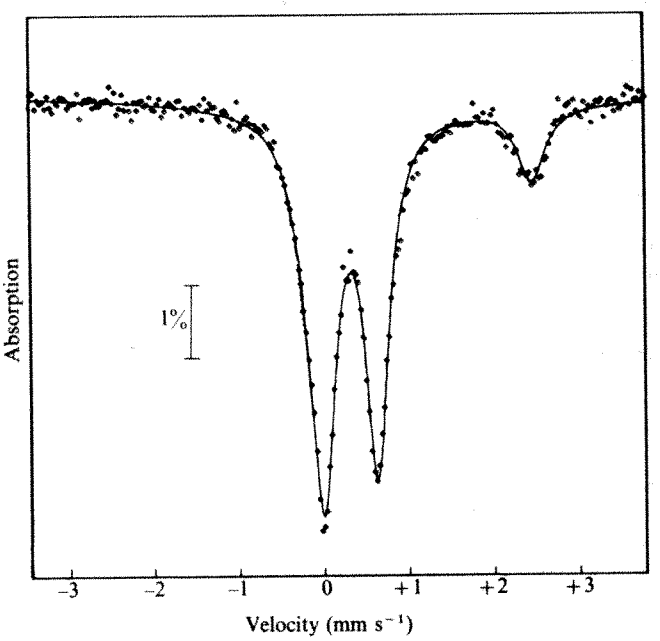


Fig. 2 Room-temperature Mössbauer spectrum of 1-2 cm fractions of Lake St George sediment, showing the two-doublet computed fit as the continuous line. χ^2 value is 339 with 240 d.f.

Selected Mössbauer spectra of sediment residuals are shown in Fig. 1. The disappearance of a 2.6 mm s^{-1} high-spin ferrous peak in spectra of the deeper fractions is clear evidence for diagenesis. Spectra of 50–52 cm, 62–64 cm and 86–88 cm fractions are virtually identical, and a one-doublet fit yielded values of isomer shift (IS) = 0.31 ± 0.01 mm s^{-1}, quadrupole splitting (QS) = 0.61 ± 0.01 mm s^{-1}, and half width (HW) = 0.285 ± 0.005 mm s^{-1}, which are clearly indicative[6] of pyrite (Table 1). Similar half widths were measured for powdered pyrite crystals from Tanybwlch Beach, Aberystwyth. Attempts to force-fit the spectra to two doublets by constraining pyrite peak positions and by constraining the peak positions and half widths for amorphous ferric compounds (using values of IS = 0.37 mm s^{-1}, QS = 0.67 mm s^{-1} and HW = 0.52 mm s^{-1} measured earlier[7] for Bay of Quinte, Ontario muds) were either unsuccessful or did not yield significantly lower values of χ^2. We estimate that >90% of total Fe is in FeS_2; based on 1.6% Fe, the S requirement is ~1.85%. However, 2.2% total S was measured.

Mössbauer spectra of 0–1 cm and 1–2 cm (Fig. 1) and 4–6 cm fractions are virtually identical. A two-doublet fit yielded hyperfine parameters for high-spin Fe^{2+} (the outer doublet) of IS = 1.15 mm s^{-1}, QS = 2.65 mm s^{-1} and HW = 0.40 mm s^{-1},

and for the inner doublet corresponding values of 0.32 mm s^{-1}, 0.62 mm s^{-1} and 0.32 mm s^{-1}, all values ±0.01 mm s^{-1}. The former set is reminiscent of octahedrally bonded Fe^{2+} in chlorite[7-9], whereas the second set is indicative of pyrite. Compromising the two-doublet scheme are the consistently poor fits on the high-energy limb of the main envelope (Fig. 2) and the significantly larger half widths of the inner doublet (0.32 mm s^{-1}) compared with the 0.285 mm s^{-1} values computed for pyrite in the deeper sections. A three-doublet fit with no additional constraints gave erratic results, so pyrite peak positions and half widths were fixed at values derived from the 50–52 cm, 62–64 cm and 86–88 cm fits. Computed hyperfine parameters and Fe distributions, showing good consistency, are listed in Table 1, together with Fe distribution for 20–22 cm and 44–46 cm fractions. The ferric parameters are in good agreement with those derived for hydrated ferric oxides in muds from other lakes[7-10].

The results suggest that pyrite forms from the reaction of H_2S with ferrous ions located in fine-grained chlorite or in an amorphous silicate and with ferric ions in mainly amorphous hydrated oxides. In contrast, chlorite-bound ferrous ions in Moira Lake, Ontario, muds (total S ~2%, total Fe ~4.5%) are not attacked[10] by H_2S. QS values measured for high-spin ferrous ions in Bay of Quinte[7] and in Lac Phillippe (P.G.M., unpublished work), Quebec, muds are 2.55 mm s^{-1} and 2.50 mm s^{-1}, respectively, suggesting significant differences in structure and/or composition of the host material; the latter value, in particular, seems outside the range for most chlorites[8]. Significant amounts of ferric ion persist for ~200 yr (~20 cm burial) in these reducing sediments, in contrast to conventional views[11]; they may represent 'aged' (polymerised?) refractory oxides. The mackinawite spectrum[12] is a single peak with a small shift of 0.2 mm s^{-1}; because of the satisfactory fits obtained with three doublets, it is considered improbable that >10% of total Fe is in mackinawite. Amorphous Fe–S compounds in the upper few cm are probably oxidised on turnover, but quantitatively similar Fe distributions were obtained in September and early-October samplings. Because such compounds probably contain high-spin ferrous ions which will absorb away from the main envelopes, we estimate that <10% of total Fe is in amorphous sulphides.

Although the upper few cm of sediment are probably well mixed on turnover, it would seem that ~50% of deposited iron is converted to pyrite within, say, 10 yr, 80% is converted over ~200 yr, and >90% over ~500 yr. The relative rates are explicable because the concentrations of organic matter and the activity of sulphate-reducing bacteria are greatest in the upper few cm. Framboids were observed in the sediment traps, indicating pyrite formation in the water column during the summer.

Table 1 Hyperfine parameters* and Fe distributions† in Lake St George sediments

Fraction, (cm)	Ferric doublet IS	QS	HW	Ferrous doublet IS	QS	HW	Fe distribution (%) FeS_2	Fe^{3+}	Fe^{2+}
0–1‡	0.41	0.73	0.44	1.14	2.71	0.42	57	24	19
1–2‡	0.39	0.75	0.53	1.14	2.66	0.39	53	27	20
4–6‡	0.44	0.67	0.46	1.14	2.64	0.40	58	16	26
20–22§							80	10	10
44–46§							>85	<15	
50–52									
62–64							>90		
86–88									

* In mm s^{-1}.
† Assumes equal recoil-free fractions for all compounds.
‡ Pyrite peak positions and half widths fixed in computations.
§ Pyrite and ferric peak positions and half widths fixed in computations.

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A new 14 Å mineral of the birnessite group in deep-sea micronodules

EXPERIMENTS by Feitknecht and Marti[1] and more recently by Giovanoli *et al.* have shown that oxidation of $Mn(OH)_2$ in NaOH solution gives rise to a Na-buserite. Drying Na-buserite converts it to Na-birnessite. If a solution of a calcium salt is shaken with Na-buserite the latter is transformed into pseudo-hexagonal Ca-buserite, characterised by a basal spacing of 10 Å. After drying this spacing is reduced up to 7 Å and buserite is converted into Ca-birnessite. We describe here a new natural Ca-bearing member of the birnessite group of unit cell parameters comparable with those of a synthetic compound described[2] as Na–Mn (II, III) manganate, belonging to the birnessite group. In ASTM (23–1046) this substance is called Na-birnessite.

Dark brown-black nodules about 0.5 mm in diameter have been found in one of the coccolith mud specimens collected in

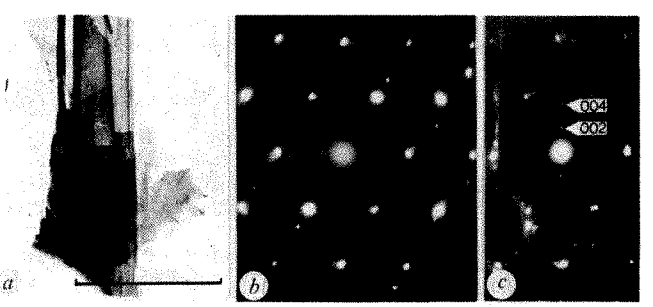


Fig. 1 *a*, Electron micrograph of 14 Å Ca-bearing birnessite. *b*, SAD-pattern of one platelet corresponding to the plane (001) of this mineral lattice. *c*, Diffraction pattern of a bent platelet edge. Arrows show the basal reflections 002, 004. Scale bar, 1 μm.

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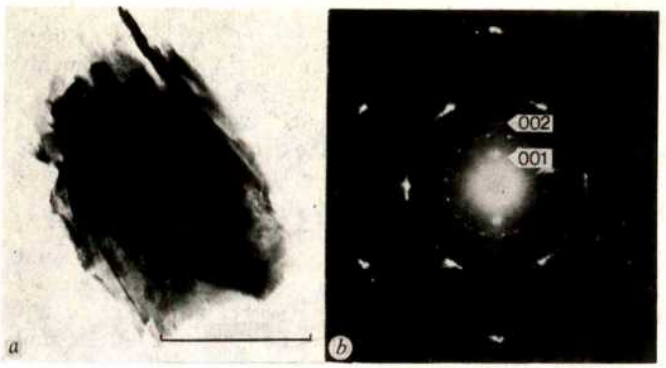


Fig. 2 *a*, Electron micrograph of 7 Å Ca-bearing birnessite. *b*, SAD-pattern of a bent edge of this mineral platelet. Arrows show the basal reflections 001, 002. Scale bar, 1 μm.

1977 from the Atlantis fracture zone (Atlantic Ocean) at a depth of 5,520 m (270–280 cm below the sediment–seawater interface), by the 24-th Expedition of RV Academician Kurchatov.

Selected area electron diffraction (SAD) and spectrometer studies of energy dispersion (Kevex) of these nodules revealed two manganese oxide minerals, one of which was named the orthorombic 14 Å Ca-bearing mineral of birnessite group. The particles of this mineral are elongated platelets (Fig. 1*a*) ranging in length from fractions of a micron to several microns. When splitting these elongated platelets into still narrower laths, the width range is greater. Many platelets display double or treble intergrowths. The SAD-patterns of single platelets normal to the beam (Fig. 1*b*) consist of a pseudohexagonal net of closely spaced point reflections. However, an analysis of the diffraction patterns has shown that the hexagonal arrangement is distorted, the extent of distortion varying from particle to particle. SAD patterns of platelets with bent edges and of platelets tilted to the electron beam have been obtained with 00*l* and *hkl* reflections respectively (Fig. 1*c*). From the diffraction pattern data averaged over a number of particles we determined the unit cell parameters as $a_0 = 8.52$ Å; $b_0 = 15.1$ Å; $c_0 = 14.1$ Å. We named this phase orthorombic 14 Å-birnessite. The established unit cell parameters are almost identical with those of synthetic Na-birnessite of the formula $Na_4Mn_{14}O_{27}.9H_2O$ having: $a_0 = 8.54$ Å; $b_0 = 15.39$ Å; $c_0 = 14.26$ Å. Slight differences in the b_0 and c_0 values can be attributed to a small variation in the degree of vacancy of the octahedral layers, and to a partial dehydration of birnessite in the vacuum conditions of the electron microscope. The mineral of birnessite group with the same unit cell parameter was synthesised in solutions of NaOH as a result of Mn^{2+} oxidation with free oxygen.

The second phase is characterised by slightly elongated laths (Fig. 2*a*) from fractions of a micron to several microns in diameter. The electron diffraction patterns of their basal planes show a hexagonal net of widely spaced *hk*0-reflections. Apart from the *hk*0 and 00*l* reflections, *hkl* space reflections have been observed (Fig. 2*b*) in many diffraction patterns of the bent edges of particles. The electron diffraction data indicate that the second phase has a hexagonal unit cell with $a_0 = 2.84$ Å and $c_0 = 7.07$ Å, both values being in accord with the parameters of common hexagonal 7 Å birnessite.

The Kevex spectrometer analysis of separate single crystal platelets has revealed that the only cations present in all the platelets of natural 14 Å birnessite are manganese and calcium. We can conclude therefore that the structural analogue of synthetic Na–Mn manganate (Na-birnessite) which we found is a 14 Å Ca-bearing birnessite.

The analysis of individual platelets of the 7 Å birnessite mineral with a Kevex spectrometer shows only manganese and calcium. Its diffraction features observed in vacuum conditions, and its chemical composition show that this birnessite is similar to rancieite. That rancieite belongs to the birnessite group (family) has been established by Bardossy and Brindley[3].

A few platelets from minerals of the birnessite group were found to contain insignificant amounts of potassium. In one micronodule the crystallites of 7 Å birnessite mineral contain minor amounts of Na in addition to Mn and Ca. Magnesium, which in principle can be detected by the Kevex spectrometer, was not found either in the 14 Å or in the 7 Å birnessite minerals of the studied micronodules.

Hence this study of micronodules from the Atlantic Ocean floor has revealed for the first time a new natural member of the birnessite group.

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New fossil finds from the Libyan Upper Neogene site of Sahabi

THE site at Sahabi, Libya, was first investigated in 1934–39[1], and geological, palaeontological and palaeoanthropological research began again in 1977, as part of the International Sahabi Research Project. Further extensive fossiliferous sediments were revealed. A vertical extent of more than 80 m of such sediments are exposed, and on the basis of the mammalian fauna their age is inferred as Late Turolian to Ruscinian, 7–4 Myr BP. We present here a report of Sahabi geology and palaeontology based on results obtained up to March 1979. More than 3,000 invertebrate and vertebrate fossil specimens have been collected, and for several taxonomic groups they fill a gap in the African fossil record.

The fort at Sahabi ('Qasr as-Sahabi') is 90 km south of the coastal town of Agedabia and investigations extend to the north and east (Fig. 1). Sixty-five localities have been established (numbered P1–P65) as stratigraphic profiles or fossil sites.

Table 1 Provisional list of invertebrates from Sahabi

Echinodermata	Bryozoa
Conoclypus	cf. *Membranipora*
Clypeaster	Arthropoda
Mollusca	Crustacea
Bivalvia	cf. *Balanus*
Anadara	Ostracoda*
Pecten	Coelenterata
cf. *Ostrea**	cf. *Montastrea*
cf. *Crassostrea**	Protozoa
cf. *Codakia*	Foraminifera
Lucina	Alveolinidae
Glycymeris	Ichnogenera
Mytilidae (cf. *Lithophaga*)	*Ophiomorpha*
Gastropoda	*Thallassanoides*
Conidae	
Strombus	
cf. *Mathilda*	
cf. *Turritella*	
cf. *Cerithium**	
Trochidae	
Naticidae	

These specimens were identified by A. Ekdale and F. Brown. *Taxa from the Sahabi Formation. Other taxa derive from 'Formation M'.

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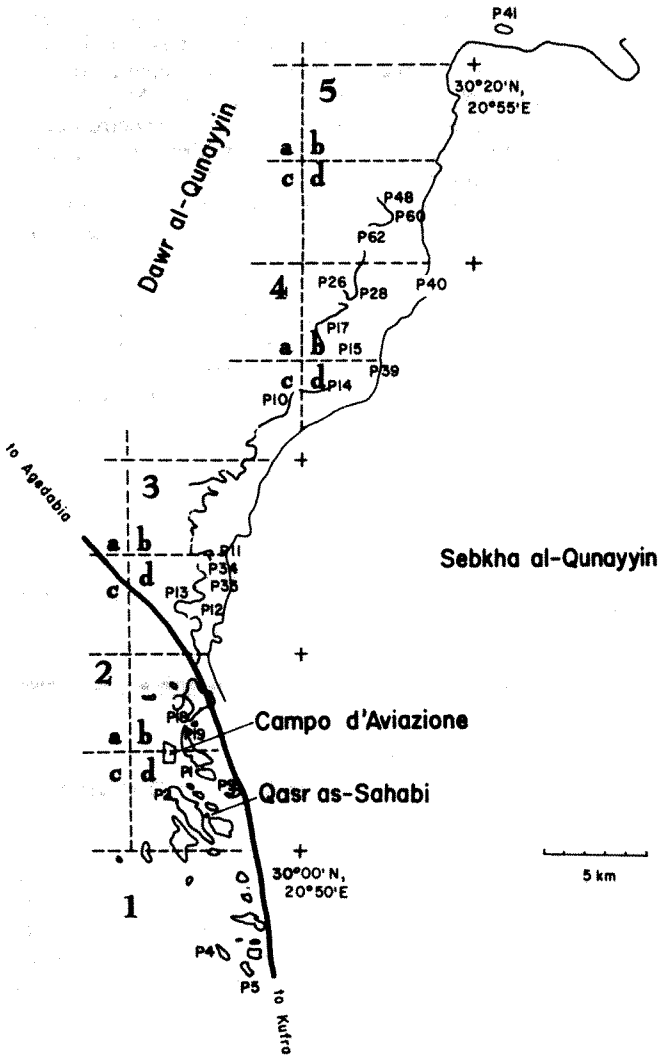


Fig. 1 Map of the major localities at Sahabi. Localities are arranged into sectors which are based on divisions of latitude and longitude. The fort ('Qasr as-Sahabi') and the 1934 Italian airfield are indicated.

De Heinzelin *et al.*[2] have defined the Sahabi Formation. It lies unconformably on a gypsiferous green clay and sand horizon known as 'Formation P', in turn overlying unconformably Middle Miocene strata ('Formation M'). P may represent deposition during the Messinian Event. Eight lithological members and sub-members (Q,R,S,T,U-1, U-2, V-1 and Z) are distinguished within the Sahabi Formation (Fig. 2). All are fossiliferous with the exception of the highest level, Z. Member Q consists of littoral and lagunal facies followed by shallow marine conditions, as indicated by the deposition of a white dolomite (Member R). Member S records the retreat of the sea by the presence of coastal facies such as lagoons, deltaic channels and freshwater basins. Shallow marine conditions return in Member T, which contains numerous mollusc shells and vertebrates (chelonians, sirenians and cetaceans). The very fossiliferous Member U comprises greenish coarse cross-bedded fluviatile sands as well as lagoonal clays.

Most fossil specimens were found on the surface, although limited excavation and sieving was undertaken. Groups thus added to the Sahabi faunal assemblage include primates, ursids, canids, sirenians, rodents, choerodonts and possibly amebelodonts. Abundant fish, reptile and invertebrate remains are being studied.

All invertebrate remains are of marine origin. Subhorizontal exposures of Formation M north of P39 (Fig. 1) preserve shallow marine, near-shore invertebrate communities, with autochthonous fossils of sea urchins, bivalves and corals. At P40 burrows referred to the ichnogenera *Ophiomorpha* and *Thallassanoides* are preserved. The gastropod *Cerithium* which lives on the bottoms of lagoons is also found in these sediments (personal communication from A. Eckdale and F. Brown). Continuing work on these fossils and on ostracods, diatoms and foraminiferans will contribute to the dating and palaeoecological reconstruction of Sahabi. Table 1 summarises the invertebrate fauna.

The vertebrate fauna is interesting for several reasons. It will provide information about the evolutionary history of several groups whose fossil records are incomplete for the period represented by these sediments. Another advantage of this fauna is that its intermediate position between Africa and Eurasia will make it possible to assess biogeographical connections between the two areas. Palaeoecological studies will be facilitated by subhorizontal exposures which preserve virtually intact fossil surfaces, frequently with *in situ* plant roots, fossil soils and footprints. Fossil wood is abundant and we are looking for pollen in samples of sediments and coprolites.

Abraded and fragmentary bones are abundant in fluviatile and deltaic facies. Rarer but more complete and semi-articulated remains occur in lagoonal and estuarine facies. Transported bone assemblages contain mixed species from different habitats: in locality P16, for example, cercopithecoids, giraffids, *Hipparion*, anthracotheres and sharks (*Carcharodon*) all occur. Table 2 summarises the vertebrate fauna from Sahabi.

The primate remains are the first from pre-Pleistocene strata in Libya. A right half-mandible (1P28A; Fig. 3*a*) and other postcranial remains are referable to a colobine, *Libypithecus* cf. *markgrafi* Stromer 1913[3]. Postcranial fossils, including a femur (1P17B) and a proximal ulna (88P36A), show close resemblances to the genus *Macaca* and are referred to cf. *Macaca*. A left clavicle (26P4A) lacking the lateral end has a large sternal articular surface, a large diameter throughout its length, a robust shaft and a strong curvature. The specimen is attributed to Hominoidea, and more precise taxonomic assessment awaits further study.

Sahabi is known for the discovery in 1934 of the type specimen of the proboscidean *Stegotetrabelodon lybicus* Petrocchi

Table 2 Provisional list of vertebrates from Sahabi Formation

Cercopithecidae	Giraffidae
Libypithecus cf. *markgrafi*	?*Samotherium* sp.
cf. *Macaca*	Hyaenidae
Hominoidea	*Percrocuta* sp.
Proboscidea	Felidae
Choerolophodon	*Machairodus* sp.
Anancus	Ursidae
Stegotetrabelodon lybicus	cf. *Agriotherium*
?*Stegodon*	Canidae
?*Amebelodon*	Dugongidae
Rhinocerotidae	*Metaxytherium* sp.
Brachypotherium cf. *lewisi*	?Hyracodontidae
Equidae	Rodentia
Hipparion sp. (large)	Ctenodactylidae
Hipparion sp. (small)	spp. as yet unidentified
Anthracotheriidae	Cetacea
Merycopotamus petrocchii	Aves
Hippopotamidae	spp. as yet unidentified
Suidae	Crocodilidae
Nyanzachoerus syrticus	*Crocodilus checchiai*
Nyanzachoerus cf. *jaegeri*	?Tomistomidae
Bovidae	Chelonia
Bovini	cf. *Trionyx*
Leptobos syrticus	Carcharidae
Boselaphini	*Carcharodon* sp.
Miotragoceros sp.	Myliobatidae
Hippotragini	*Sphyrna* sp.
cf. *Hippotragus* sp.	Centropomidae
Reduncini	*Lates* sp.
Kobus sp.	

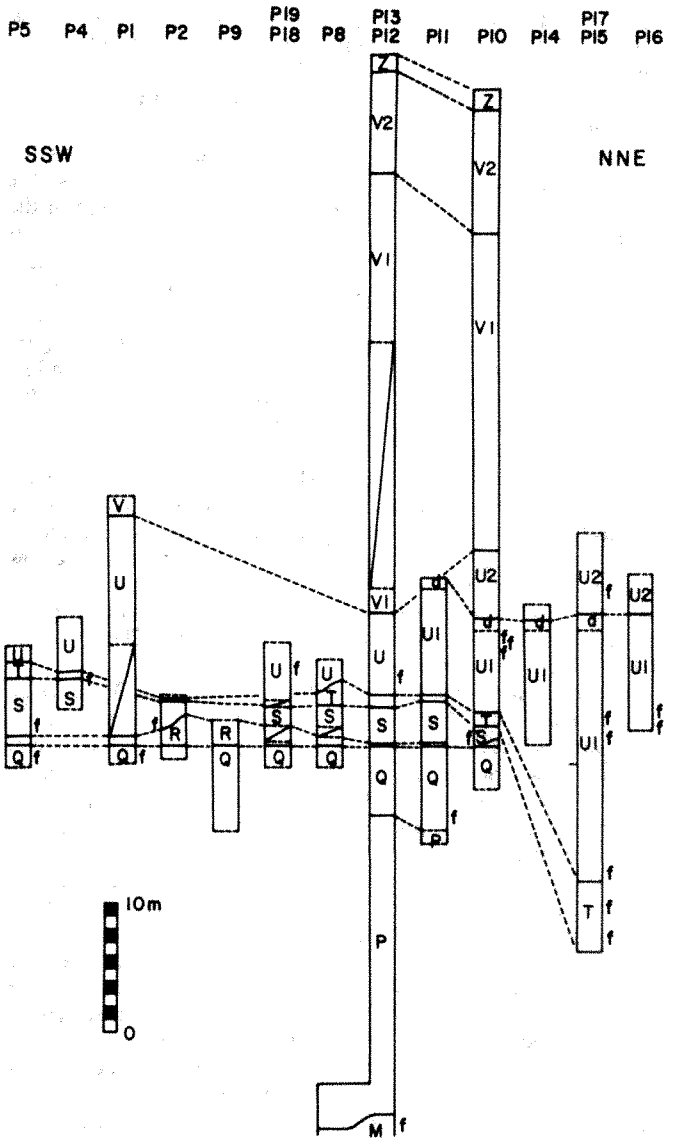


Fig. 2 Stratigraphic sections from selected localities showing 'Formation M; Formation P', and the members of the Sahabi Formation. Major fossiliferous horizons are indicated (after ref. 2). d, Dune; f, fossiliferous horizon.

1941[4]. Diverse proboscideans (Table 2) are now represented by cranial, tusk, molar and postcranial remains. *Stegotetrabelodon* is represented by a complete cranium (1P26A) and fragmentary remains. Upper tusks have almost circular cross-sections and lower tusks have oval cross-sections. The minimum length of a lower tusk is 180 cm. *Anancus* is represented by upper straight tusks with circular cross-sections, and probably also by a right lower third molar with six lophs and a bunodont structure. This specimen resembles that which Petrocchi[5] ascribed to *Pentalophodon sivalensis*.

Locality P25 yielded the remains of a proboscidean cranium, dentition and upper tusks, the latter being flat in cross-section and with 'rod-cones'[6,7]. These remains may represent *Amebelodon*. Several bunodont molars with three lophs can be referred provisionally to *Trilophodon* sp. A fragmentary proboscidean molar with only two lophs and no tubercles in the anterior valley could be from a primitive stegodont (*Stegodon*). Another proboscidean molar germ of small size with two lophs has similarities to those of choerodonts and ptychodonts. It resembles molars ascribed to *Choerolophodon* from Anatolia[7] and is assigned to *Choerolophodon* sp.

Anthracotheres represent one of the most abundant taxa in these sediments. We have provisionally assigned these specimens to *Merycopotamus petrocchii*, the name given by Black[8] to specimens found to the north of Sahabi which may or may not be of the same age as those found at Sahabi. The occurrence of hippopotamids of undiagnosed affinities together with anthracotheres is interesting as the latter have been suggested to be ancestral to hippopotamids.

Cooke[9] has discussed briefly the suids already known from Sahabi and referred them to *Nyanzachoerus syrticus* and *Nyanzachoerus jaegeri*: our discoveries are referable to the same genus. The discovery of an ursid (Fig. 3*b*) and a canid at locality P28A (U-1) relates Sahabi not only to Eurasian sites but to the South African site of Langebaanweg, dated at approximately 4 Myr, and from which *Agriotherium* and species of Canidae are known[10]. The Sahabi forms probably pre-date the South African by at least 1 Myr.

The overall complexion of the Sahabi fauna is African but several taxa indicate Eurasian affinities. These include the boselaphine *Miotragoceros*, the ursid cf. *Agriotherium*, and perhaps the proboscideans *Choerolophodon* and ?*Stegodon* and the cercopithecine cf. *Macaca*.

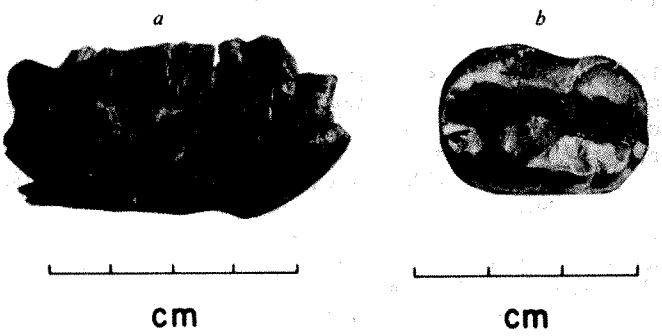


Fig. 3 *a*, Lateral view of specimen 1P28A, a right half-mandible ascribed to *Libypithecus* cf. *markgrafi*. *b*, Occlusal view of specimen 184P28A, an ursid lower molar, tentatively referred to cf. *Agriotherium*.

The nature of the terrestrial fauna indicates marshy to riverine-fringe, wooded environments proximal to the sites of deposition, and more open savanna habitats farther away. Abundant fossils of tree trunks and initial sedimentological findings confirm this reconstruction. Compared with other sites of Turolian to Ruscinian age in Eurasia and Africa, Sahabi presents a high proportion of forest, woodland or water-living forms, including abundant anthracotheres, dugongids, proboscideans, hippopotamids and crocodilids. Taxa more characteristic of open savanna conditions, such as bovids, giraffids, rhinocerotids and equids are less numerous than at contemporary sites[11]. Cercopithecids and hominoids probably belong to the water-tied or woodland group[12,13], which agrees with their absence or rarity at sites with more open habitats lacking surface water, such as Maragheh[11].

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Insecticide-resistant *Myzus persicae* as an example of evolution by gene duplication

DUPLICATION of structural genes is believed to be a prerequisite for evolution because it allows forbidden mutations of the redundant copy while preserving the advantageous parental gene[1,2]. Evidence for this phenomenon is derived from studies of proteins with closely related structures which fulfil either slightly or very different roles in metabolism. The first stage of the process, duplication without subsequent mutation, leads to the production of more of a particular gene product. This is especially advantageous during development, when large amounts of ribosomal and transfer RNA are required[3]. However, so far the only examples of this mechanism causing increased production of specific proteins in response to changing environmental conditions are from studies of bacteria[4], yeast[5] and eukaryotic cell cultures[6]. Using insecticide-resistant *Myzus persicae*, we provide evidence here for gene duplication without subsequent mutation, conferring a definite selective advantage to intact higher organisms in adverse environmental conditions.

Needham and R.M.S.[7] showed a positive correlation between resistance to insecticides of the peach-potato aphid, *M. persicae*, and total carboxylesterase activity measured by the *in vitro* hydrolysis of 1-naphthylacetate. Further work demonstrated that increased esterase activity arises mainly from changes in a single enzyme, E4 (ref. 8). The increased activity is caused by the presence of more E4 rather than a more active mutant enzyme, as shown by measuring the catalytic centre activity of the enzyme from different variants[8]. As E4 hydrolyses insecticidal esters in addition to artificial substrates such as 1-naphthylacetate, it enables resistant aphids having larger amounts of the enzyme to survive otherwise lethal doses by degrading more insecticide[9].

Genetic studies of increased enzyme production gave ambiguous results[10,11], and were hampered by difficulties in breeding the aphids through their sexual cycle. Biochemical studies have now clarified the nature of this phenomenon.

Seven variants of *M. persicae* can be distinguished by esterase assays of either crude aphid homogenates or E4 after electrophoretic separation from other carboxylesterases. Although total esterase assays show a progressive increase in enzyme activity with increasing resistance, quantitative relationships are affected by the hydrolysis of 1-naphthylacetate by esterases other than E4; enzyme staining after electrophoresis only gives an approximate measure of E4 activity. However, the amount of E4 in crude homogenates is readily determined by radioassay with the insecticide paraoxon[8] (diethyl *p*-nitrophenyl phosphate prepared by oxidation of [*ethyl*-^{14}C]parathion (Radiochemical Centre)) as substrate, because only E4 hydrolyses paraoxon[8].

Weighed groups of adult apterous aphids were homogenised in 0.02 M phosphate buffer, *p*H 7.0, and incubated at 25 °C with 1.25 μM [*ethyl*-^{14}C]paraoxon, a concentration giving V_{max} for the enzyme. The hydrolysis product, diethyl phosphate, was measured at intervals by liquid scintillation spectrometry of 1-ml samples of the aqueous phase after extracting the unchanged paraoxon into 3 × 1 ml of chloroform. E4 activity (pmol per h per mg aphid) was calculated from these data and the known specific activity (40 mCi mmol^{-1}) of the paraoxon. The results showed that the rate of hydrolysis increased by the same factor between each of the seven variants. Because the catalytic centre activity of E4 for paraoxon is the same in the different variants (0.6 h^{-1})[8], the rates give a direct measure of its amount in each variant (Table 1). Statistical analysis[12] using a maximum likelihood computer program confirmed that the amounts in the seven clones form a geometric series with a factor of 1.91 and fiducial limits 1.77 and 2.12. This doubling of E4 throughout the series is best explained by a succession of tandem duplications of the structural gene for E4, with susceptible aphids having one copy, 240N two copies, and so on to G6 with 64 copies of the gene.

Excess production of E4 by *M. persicae*, in common with bacteria[4], yeast[5] and eukaryotic cell cultures[6] showing gene duplication, is unstable in the absence of selection. The two most resistant variants (PirR and G6) spontaneously revert to low esterase activity with concomitant loss of resistance when insecticide selection is removed. This is not observed with the moderately resistant variants (up to and including TIV) in which E4 production is stable.

The three most resistant variants (TIV, PirR and G6) have an A1,3 translocation[13], unlike the susceptible and the three least resistant variants (with up to eight times as much E4) where karyotype is normal. It has been suggested that this translocation, that is, an exchange between non-homologous chromosomes, or the related dissociation, is responsible for the increased production of E4 by altering interactions between genes on these two chromosomes[14]. This is reasonable in view of the location on different chromosomes of some structural genes and their corresponding regulatory genes in *Drosophila*[15,16]. The clear evidence for gene duplication as the mechanism for increased enzyme production suggests that in this case translocation is more likely to be a mechanism giving rise to tandem duplications. Other mechanisms such as exchanges between homologous chromosomes (unequal crossing over) cannot be excluded, as the karyotype of the less resistant variants is normal. Thus, resistance and the translocation do not show complete linkage as previously suggested[14].

The increased production of E4 clearly confers a selective advantage on *M. persicae* in the presence of insecticides. In Britain, the susceptible variant is now rare, almost certainly as a result of the extensive use of insecticides. It has been almost completely replaced in the field by the MSIG (R_1) variant, with four times as much E4 (ref. 9), and in some areas the even more resistant TIV (R_2) variant is common. So far, the two most resistant variants (with 32 and 64-fold increase in E4) have been widespread only in glasshouses where insecticidal application is much more intensive. These aphids are well adapted to their

Table 1 Concentrations of the insecticide-hydrolysing esterase, E4, in seven aphid variants

Variant	Clone	pmol E4 per mg aphid* ± s.d. (no. of independent determinations)
V1(S)	USIL	0.37 ± 0.20(9)
V2	240N	0.85 ± 0.18(2)
V4	MSIG	1.78 ± 0.75(7)
V8	French R	4.80 ± 0.60(3)
V16†	TIV	6.70 ± 0.70(2)
V32†‡	PirR	11.80 ± 1.20(5)
V64†‡	G6	24.70 ± 0.20(2)

* Calculated from the rate of paraoxon hydrolysis and the catalytic centre activity of E4 for this substrate.
† Karyotype with A1,3 translocation.
‡ E4 reverts to low activity in the absence of insecticide selection.

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environment; they have the ability both to replicate the E4 gene enabling them to survive treatment with insecticides, and to avoid excessive E4 production in the absence of insecticides because the mutational change is unstable[17]. This nullifies any possibly adverse effect on fitness through the production of very large amounts of an apparently redundant enzyme.

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Nerve growth factor alters the fate of embryonic neuroblasts

RECENT work from this laboratory has documented the initial expression of the noradrenergic phenotype in autonomic neuroblasts of the rat embryo *in vivo*. Tyrosine hydroxylase (T-OH) (rate-limiting enzyme in catecholamine biosynthesis)[1], dopamine-β-hydroxylase (DBH) (which converts dopamine to noradrenaline) and the catecholamine (CA) transmitters appear simultaneously in sympathetic ganglion primordia at 11.5 days of gestation[2,3]. In addition, these noradrenergic characters transiently appear in a population of presumptive neuroblasts within the primitive gut mesenchyme[2,3]. The noradrenergic gut neuroblasts appear at 11.5 days and increase in numbers over the ensuing 24 hours. Subsequently, however, numbers decrease progressively so that by 14.5 days of gestation virtually no cells exhibiting noradrenergic traits are detectable[2,3]. Elucidation of the fate of these neuroblasts may help define mechanisms influencing neuronal phenotypic expression and/or the factors which govern selective neuronal death during ontogeny. In this report we show that treatment with the trophic protein macromolecule, nerve growth factor (NGF), increases the number of catecholamine (CA)-fluorescent cells and delays the ontogenetic disappearance of CA from the gut.

NGF is present in various sympathetic target organs, and is necessary for normal survival and growth of sympathetic neurones *in vivo* and *in vitro* (see refs 4 and 5 for reviews). Conversely, treatment of neonates with antiserum to NGF results in widespread destruction of sympathetic neurones throughout the animal[6,7]. As NGF has a critical regulatory role in sympathetic ontogeny, it was logical to define the effect of this agent on the noradrenergic neuroblasts of the embryonic rat gut *in vivo*.

At 11.5 days of gestation embryos were injected with NGF and the gut was examined for CA-containing neuroblasts. The β subunit of NGF was purified as before[8]. Pregnant Sprague-Dawley rats were anaesthetised[9], and trans-uterine injections of embryos were performed by opening the mother's peritoneum and exposing and trans-illuminating the uterus. The visualised embryos were injected using a 33-gauge needle with either β-NGF or control buffer. Animals were killed at daily intervals thereafter, from 12.5 to 15.5 days, and CA was detected by formaldehyde-induced fluorescence[10].

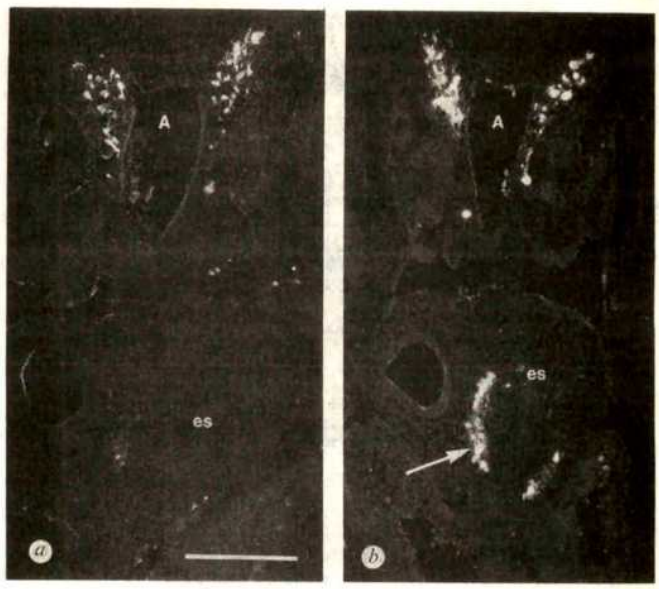


Fig. 1 Early effects of NGF treatment on CA-containing neuroblasts of the gut. Embryos were injected with either vehicle (*a*) or 50 BU of β-NGF in 5 μl (*b*) at 11.5 d of gestation; 2 d later the embryos were processed for the histochemical demonstration of CA. a, Mid-thoracic transverse section of control (vehicle-treated) embryo. CA-histofluorescence is evident in sympathetic primordia located on either side of the aorta (A). Note a small number of faintly fluorescent cells in the gut mesenchyme, adjacent to the oesophageal epithelium (es). *b*, NGF-treated embryo. Transverse section at same anatomical level as in *a*. The number and fluorescence intensity of CA-containing cells in the gut mesenchyme have increased dramatically (arrow). The neuroblasts are aggregated in ganglion-like clusters surrounding the oesophageal epithelium (es). The ganglion primordia adjacent to the aorta (A) are not significantly altered. Scale bar, 200 μm.

Treatment with NGF was associated with a dramatic increase in the number and fluorescence intensity of noradrenergic neuroblasts in the gut. These effects were most striking 2 days after injection, at 13.5 days of gestation (Fig. 1). In the oesophagus, at the level of the gastro-oesophageal junction, neuroblast numbers were markedly increased by NGF, and cells aggregated to form ganglion-like clusters (Fig. 1*b*). In contrast, far fewer CA-containing cells were detected in vehicle-treated controls, and fluorescence intensity was diminished (Fig. 1*a*). NGF induced similar increases in fluorescent neuroblast numbers and in cellular aggregation in the proximal small intestine. Although effects decreased progressively with time after injection, changes were still evident in 15.5-day embryos, the oldest specimens examined. NGF treatment caused persistence of scattered fluorescent neuroblasts in the gut of 15.5-day embryos, whereas control guts were devoid of fluorescent cells (Fig. 2). Preliminary observations suggest that the gut neuroblasts are sensitive to NGF only during a restricted period of ontogeny; treatment at 10.5 or 13.5 days of gestation had little effect.

In addition to increasing fluorescent cell numbers in the gut, NGF treatment increased the number of apparently ectopic neuroblasts in unusual locations. Fluorescent neuroblasts were observed in the liver parenchyma, the heart primordium, the somatopleural mesenchyme of the body wall and in some instances, in the limb bud mesenchyme (Fig. 3) after NGF administration.

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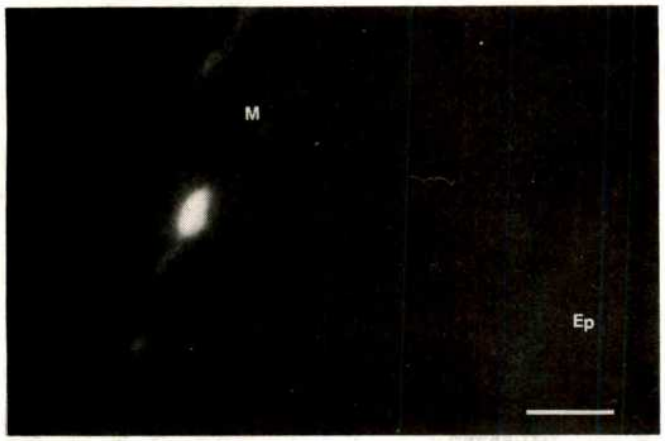


Fig. 2 Late effects of NGF treatment. Embryos were injected with vehicle or β-NGF at 11.5 d of gestation as in Fig. 1, but were examined 4 d later, at 15.5 d of gestation. An isolated fluorescent cell with a fluorescent cytoplasmic process was observed in the developing circular muscle layer of the intestine (M). Such isolated, CA-containing cells were present throughout the intestines of NGF-treated embryos, whereas the guts of controls were completely devoid of fluorescent cells. Ep denotes epithelium of the gut. Scale bar, 20 μm.

In contrast to effects elsewhere, NGF had little apparent effect on neuroblasts in the sympathetic ganglion primordia (Fig. 1). Treatment did not alter neuroblast numbers and had inconsistent, minimal effects on fluorescence intensity in the primitive ganglia. However, embryonic ganglia are responsive to NGF[11]. Consequently, our observations suggest that the exogenous dose of NGF may have been too small, compared with local endogenous concentrations, to elicit changes.

NGF may have increased the number of noradrenergic gut neuroblasts by (1) increasing the number of undifferentiated precursors that acquire noradrenergic traits; (2) causing persistence of noradrenergic traits in neuroblasts normally destined to acquire and then lose these characters; (3) causing mitosis of noradrenergic neuroblasts; (4) lengthening survival times of noradrenergic neuroblasts; (5) increasing the CA content of noradrenergic cells to the level necessary for visualisation. Alternatives (1) and (2) imply that NGF regulates phenotypic expression directly, whereas (3)–(5) suggest that NGF regulates cell number, and/or CA content, but not phenotypic expression

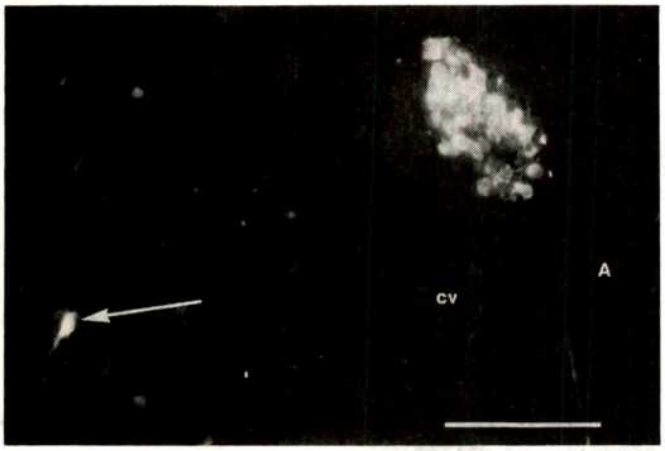


Fig. 3 Appearance of ectopic cells after NGF treatment. High thoracic transverse section of a 13.5-d embryo. An isolated fluorescent cell (arrow) is observed in the mesenchyme of the proximal portion of the limb bud. The sympathetic ganglion primordium, located between the aorta (A) and the cardinal vein (cv), is a great distance from the ectopic cell. NGF treatment increased the number of ectopic CA-containing cells in a variety of unusual locations. In contrast, ectopic fluorescent neuroblasts were very rarely encountered in controls. Scale bar, 100 μm.

per se. It is certainly well documented that NGF enhances sympathetic neurone survival during development[12,13]. However, the protein does not apparently alter transmitter phenotypic expression. Rather, NGF increases acetylcholine synthesis in cholinergic neurones, and noradrenaline synthesis in noradrenergic neurones[14–17]. It remains to be determined whether NGF influences gut neuroblasts in the rat embryo in an analogous manner. Regardless of the mechanism involved, our observations suggest that NGF can increase the number of noradrenergic neuroblasts which can be visualised in the gut and ectopic locations, and cause persistence of such cells. It is possible, consequently, that local concentrations of endogenous NGF normally determine which neuroblasts will survive and/or continue to express noradrenergic characters.

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Appearance of H–W (H–Y) antigen in the gonads of oestradiol sex-reversed male chicken embryos

THERE is evidence that H–Y antigen has a major role in testis differentiation in mammals[1–3]. Presumably, this antigen is the essential factor for the development of the undifferentiated gonadal anlage into a testis. Antiserum to H–Y antigen, raised in highly inbred strains of mice and rats, exhibits cross-reactivity with cells derived from non-mammalian vertebrates like birds, amphibians and fish[4,5]. In these animals, the cross-reaction is strong in the heterogametic sex. This means that in birds with the ZZ/ZW mechanism of sex determination the female types as positive, as also occurs in the toad *Xenopus laevis*; on the other hand, in the frog genus *Rana* and the cyprinodont fish *Lebistes* and *Xiphophorus*, which have the XX/XY mechanism of sex determination, the male types as positive. This correlation with the heterogametic sex favours the assumption that the cross-reacting antigen 'H–Y' or 'H–W' in non-mammalian vertebrates

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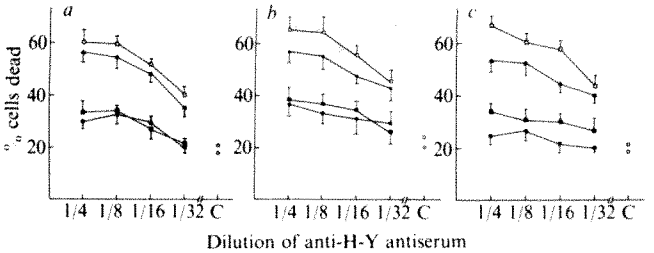


Fig. 1 Cytotoxicity of anti-H–Y antiserum in the presence of rabbit complement after absorption by 25 mg wet weight of pooled embryonic chick gonads. Mean and standard deviation of six determinations each are shown. □, Dead cells at different dilutions of anti-H–Y antiserum; ▲, dead cells at different antiserum dilutions after absorption by left control testes; ■, dead cells at different antiserum dilutions after absorption by oestradiol sex-reversed left ovotestes; ●, dead cells at different antiserum dilutions after absorption by left control ovaries; ○, complement control. *a*, 13-d-old embryonic gonads; *b*, 14-d-old embryonic gonads; *c*, 16-d-old embryonic gonads.

has a similar function to that occurring in mammals for the differentiation of the heterogametic gonad, that is, the ovary in ZW, and the testis in XY animals. In contrast to placental mammals, in the other vertebrates it is possible to influence gonadal differentiation by sex steroid hormones (for review, see ref. 6). In the embryonic male (ZZ) bird, application of oestrogens leads to the development of an ovotestis, in amphibians and fish, treatment with steroid hormone of the opposite sex causes complete sex reversal. If H–Y (H–W) is the differentiation antigen for the heterogametic gonad, and if on the other hand sex reversal can be achieved by the steroid hormone of the opposite sex, the question arises whether the hormone acts by way of the H–Y (H–W) antigen. Therefore, we have converted embryonic male chickens using oestrogen to develop an ovotestis, and then tested them for the presence of cross-reactivity with rat anti-H–Y antiserum. We report here that, indeed, the normally antigen-negative testis becomes antigen-positive after hormonal treatment. Thus, the gene for this antigen must be present in the genome of both sexes.

Embryos of white Leghorn chickens were used for the experiments. Eggs were injected with 100 μg oestradiol benzoate on day 4 of incubation as described, for example, by Akram and Weniger[7], and killed on days 13 or 16. Male (ZZ) converted gonads were definitely distinguished from female ones by morphological criteria. The feminised testis has the flattened form of the left ovary, but it is much smaller and its inner and outer borders have indentations, in contrast to the smooth aspect of the left ovary. The feminised right testis is longer and thinner than the right female gonad. The müllerian ducts of the feminised embryo resemble those of the female embryo, except that the left one lacks a shell gland. All these criteria taken together make the diagnosis of the genetic sex quite definite. In only three out of more than 100 cases was a decision difficult; these three embryos were discarded. Thus, gonads regarded as feminised testes were without doubt of ZZ constitution. In an additional experiment, eggs of the Ross Brown breed, which shows sex-linked differences in feather colouration, were used. Thus, this breed offers an independent criterion for sexual assignment. The plumage is brownish in females and white in males. These characters are already clearly distinct in 12-d-old embryos, and allow the genetic sex of the oestrogen-treated embryos to be determined. Sex ratios obtained according to the above criteria were:

16 d, I (Leghorn):	19♂ 24♀
16 d, II (Leghorn):	6♂ 7♀
13 d, I (Leghorn):	12♂ 15♀
13 d, II (Leghorn):	16♂ 12♀
14 d (Ross Brown):	30♂ 26 ♀

Gonads were collected in liquid nitrogen immediately after preparation and weighing, and stored at −80 °C. Untreated left testes and ovaries of embryos of the same ages served as controls. Only left gonads were used throughout the experiments, as in the female the right gonad is rudimentary. H–Y antiserum was raised in female isogeneic Lewis rats by several injections of male spleen cells as described earlier[8]. Cytotoxic testing for the detection of H–W antigen was carried out essentially according to Scheid *et al.*[9]: 20 μl 1/4 diluted antiserum was absorbed with 25 mg wet weight of pooled gonads derived from the respective type of embryo and tested for cytotoxicity on male rat epidermal cells. For each test 10 μl absorbed antiserum were used, the remainder being stored at −80 °C and tested for confirmation later on. Five experiments were carried out, two experiments each for yielding 13- and 16-d-old embryonic gonads, respectively, and another one for 14-d-old gonads of the Ross Brown breed. For each experiment about 80 eggs were used, about 50 of which were treated with oestrogens.

Figure 1*a*, *b* and *c* demonstrates the results of conversion experiments finished on days 13, 14 and 16, respectively. Converted gonads of 13- and 14-d-old embryos absorb almost as much antiserum as do normal female gonads, whereas those of 16-d-old embryos absorb a little less than do control ovaries. No elevated titres of H–W antigen were found in oestrogen-treated ovaries.

These findings clearly indicate that, after treatment with oestrogens, H–Y (H–W) antigen becomes expressed in the male gonad. This suggests that, analogous to the well studied situation in mammals, in birds the presence of H–W antigen is responsible for the formation of an ovary; if this is true, oestrogen-induced sex reversal, which is well known (for review, see ref. 6), is only an indirect effect, mediated by H–W antigen. In mammals, attempts to induce H–Y antigen by testosterone have been reviewed by Erickson[10]. Evidently, however, the primary differentiation of the gonadal anlage is independent of sex steroids in mammals (for review, see ref. 6).

It may be significant that hormone-induced sex reversal is not complete in the chick. In general, an ovotestis develops which gradually regresses towards hatching. Although, we were not able to study H–W antigen during the entire course of embryonic development, in the gonads of 13- and 14-d-old embryos, the antigen titre was clearly stronger than in those at 16 d. Therefore, there does seem to be a correlation between morphogenetic changes and the H–W titre in the gonad. Apparently, the 'induction' of H–W antigen production by oestrogen does not function by means of a switch-on mechanism resulting in the continuous production of the antigen (there must be some interaction with a repressing or degrading factor).

The results obtained in this study also have implications for the sex specificity of the H–W structural gene. Induction of H–W expression in the male bird presupposes the existence of the respective gene in the 2A, ZZ genome. Therefore, W-linkage of the H–W structural gene is excluded; this gene must be shared by both sexes. In mammals, however, the H–Y structural gene is assumed by many authors to be Y-linked[11] and consequently absent in the female. This question, however, cannot be considered as settled[12]. In addition, because in lower vertebrates sex reversal can be achieved by appropriate treatment, the gene responsible for the primary differentiation of the heterogametic gonad must be present in both sexes. From an evolutionary point of view, we believe therefore that in mammals also, this gene is not sex specific, and only its state of activity determines whether the undifferentiated gonadal anlage becomes a testis or an ovary.

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Preimplantation lethality of monosomy for mouse chromosome 19

IT remains a mystery why autosomal trisomy is found more frequently than autosomal monosomy in man. Several surveys of spontaneous abortions have revealed these chromosomal abnormalities in proportions of 26% (ref. 1) and 0.1% (refs 1, 2), respectively, while a prospective study of 47,000 newborn babies revealed no full monosomy and only five cases in which parts of chromosomes were missing[3]. Similarly, in mouse embryos from translocation stocks bred to produce aneuploid progeny, high frequencies of trisomy but no monosomy are found after 12–13 days[4,5]. This is hard to understand because most autosomal aneuploidy is assumed to result from meiotic nondisjunction, so that for every gamete carrying two copies of a particular chromosome there should be a complementary gamete carrying none. However, the deficiency of monosomic embryos could be because nullisomic gametes are less able than disomic gametes to participate in fertilisation, or because the resulting monosomic embryos are less viable early in gestation. We report here the investigation of these possibilities in a mouse system which is known to produce a high frequency of nondisjunction. Equal numbers of monosomic and trisomic progeny were found early in gestation, but while some monosomies were compatible with survival to implantation, monosomy for the smallest mouse autosome, chromosome 19, was lethal at the early to mid-blastocyst stage. This lethality did not seem to be the result of the expression of recessive lethal genes.

Table 1 Frequency of aneuploid preimplantation mouse embryos

Chromosome	Day	No. of embryos		
ICR females		Total	Monosomic	Trisomic
1	3	19	3(16%)	3(16%)
	4 all	54	5(9%)	9(17%)
	in vivo	13	1(8%)	2(15%)
	in vitro	41	4(10%)	7(17%)
12	3	72	10(14%)	11(15%)
	4 *in vitro*	38	5(13%)	5(13%)
17	3	23	2(9%)	5(22%)
	4 *in vivo*	48	4(8%)	6(13%)
19	3	116	21(18%)	23(20%)
	4 all	194	3(2%)	46(24%)
	in vivo	65	0(0%)	15(23%)
	in vitro	129	3(2%)	31(24%)
C57BL/6J females				
19	3	55	9(16%)	11(20%)
	4 all	84	1(1%)	16(19%)
	in vivo	16	0(0%)	4(25%)
	in vitro	68	1(1%)	12(18%)

Aneuploid embryos were generated by mating males doubly heterozygous for two Robertsonian translocations[5,6] to superovulated ICR-Swiss or C57BL/6J females. The following male stocks were used: Rb1Bnr/Rb10Bnr for aneuploidy of chromosome 1, Rb5Bnr/Rb9Bnr for chromosome 12, Rb11Rma/Rb1lem for chromosome 17 and Rb 163H/Rb1Ct for chromosome 19. Preimplantation embryos were obtained on the third (day 3) or fourth (day 4 *in vivo*) day after observation of the plug (day 0). Day 4 *in vitro* embryos were obtained by culturing[9] day 3 embryos for 24 h. The embryos were karyotyped as before[17].

Table 2 Development of aneuploid day 3 embryos from ICR × Rb163H/Rb1Ct matings

Karyotypes	Total no.	Stage of development reached: 8–16 cells	Late morula	Early blastocyst	Blastocyst
Normal	18		9	8	1
Trisomy 19	6	1	2	3	
Monosomy 19	6			3 2	1
Undeterminable	10		7	1 2	

Day 3 embryos were scored for development and then karyotyped as previously described[17].

Mouse embryos aneuploid for chromosomes 1, 12, 17 and 19 were generated by the double metacentric technique[5,6]. Because of indications[5,7] that most monosomic embryos do not survive much beyond implantation, we used preimplantation embryos for our initial studies. The frequencies of aneuploid embryos were analysed on days 3 (late morula/early blastocyst) and 4 (late blastocyst) of development (Table 1). In each case, the doubly heterozygous parent was the father. For each of chromosomes 1, 12 and 19, the frequencies of monosomy and trisomy were virtually the same on day 3 and varied from 14 to 20%. Therefore, for these three mouse chromosomes we conclude that there is no selection against nullisomic sperm, that equal numbers of monosomic and trisomic gametes are produced at fertilisation and that the survival of monosomics to day 3 is relatively normal.

The situation was different on day 4. While the frequencies of trisomies 1, 12 and 19 and monosomy 12 each remained about the same as on day 3, the frequency of monosomy 1 had decreased somewhat and that of monosomy 19 had decreased considerably, from 18 to 2%. This loss of embryos lacking chromosome 19 occurred both *in vivo* and *in vitro*. When day 3 embryos were cultured overnight, a significant number of them died and the frequency of monosomy declined to 2% (Table 1). However, no viable monosomics were found among embryos obtained directly on day 4 (Table 1), and the inviability of monosomy 19 cannot therefore be attributed to some increased sensitivity to *in vitro* culture conditions. There were too few chromosome 17 aneuploids to draw firm conclusions about their overall viability. Nevertheless, the data suggest that at least some monosomics are viable to the late blastocyst stage.

The day 3 embryos listed in Table 1 were a mixture of late morulae and blastocysts. Because the transition between morula and blastocyst is particularly sensitive to various agents, including α-amanitin[8] and bromodeoxyuridine[9], and is the time when development stops in embryos homozygous for the t^{12} mutation[10], we investigated whether it was affected by monosomy 19. Day 3 embryos were scored visually and then karyotyped (Table 2). Both normal and trisomic embryos consisted of approximately equal numbers of late morulae and early blastocysts. Similarly, half of the identified monosomics had also blastulated, while it was not clear whether the rest had blastulated. Thus

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blastulation itself does not appear to be the vulnerable step in monosomy 19, but the period immediately after does.

One possible explanation for the very early lethality of monosomy 19 is that a recessive allele, able to cause death before implantation, becomes uncovered in the monosomic state. Although the only chromosome 19 present in the embryos is that derived from the normal but outbred ICR Swiss parent, this does not seem likely. A very high frequency of such a lethal allele would be necessary to explain the death of all the embryos monosomic for chromosome 19. This would, in turn, result in the death of many normal embryos at the blastocyst stage, but there was no evidence for significant lethality among the hundreds of day 3 ICR blastocysts surviving in culture to day 4. Nevertheless, to obtain further evidence against the recessive lethal hypothesis, double metacentric-bearing males were mated with inbred C57BL/6J females and the progeny were scored as before (Table 1). Again, approximately equal numbers of monosomics and trisomics were observed on day 3, but as before, virtually no monosomic embryos survived to day 4. Therefore, we conclude that the lethality of monosomy 19 is an inherent property of the aneuploid state itself and does not depend on the hemizygous expression of recessive lethal alleles.

Data complementary to ours have been published by Dyban and Baranov[11]. After 3 days of development, no embryos monosomic for chromosome 5 or 17 were found. The proportions of embryos monosomic and trisomic for chromosomes 15 or 19 were the same, but both types of monosomics were retarded in development and had low cell numbers.

Chromosome 19, which comprises only 2.7% of the haploid set by length, is the smallest of the mouse chromosomes, and trisomy 19 is the trisomy associated with the greatest survival and the only one compatible with viability beyond term (Table 3)[12]. Survival of trisomies 1, 12 and 17 is much more limited[13]. By contrast, of the four monosomies studied, monosomy 19 is clearly the most lethal. Therefore, there does not seem to be any reciprocity between the viability of the monosomies and of the corresponding trisomies. Likewise, from the data in Table 3 and the more extensive data obtained by others from human trisomic embryos[14,15], there is no obvious relationship between the overall size of a chromosome and the survival of embryos which are aneuploid for it[16]. Although it has been suggested that there is an inverse correlation between the viability of specific human trisomics and the amount of active chromatin, as judged from Giemsa negative or R-band positive regions in the chromosomes involved[14], such a relationship does not exist for the mouse aneuploids discussed here (Table 3). We conclude therefore that the ultimate effect of each specific aneuploidy, and particularly of the monosomies, is as much, if not more, a function of the particular loci affected than of the total number of genes or amount of chromatin present on the chromosomes.

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Table 3 Relationship of survival of aneuploid mouse embryos to chromosome length and Giemsa light banding content

Chromosome	Length (% of haploid set) Total	Giemsa light bands	Survival (d) Monosomics	Trisomics
1	7.2(2.7)	3.1	≥4	14–15
12	4.6(1.8)	2.2	≥4	14–17
17	3.7(1.4)	1.3	≥4	10–12
19	2.7(1.0)	1.0	3.5	≥19

Chromosome lengths are as reported by Nesbitt and Francke[18], and the lengths of the Giemsa light band regions are calculated from Fig. 1 of the same report. The figures in parentheses are the ratios of chromosome lengths to the length of chromosome 19. The survivals of the trisomic embryos are as reported by Gropp *et al.*[13] and White *et al.*[12], and of the monosomics as reported here.

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Selective suppression of IgE antibody responsiveness by maternal influence

IMMUNOGLOBULIN E antibodies are produced in two circumstances: by all normal animals in infections with helminth parasites[1,2], and by certain individuals against common environmental substances[3]. In these latter 'atopic' individuals, immediate hypersensitivity reactions mediated by IgE are responsible for various disorders, which in man include hay fever, allergic asthma and allergies to foods and drugs. It is known that the antibody response of the young animal may be reduced or enhanced by maternal immunisation[4–9]. We have examined the antibody response of rats immunised with egg albumin (EA) and show here that both these effects can occur together: suppression of the IgE antibody response may occur against a background of enhanced total antibody responsiveness in the offspring of immunised mothers.

Our study was originally stimulated by observations that animals including man can occasionally be born hypersensitive or with specific IgE antibody in the cord blood[10–13]. As IgE does not cross the placenta[14] its presence in the neonate indicates fetal synthesis following *in utero* stimulation with antigen. We reasoned that suppression of IgE response could also result from such an antigenic stimulus and might in fact be a more frequent occurrence. Consequently, we fed or injected rats during pregnancy and lactation with different quantities of EA given without adjuvant. In this way, the mother would act merely as a vehicle for conveying antigen to the fetus without herself producing an IgE response, as this requires that the inducing dose of antigen be administered together with an adjuvant[15]. We found that such treatment of the mothers did not have any influence on the subsequent IgE responsiveness of the offspring.

These experiments, however, led to others in which we examined the effect of immunising rats with antigen and adjuvant long enough before a pregnancy to allow the development of an immune response. Prospective mothers were immunised with either 10 mg or 10 μg EA together with *Bordetella pertussis* as adjuvant 1 month before mating (Fig. 1). These doses of antigen had previously been shown to generate two different types of response: namely, specific IgE response in the rats

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immunised with the lower dose and superimposed specific IgE suppression in those immunised with the higher dose[15,16].

The results show that the offspring of the mothers immunised with either dose had a greatly depressed IgE response to EA. Feeding EA during pregnancy did not influence the subsequent IgE responsiveness of the progeny. Similarly, injection of the mothers with adjuvant alone was without effect.

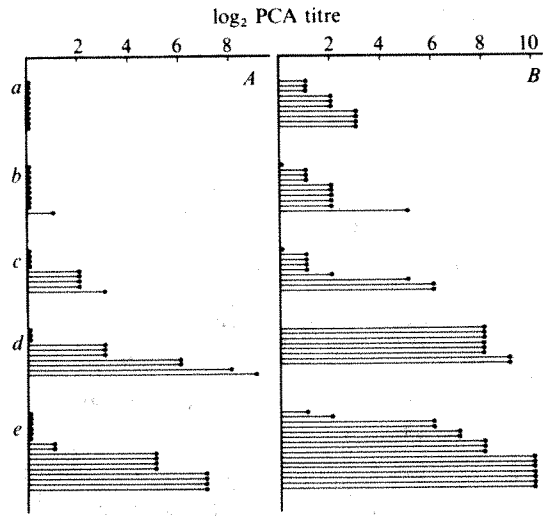


Fig. 1 The effect of preimmunisation of female rats or of feeding antigen during pregnancy on the specific IgE antibody responsiveness of the offspring. Outbred female Hooded Lister rats (Animal Suppliers) weighing 150–200 g were immunised 1 month before mating with the indicated amounts of egg albumin (EA) (Sigma grade V) injected intraperitoneally in 0.15 M NaCl together with a suspension of 10^{10} heat-killed *B. pertussis* as adjuvant. In addition the rats of groups *b*, *c* and *d* received EA in the drinking water at 10 μg ml^{-1} for 10 d starting 9 d after mating. Animals of a group immunised with 10 μg EA without additional antigen feeding did not become pregnant. The female offspring (two to three litters per group), which were born within 5 d of each other, were immunised with 10 μg EA + *B. pertussis* 6 weeks after the birth of the last litter and were challenged with 1 μg EA 35 d later. Blood for antibody assay was collected by severing the tip of the tail. IgE antibodies were estimated by the passive cutaneous anaphylaxis technique as previously described[16]. The figure shows the primary and booster IgE responses of the individual animals 12 d after immunisation (*A*) and 4 d after challenge (*B*). The times of bleeding were determined by previous observations on the kinetics of these responses in Hooded Lister rats[15]. Treatment of mothers: *a*, Immunised with 10 mg EA 1 month before mating. *b*, Immunised with 10 mg EA 1 month before mating and EA in the drinking water during pregnancy. *c*, Immunised with 10 μg EA 1 month before mating and EA in the drinking water during pregnancy. *d*, EA in the drinking water during pregnancy. *e*, No treatment.

The suppression of IgE responsiveness was antigen specific. This was demonstrated by inoculating the offspring of EA-immunised or normal mothers with both EA and keyhole limpet haemocyanin (KLH). The IgE response to EA but not to KLH was depressed in the offspring of the EA-immunised mothers.

To determine whether the suppressive effect was transferred transplacentally or in the milk, the offspring of EA-immunised and untreated mothers were exchanged on the day of birth. The IgE response of these rats following immunisation at 5–6 weeks of age was compared with that of rats reared by their natural mothers. As shown in Fig. 2 the IgE response to EA was suppressed in the offspring of normal rats if suckled by previously immunised mothers whereas the IgE response was normal in the offspring of immunised mothers fostered to non-immunised mothers. This indicated that the factors responsible for suppression were transferred in the milk. When further litters were cross-fostered at 1 or 2 weeks of age, specific suppressive factors from the immunised mothers were found to be transferred during the entire period of lactation. Thus, if the offspring of normal mothers were suckled by immunised mothers for even 1 week (from days 15–21 of lactation) a significant degree of suppression was transferred.

Assessment of diminished antibody response in the progeny of immune mothers—by measuring total antibody or numbers of antibody-producing cells, or by observing functional effects such as vaccination failure—has hitherto been made without reference to possible differential action on antibody class. To determine whether the suppression we had observed in the offspring of immunised mothers was selective for IgE or merely a facet of a generally suppressed response, we measured the IgG response and total antigen-combining capacity for EA in the sera of normal and IgE-suppressed offspring. The results in Table 1 show that higher levels of IgG and total antibody were produced in the primary response to antigen by the offspring of immunised mothers. Examination of the sera of progeny before immunisation showed that antibodies to EA (excluding IgE) were passively transferred from the immunised mother, but declined to scarcely detectable levels by 6 weeks. The additive effect of residual maternal antibody could therefore not explain the higher level of primary response seen in the progeny of immunised mothers.

Table 1 Comparison of EA IgG and total antibody response with EA IgE levels in the offspring of normal (*A*) and immunised (*B*) mothers

	IgE (geometric mean PCA titre)		IgG (mean c.p.m. $\times 10^{-3}$) (±s.d.)		Total antigen-binding capacity (mean $\log_{10}$ μg EA bound per ml serum ±s.d.)	
	A	*B*	*A*	*B*	*A*	*B*
Day 20 after immunisation	9.18	All negative	1.92 (pool)	2.93 (pool)	0.97 (0.15	1.35 (0.19)
Day 4 after challenge	1,175	All negative	4.45 (2.12)	4.71 (1.43)	1.39 (0.20)	1.47 (0.18)
Day 21 after challenge	278	All negative	3.39 (1.05)	3.72 (2.62)	1.71 (0.36)	1.49 (0.20)

IgG response to EA was measured by a paper radioallergosorbent test in which paper disks to which EA had been coupled were reacted first with serum and then with ^{125}I-labelled anti-rat IgG. The method is fully described elsewhere[19]. The antigen-binding capacity was measured by the Farr technique[20] as modified by Mitchison[21,22]. Iodination of EA was carried out with the labelled ester Bolton and Hunter reagent[23] (*N*-succinomidyl-3-(4-hydroxy, 5-^{125}I-iodophenyl) propionate; Radiochemical Centre using 5 mg EA and 1 mCi of the labelled reagent. The measurements shown were made on five serum samples from each of groups *a* and *b* rats in the experiment shown in Fig. 2. The difference between groups *A* and *B* on day 20 after immunisation in the antigen-binding assay is significant ($P < 0.01$).

Reduced antibody production in the offspring of immunised mothers has usually been attributable to maternal antibody

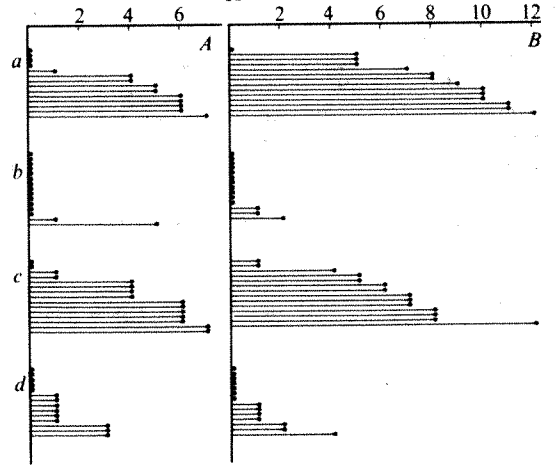


Fig. 2 Effect of cross-fostering on the transfer of the IgE suppressive effect. The immunised mothers were injected with 1 mg EA and 10^{10} *B. pertussis* 1 month before mating; control mothers were untreated. At birth the offspring were either left with their own mothers or were exchanged so that those born of immunised mothers were suckled by non-immunised mothers and vice versa. These exchanges were carried out as soon as possible and not more than 1–2 h after birth. Each of the groups *a*–*d* consisted of the female rats of four litters. These rats were immunised at 5–6 weeks with 10 μg EA and *B. pertussis* and were challenged 1 month later with 1 μg EA alone. The results shown are the primary and booster EA IgE antibody responses of individual rats. 20 d after immunisation (*A*) and 4 d after challenge (*B*). Groups: *a*, offspring of normal mothers; *b*, offspring of immunised mothers; *c*, offspring of immunised mothers fostered to normal mothers; *d*, offspring of normal mothers fostered to immunised mothers.

passively transferred transplacentally and/or in the milk according to species[17,18], although tolerance induction following encounter with antigen by the same routes has also been suggested[5,6]. We do not know the mechanism of suppression of IgE responsiveness nor whether it is related to the enhanced total antibody response. However, because a maternal immune response is necessary the phenomenon must be mediated by immune factors.

Maternal immunity transferred to the offspring for protection against infection may also interfere with the development of active immunity. The latter can be seen as a side effect, a price to be paid to ensure the survival of the neonate. However, transfer of IgE suppression from mother to young can be regarded as an objective. For, whatever the biological role of IgE, a response to substances such as pollens or foods apparently leads only to a deleterious hypersensitivity. To control such aberrant production, elaborate and sensitive immunoregulatory mechanisms have been evolved. The existence of an allergy-protective device involving specific IgE suppression by maternal influence is an additional possibility which must now be considered.

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T cells primed by influenza virion internal components can cooperate in the antibody response to haemagglutinin

THE fact that nude mice produce significantly lower titres of antibody to influenza haemagglutinin than do normal mice implies that this response is T-cell dependent[1,2]. Further evidence of cooperation between T and B cells has been provided by the immune response of mice to purified haemagglutinin[3]: a greater production of strain-specific, but not of cross-reactive, antibody was observed in the presence of haemagglutinin-primed T cells. We report here that the antibody response to haemagglutinin can also be enhanced by T cells primed by the internal components of the influenza virus. This may provide an explanation for previous findings[4–6] that infection with one influenza subtype may influence the subsequent response to another which does not cross-react serologically.

Table 1 X31 HAI antibody response of mice after antigen priming

Priming antigen	Boosting antigen	$\log_2$ serum HAI titre ± s.e.m. Day 4	Day 10
—	X31 Virus	≤1.58*	2.38 ± 0.49
X31 Spikeless particles	X31 Virus	4.98 ± 0.93	5.38 ± 1.07
—	X31 Monomer HA	≤1.58	2.33 ± 0.75
X31 Spikeless particles	X31 Monomer HA	≤1.58	≤1.58
X31 Spikeless particles	X31 Spikeless particles	≤1.58	≤1.58

Groups of five CBA/T6 mice were primed by i.p. injection of 100 μg *B. pertussis* plus 10 μg antigen as stated. After 28 d the mice were boosted by i.p. injection of 10 μg antigen and bled 4 and 10 d later.
* Limit of assay: no antibody detectable.

Treatment of the X31 strain of A/Hong Kong/1/68 influenza virus (ref. 7) with the protease bromelain removes the haemagglutinin and neuraminidase subunits from the surface of the virion and leaves the particles smooth-surfaced[8]. Spikeless particles prepared in this way were injected intraperitoneally (i.p.) into 12-week-old CBA/T6 female mice together with *Bordetella pertussis* (Wellcome). When subsequently injected i.p. with purified X31 virus, mice primed with spikeless particles gave a much higher and earlier haemagglutination inhibition (HAI) antibody response than did controls that had been injected with *B. pertussis* alone (Table 1). In contrast, the response to bromelain-extracted monomer haemagglutinin[8] was not enhanced by priming with spikeless particles. There was also no HAI antibody response to a second injection of spikeless particles. Similar results were obtained from preliminary experiments using deoxycholate-treated virus as the priming antigen. This preparation lacks both envelope lipid and surface glycoproteins but contains all the internal proteins and RNA of the virus[9]. These results suggest that the priming effect is unlikely to be mediated by otherwise undetectable traces of haemagglutinin remaining in the X31 spikeless particle preparation.

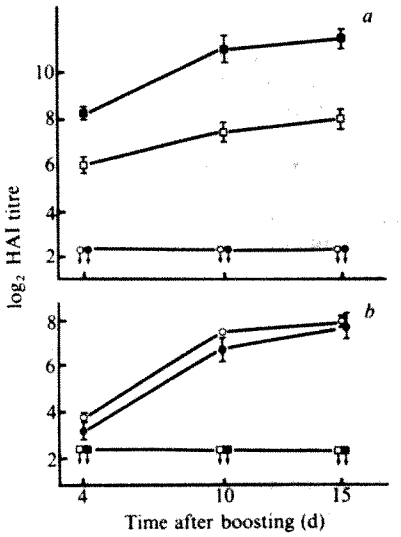


Fig. 1 *a*, A/PR/8/34 HAI antibody response; *b*, B/Victoria/70 HAI antibody response. Geometric mean serum antibody titres in groups of five mice. Bars indicate standard errors. □, Normal mice immunised with 10 μg A/PR/8/34; ■, mice primed with 10 μg X31 spikeless particles and boosted with 10 μg A/PR/8/34; ○, normal mice immunised with 10 μg B/Victoria/70; ●, mice primed with 10 μg X31 spikeless particles and boosted with 10 μg B/Victoria/70.

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Mice primed with X31 spikeless particles and then injected with A/Puerto Rico/8/34 virus gave an enhanced HAI antibody response to this subtype (Fig. 1*a*), even though the H_3 haemagglutinin of X31 is antigenically distinct from the H_0 haemagglutinin of A/PR/8/34. The HAI antibody response of mice to B/Victoria/70 was not influenced by priming with X31 spikeless particles (Fig. 1*b*), indicating that the priming is type specific.

The cell type responsible for the priming effect was investigated using a spleen cell transfer technique (Table 2). Recipient mice given 10^8 unfractionated spleen cells from primed donors were able to mount an enhanced response after i.p. injection of X31 virus. Ig-negative spleen cells separated by the rosette method, which removes more than 97% of Ig-bearing cells as detected by autoradiography[10], were also effective; 2×10^7 Ig-negative spleen cells were capable of substituting fully for 10^8 unfractionated cells. Smaller quantities were progressively less effective, but still produced a measurable improvement in antibody response. We have not found it necessary to irradiate the recipient mice in our adoptive transfer experiments. This may be because relatively few primed cells are required. It may also reflect a more general property of the virus, as successful adoptive transfer of influenza immunity using normal recipients has previously been reported[11,12].

Our findings strongly suggest that cell cooperation analogous to that seen in the carrier–hapten phenomenon[13–15] occurs in the antibody response to influenza haemagglutinin. Thus, T cells recognising some internal component of the virion are capable of cooperation with B cells in the production of HAI antibody. Work in progress indicates that of the internal components at least the matrix protein is recognised by cooperating cells (Table 3). The mechanism by which associative recognition of the haemagglutinin and internal proteins can occur is unclear. The haemagglutinin spikes are present on the virus surface and do not seem to penetrate the lipid envelope, whereas the matrix protein, the outermost of the internal components, is contained within the lipid envelope and is thought not to penetrate the bilayer to the virion surface[16]. The two proteins are therefore connected and also separated by the lipid bilayer. Thus, for recognition and cell cooperation to occur, either the matrix protein is more accessible than described above or some processing step may be necessary to present both proteins in association on the surface of a macrophage or other cell.

In this latter case, the mechanism of help is similar to that of the associative recognition of intrinsic cell surface proteins described by Lake and Mitchison[17]. In their terminology this type of effect could be described as intermolecular, intrastructural help, as both helper and principal determinants are carried by different proteins. Our data then provide an example of cell-surface associative recognition in which both antigens are of viral origin.

It has been reported that the HAI antibody response of ferrets, hamsters and mice to inactivated influenza vaccines can be enhanced by prior heterotypic infection[4,5] and even that infection can provide a partial heterotypic immunity[6]. Our results may help to explain these otherwise puzzling phenomena.

Table 2 Adoptive transfer of the priming effect by Ig-negative cells

Donor cells	$\log_2$ serum HAI antibody titre ± s.e.m. Day 4	Day 10
—	≤1.58*	5.78 ± 0.58
1×10^8 Normal spleen cells	≤1.58	4.78 ± 0.37
Primed spleen cells:		
1×10^8 Unfractionated	3.98 ± 0.24	5.98 ± 0.24
2×10^5 Ig-negative	3.58 ± 0.00	5.98 ± 0.51
2×10^6 Ig-negative	3.78 ± 0.20	5.78 ± 0.37
2×10^7 Ig-negative	4.78 ± 0.20	6.33 ± 0.25

Groups of five normal CBA/T6 female mice were injected with spleen cells from donors primed 28 d earlier with 10 μg X31 spikeless particles. Spleen cells were fractionated by the rosette method[10]. Recipients were boosted with 10 μg X31 virus and bled 4 and 10 d later.
* Limit of assay: no antibody detectable.

Table 3 Ability of purified matrix protein to prime helper cells

Priming antigen	$\log_2$ serum HAI antibody titre ± s.e.m. Day 4	Day 11
—	≤1.58*	4.58 ± 0.77
X31 Spikeless particles	4.30 ± 1.12	6.38 ± 0.37
Matrix protein	3.98 ± 0.68	7.18 ± 0.51

Groups of five CBA/T6 mice were primed by i.p. injection of 100 μg *B. pertussis* plus 10 μg antigen as stated. After 28 d the mice were boosted by i.p. injection of 10 μg purified X31 virus and bled 4 and 11 d later. Matrix protein was derived from A_2/Jap/305/57 × A_0/Bel/42 (H_2N_1), prepared by the method of Laver[18]. SDS-polyacrylamide gel electrophoresis gave a single distinct protein band.
* Limit of assay: no antibody detectable.

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Use of cyclosporin A in allogeneic bone marrow transplantation in the rat

ALLOGENEIC bone marrow transplantation (BMT) has become the therapy of choice for the treatment of severe aplastic anaemia and severe combined immunodeficiency diseases and is considered beneficial for the treatment and possible cure of malignant diseases, especially lymphohaematopoietic malignancies[1]. Several serious complications, however, prevent the full realisation of the therapeutic potential of this procedure. To accept an allogeneic marrow graft, patients (even those treated for aplastic anaemia) have to be conditioned with cytoreductive agents like cyclophosphamide (Cy) or total body irradiation (TBI), agents that have relatively low therapeutic indices for immunosuppression and marked toxicities. Furthermore, patients, even if matched with their respective donors at the major histocompatibility complex (MHC), have after engrafting successfully, a high incidence of potentially fatal graft-versus-host disease (GvHD). Third, patients, who successfully engraft have a severe immunodeficiency for several months after transplantation that apparently contributes to potentially fatal

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Table 1 Use of Cs A as an immunosuppressant to establish engraftment in BU-treated rats

Group	Treatment at day −8	−1	0	Days after grafting 7 Mort.	7 Chim.	7 GvHD	14 Mort.	14 Chim.	14 GvHD	21 Mort.	21 Chim.	21 GvHD	28 Mort.	28 Chim.	28 GvHD
1		BU	—	0/8	—	—	8/8	—	—	—	—	—	—	—	—
2		BU	Lewis	0/8	—	—	0/8	—	—	0/8	—	—	1/8	—	—
3		BU	ACI	0/8	—	—	8/8	—	—	—	—	—	—	—	—
4	RARTS	BU	ACI	1/8	0/7	0/7	2/8	6/6	4/6	4/8	4/4	4/4	7/8	1/1	1/1
5		BU+Cy	ACI	0/8	0/8	0/8	0/8	8/8	3/8	3/8	5/5	5/5	5/8	3/3	3/3
6	Cs A	BU	ACI	0/8	0/8	0/8	0/8	NT	0/8	0/8	8/8	0/8	1/8	7/7	0/7

Groups of Lewis rats were treated with busulphan 30 mg per kg i.p. alone (groups 1, 2 and 3) or together with cyclophosphamide 150 mg per kg i.p. (group 5), a short course of rabbit anti-rat-thymocyte serum i.p. (RARTS, 1.5 ml daily from day −8 to day −2) (group 4) or Cs A (25 mg per kg given subcutaneously daily from day −8 to day +18) (group 6). One day after busulphan administration 60×10^6 nucleated marrow cells were given intravenously. Cumulative mortality, fraction of animals proven to be chimaeras and fraction of animals with clinical signs of GvHD are recorded. Chimaerism was established by typing the peripheral lymphocytes with strain-specific cytotoxic antisera. NT, Not tested.

opportunistic infections in the post-grafting period[2]. Attempts to overcome these complications have had only limited success. Cyclosporin A (Cs A), an undecapeptide of fungal origin[3], has been reported to have very profound antilymphocytic activities with very low degrees of myelotoxicity[4]. It has been shown to suppress a variety of humoral and cellular immune phenomena[5], including the rejection of renal allografts in rabbits.[6] It seemed reasonable, therefore, to study its effects in an animal model of bone marrow transplantation. Cs A permits the full establishment of a rat marrow graft across the major histocompatibility barrier. Given post grafting it prevents GvHD in an AgB mismatched donor–recipient combination and allows recovery of T-dependent immune functions as early as 28 d after transplantation. Thus, Cs A seems to be a promising agent for use in clinical marrow transplantation.

We have chosen an allogeneic rat system that we have found particularly useful as a preclinical model for human BMT[7]. Lewis rats (Ag-B1) were given a lethal dose of busulphan (BU), a drug that is not immunosuppressive in this species but myelotoxic, leading to the death of the animals from marrow failure unless protected with syngeneic marrow[8]. ACI (Ag-B4) marrow which is different at the MHC, will not be accepted by these BU-treated animals, unless they are immunosuppressed with high doses of Cy or rabbit anti-rat thymocyte serum (RARTS)[9]. If Cs A is substituted for Cy or RARTS and given as a daily injection of 25 mg per kg subcutaneously for 28 d, the animals will accept a bone marrow graft across the major histocompatibility barrier and become healthy chimaeras as evidenced by typing on day 21 (Table 1). The animals furthermore accept skin grafts from donor- and recipient-type animals (ACI and Lewis) but reject a third party (BN) skin graft. These data suggest that Cs A is able to provide the necessary immunosuppression to allow the establishment of a functional, histoincompatible marrow graft in an animal that suffers from a presumed lethal, BU-induced marrow aplasia.

An immunosuppressive drug like Cs A, that has been claimed to induce specific immunological tolerance[10], should have a marked potential for the prevention and treatment of GvHD, a disease considered to result from an immunological attack of immunocompetent donor T cells against host target tissues[11]. This disease can be studied with relative ease in the rat bone marrow transplantation model, where a clinical syndrome consisting of weight loss, marked dermatitis, diarrhoea and general unkempt appearance can be easily recognised, especially when a histoincompatible donor–recipient combination is used[12]. All Lewis rats, conditioned with 1,000 rad TBI and infused with AgB-incompatible ACI marrow cells, developed severe GvHD within 12 d and showed a mortality of 75% (6 out of 8) by 35 d post-transplantation. Subcutaneous daily injections of Cs A starting 1 d post-transplantation and maintained until day 18 completely prevented the development of GvHD as judged by clinical observation, and by histological examination of the skin on days 14, 17, 21, 28, 35, and of tongue, liver and intestine on days 21 (Fig. 1), 28 and 35. These Cs A-treated animals have now survived more than 50 d post-transplantation without any signs of clinical GvHD and seem to be in excellent health. The peripheral lymphocytes of these chimaeras were shown to be of donor (ACI) origin with the use of specific cytotoxic typing sera[12]; furthermore, they accepted donor- and recipient-type skin grafts but rejected third party (BN) skin allografts in a normal fashion, fulfilling the criteria for operational tolerance.

Borel *et al.*[5] have used Cs A to affect GvHD in a rat bone marrow transplant model, and have shown that oral administration of Cs A merely delayed the onset of lethal GvHD in a parent-to-hybrid situation. The complete prevention of GvHD clinically and histologically seen in our study could be due to the different donor–recipient combination (although we used the most histoincompatible one available to us) but might alternatively be due to the higher doses of Cs A applied, the route of administration (parenteral compared with oral), or the duration of administration.

In previous studies[13] we have evaluated the immune status of rat chimaeras transplanted with allogeneic histoincompatible bone marrow and noted a marked immunodeficiency. Such animals failed to demonstrate delayed type hypersensitivity (DTH) at 40 d post-transplantation and showed poor antibody responses to sheep red blood cells (SRBC)[14]. This defect has been described in other animal systems and in man[15] and is

Table 2 Immune recovery of marrow transplant recipients after Cs A treatment

Donor–recipient combination	ACI*/Lewis	ACI*/Lewis, Cs A†	Lewis‡/Lewis
Relative reconstitution of lymphoid tissue	($n = 4$)	($n = 4$)	($n = 4$)
Thymus			
Cortex	all 1+	all 3+	3+, 3+, 3+, 4+
Medulla	all 1	all 2+	all 4+
Spleen			
T-cell region	all 1+	all 3+	3+, 3+, 4+, 4+
B-cell region	all 0	all 3+	all 4+
Lymph node			
T-cell region	all 1+	all 2+	3+, 3+, 4+, 4+
B-cell region	all 0	all 2+	3+, 3+, 4+, 4+
Delayed type hypersensitivity to DNCB	all 0	all 2+	all 3+
Antibody to SRBC ($\log_2$)§	all 1	4,4,4,5	6,7,7,7

Groups of Lewis rats (16 per allogeneic group, 8 per syngeneic group), were given 1,000 rads TBI and injected with 60×10^6 nucleated ACI* or Lewis‡ marrow cells i.v. One group of rats† was, in addition, given Cs A 25 mg per kg s.c. daily for 18 d starting 1 d after marrow infusion. Four animals out of each group were killed at day 21 (days 28 and 35 in the Cs A-treated group) and the lymphoid organs examined histologically. The relative number of lymphocytes in the lymphoid tissues are rated on a 0 to 4+ scale with 4+ being that of a normal Lewis rat. Surviving animals were immunised i.v. with 1 ml 10% solution of SRBC and the saline agglutination titres determined 1 week later.§ In addition, the animals were sensitised to DNCB (600 $\mu g\ ml^{-1}$ over 1 cm^2 skin) on day 28, challenged on day 42 (60 $\mu g\ ml^{-1}$ over 1 cm^2 skin) and biopsied on day 44. The delayed type hypersensitivity reation was graded on a 0–4+ scale, 4+ being the reaction of a normal Lewis rat.

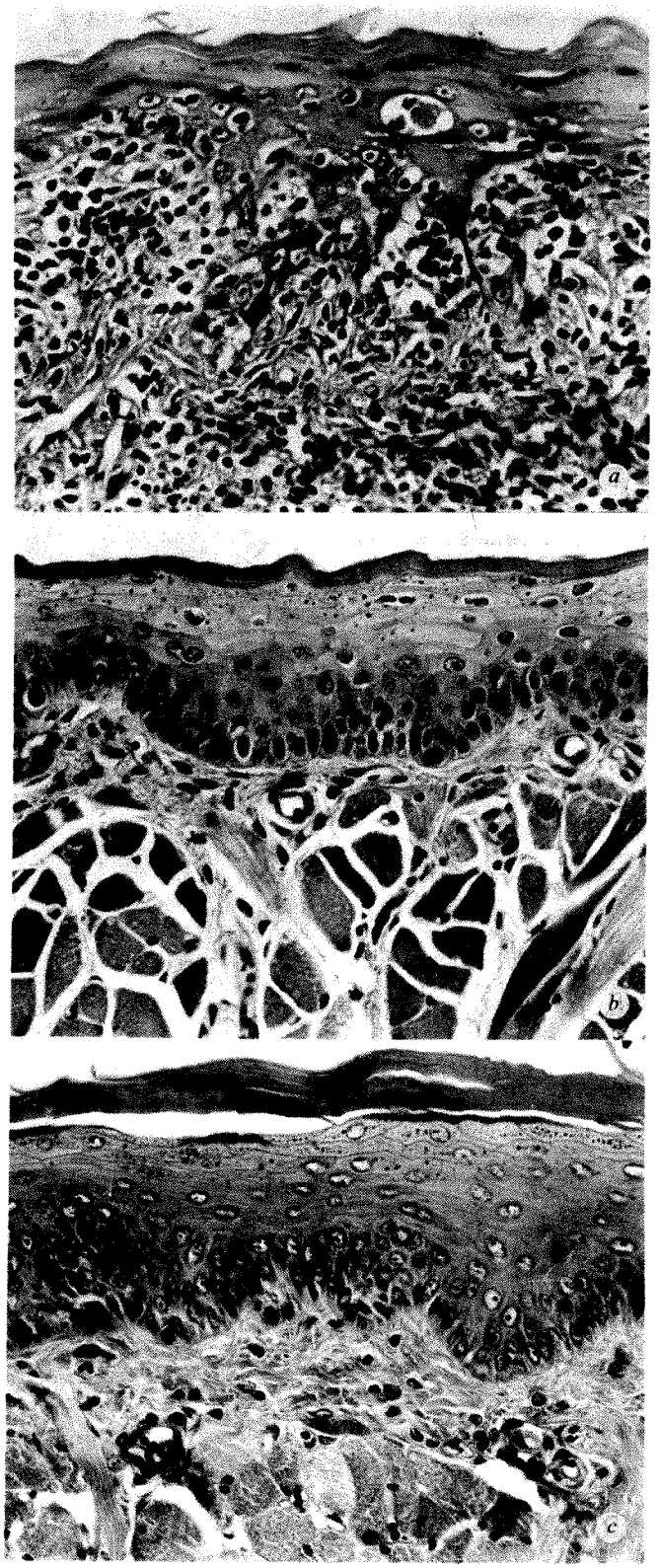


Fig. 1 Effect of Cs A on the development of GvHD in the rat. Lewis rats were given 1,000 rad total body irradiation (dual source ^{137}Ce small animal irradiator delivering 136 rad per min) followed in 24 h by infusion of 60×10^6 ACI (groups *a* and *b*) or Lewis nucleated marrow cells (*c*). Group *b* was treated in addition with Cs A given daily subcutaneously in a dose of 25 mg per kg from day +1 through day +18 post-transplantation. Four animals of each group were killed 28 d post-transplantation and autopsies performed. A representative section of the tongue is shown (haematoxylin and eosin, ×400). A graft-versus-host reaction is recognised in the allogeneic group (*a*), which did not receive Cs A. No histological difference can be seen between Cs A-treated allogeneic chimaeras (*b*) and rats given syngeneic marrow (*c*).



Fig. 2 Effect of Cs A on lymphocyte repopulation in the thymus of allogeneic marrow graft recipients. Lewis rats, conditions with 1,000 rad total body irradiation and infused with ACI (*a* and *b*) or Lewis (*c*) marrow, were either non treated (*a* and *c*) or treated (*b*) with Cs A (see Table 2). The thymuses of the animals were examined histologically (stained with haematoxylin and eosin, ×200). Cs A-treated rat chimeras show good reconstitution of the thymic cortex (*b*), with medium and small lymphocytes, occasional lymphoblasts and mitotic figures. These lymphoid cells were shown by cytotoxic typing analysis to be of ACI (donor) origin. The untreated syngeneic recipients have reconstitution of the cortex with small lymphocytes and have a distinct corticomedullary junction.

considered to contribute to a significant degree to the impaired defence of the host against opportunistic infections post-transplantation[16], especially those leading to an often fatal interstitial pneumonitis[17]. The aetiology of this immune defect is poorly understood, but might be related to graft-versus-host reactions, as the degree and length of immunodeficiency post-transplantation correlate with the degree of histoincompatibility between donor and recipient[18].

As GvHD was prevented in our rat model by Cs A we evaluated the immune status of the resulting chimaeras morphologically and functionally. Lewis rats were conditioned with TBI and given syngeneic marrow, allogeneic (histoincompatibile ACI) marrow and allogeneic (ACI) marrow plus Cs A daily for 18 d, starting on day 1 post-transplantation. Representative animals out of each group were killed on day 21 (days 28 and 35 in the Cs A-treated group) and the lymphoid organs examined histologically. The striking repopulation of the T-cell areas in the lymphoid organs of allogeneic Cs A-treated chimaeras (Fig. 2) correlates with a recovery of immunological function (Table 2). Cs A-treated chimaeras immunised with DNCB and injected with SRBC were capable of mounting an immune response against SRBC and showed DTH, in striking contrast to the immunodeficient allogeneic chimaeras not treated with Cs A.

At present it is unclear how Cs A induces specific tolerance without interfering with the immune reconstitution. Studies by Green and Allison[6] have shown that Cs A seems to act on lymphoblasts with both T- and B-cell markers and presumably eliminates or suppresses clones of lymphocytes responding to the antigens in question. The operational tolerance which we have in our Cs A-treated allogeneic chimaeras would then have been caused by a specific deletion or inactivation of clones of donor cells responding to host target antigens according to the clonal selection theory of transplantation tolerance. On the other hand, we and others have shown that transplantation tolerance might be induced and maintained by donor-type suppressor cells[14,15,19,20]. It is tempting to speculate that Cs A acts by accelerating the appearance of suppressor cells, and indeed we have found in preliminary experiments that Cs A-treated allogeneic chimaeras possess cells in the spleen as early as 21 d post-transplantation, which suppress mixed lymphocyte reactions between donor- and recipient-type cells (data not shown). If these findings can be confirmed in future experiments, we might have a better understanding of the mechanism of action of this new drug and of the mechanism of transplantation tolerance as well.

Our studies seem to indicate that the optimism for Cs A generated from studies in other animal systems is justified. In our rat system this drug proved as immunosuppressive as Cy or RARTS, prevented GvHD and allowed early recovery of the immune function post transplantation. Cs A therefore seems to be a promising agent for use in clinical BMT.

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Degranulation capacity of cultured mast cells in the presence of histamine releaser

A PURE population of mast cells develops when suspensions of lymphoid cells prepared from mice pre-immunised with horse serum are grown on embryonic mouse skin fibroblast monolayers[1]. These *in vitro* matured mast cells are capable of undergoing repeated cycles of degranulation and regeneration[2]. Both IgG_1 antibody and the corresponding antigen and the compound 48/80 (ref. 3) trigger degranulation of more than 90% of the mast cells, releasing 80 to 90% of their histamine content[2]. This produces an immediate effect on the cells in the fibroblast monolayer. The well-stretched cytoplasm is displaced, opening numerous 'windows' scattered over the whole monolayer[2]. These capacities of cultured mast cells raise questions on the relationships between them, the degranulating agent and the degranulating products released into the medium. Using our tissue culture, mast cell behaviour can be studied in the continuous presence of the degranulating agent or of the degranulating products. We show here that mast cells regenerate degranulation irrespective of the continuous presence of the histamine releaser compound 48/80, but they become unresponsive to its degranulating effect, although they are totally degranulated by IgG_1 and the corresponding antigen.

The first experiments compared the behaviour of the mast cells grown: (1) in the continuous presence of 48/80 and the degranulating products; and (2) in similar cultures but from which the medium containing the 48/40 and the degranulating products was replaced 15 min later by a fresh 48/80-free medium. No difference was observed between the two groups. After 3–5 h, the fibroblasts stretch back, the windows close and the monolayer resumes its former confluent texture, with a large number of granules spread over it. The degranulated cells start immediately to synthesise histamine at an accelerated rate, and in 3 d the amount of histamine is totally replenished (manuscript in preparation). In some cases it even surpasses the level of the control undegranulated cultures (Table 1).

To see whether the regeneration capacity and lack of degranulation activity is due to decomposition of 48/80 we transferred medium containing 48/80 after further 4 days' incubation of the mast-cell cultures (91% mast cells degranulated, and 83% of the histamine was released 15 min after addition of the 48/80) to parallel control plates containing mast cells maintained with ordinary medium. Histamine was released and windows were formed (Table 1, group B). This clearly shows that 48/80 preserves its degranulating capacity over a period of 4 d. In further experiments 48/80 was transferred every 24 h for 4 d to new mast cell plates. At the fourth transfer the percentage of degranulated mast cells dropped from 73 to 45%. To confirm that the regenerated mast cells are refractory to triggering by 48/80, we replaced the 4-d-old medium with fresh medium

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containing 48/80 and incubated the plates for 15 min. No degranulation occurred, and a negligible amount of histamine was found in the medium (Table 1, group A). However, when the medium containing fresh 48/80 was replaced by anti-horse serum IgG_1 plus antigen there was intensive degranulation (91%) with abundant window formation and 90.3% release of histamine (Table 1, group A).

This desensitisation course was studied further by exposure to serial dilutions of 48/80. Half of the plates of each dilution were assayed for histamine release after 20 min of exposure (Fig. 1), while the other half was incubated for an additional 3 d and then re-exposed to 15 μg 48/80 for 20 min (Fig. 2). There was complete unresponsiveness in cultures incubated with ≥10 μg per ml. (Cell counts showed that the unresponsiveness was not a result of diminishing number of mast cells in the cultures.) However, mast cells degranulated by 15 min incubation with 48/80 and then maintained further for 4 d with ordinary medium were degranulated thoroughly (87%) by fresh 48/80–like the control plates maintained with the ordinary medium alone (91% degranulation) (see also Table 2).

The persistence of refractoriness of regenerated mast cells after removal of 48/80 was examined by two methods. In the first method, cultures were exposed to 10 μg of 48/80 per ml (79.8% histamine release) either for 3 d, or for only 20 min to allow the degranulation to terminate followed by fresh 48/80-free medium for 3 d. Control cultures received only fresh medium. The old medium was then removed, cultures were washed twice and fresh 48/80-free medium was added. At intervals, 1 ml of medium containing 10 μg 48/80 or IgG_1 + 1% horse serum (see Table 1 legend), was added and the percentage of histamine release was determined. The results are shown in Table 2. In this culture, incubation with 10 μg 48/80 did not produce complete desensitisation (25% histamine release). Nevertheless, it can be seen that the responsive capacity was only slightly increased after removal of 48/80 (33% and 45% release). On the other hand, the response to IgG_1 was not affected by pretreatment with 48/80 and remained high in all the experimental groups.

In the second method mast cells were degranulated by 48/80 and then maintained further in the same degranulating medium for 3 d while control plates were maintained with ordinary medium. Both groups were passaged by trypsinisation to make 3.5 new plates from each older plate. (Note that trypsin releases about 70% of the histamine[2,5].) The degranulation capacity was

Table 1 Histamine release by 48/80 and IgG_1 in mast-cell cultures grown continuously with 48/80

Experimental group	Treatment of cultures	Free histamine (μg per plate)	Histamine in cells (μg per plate)	Histamine release (%)
A	4 d with 48/80; old medium removed, fresh medium + 48/80 added for 15 min	0.72	8.44	7.9
	Anti-horse serum (IgG_1) + horse serum	7.62	0.82	90.3
B	Recipient of old medium with 48/80 from group A	10.36	3.71	*
C	Maintained 4 d with 48/80	—	8.28	—
D	Maintained without 48/80	—	7.03	—

To obtain clonal differentiation of the mast cells, lymphoid cell suspensions (prepared from mice immunised with horse serum[1,2]) were grown on fibroblast monolayers. BALB/c mice, 2–4 months old, were injected with 0.2 ml horse serum twice a week for 4 weeks. The animals were bled 6–12 d later and the serum was collected and frozen. This preparation, heated to 56 °C for 30 min to inactivate IgE, was used in inducing degranulation. The suspensions were prepared as described previously[2,5]. Cells 10^7 in 2 ml Dulbecco's medium, supplemented with 15% heat-inactivated horse serum, were plated on 35-mm plastic Petri dishes containing fibroblast monolayers, prepared by plating 2.5×10^5 embryo skin cells in 2 ml Waymouth's medium supplemented with 10% calf serum and ready for use 4–6 d later. In these cultures mast-cell clones attained maximal growth at ~15–25 d. Cultures were normally passaged once or twice by trypsinisation between the 10th and 24th days, with the trypsinised cell suspension plated to make 2 new plates from each old plate. Within 4–6 d, mature mast cells spread confluently over the monolayer. On the 19th day of culture, 20 μg of 48/80 (sigma) in 2 ml Dulbecco's medium were added to half the plates while the other half received fresh medium only. 91% of the mast cells degranulated. The cultures were incubated further for 4 d. The plates maintained with 48/80 contained 1.1×10^6 mast cells and those without it 0.9×10^6 mast cells. Histamine was titrated by Snyder's radioenzymatic assay as described previously[2]. The fluid, removed from the plate, was spun at 2,000 r.p.m. for 10 min. The supernatant was frozen pending assay of the free histamine in the medium; to the sediment, 2 ml of 0.1 M HCl was added and the whole content transferred to the plate containing the monolayer. The plates were frozen at −20° C pending assay of the histamine in the cells.

*As the medium already contained histamine, the extent of degranulation is shown by the amount of histamine retained in cells.

Table 2 Degranulation capacity of mast cells in culture after 3-d-old 48/80-containing medium was removed

Time after removal of 48/80 (h)	3 days with 48/80		Without 48/80		15 min with 48/80	
	Second Challenge					
	48/80	IgG_1	48/80	IgG_1	48/80	IgG_1
	Histamine release (%)					
0	25.1	76.4	70.2	—	65.1	78.2
2	26.3	—	82.3	—	74.5	—
4	26.0	80.4	84.4	70.6	—	—
8	45.3	—	—	—	—	—
16	39.1	—	—	—	—	—
24	45.0	78.9	79.0	75.4	66.4	78.2
48	27.3	—	75.4	—	65.9	—
72	33.5	71.5	80.0	60.0	76.6	70.6

Culture was established by plating 10^7 lymph node cells prepared from 4 month old BALB/c mice, 1 d after booster injection of horse serum. Culture was passaged 10 d later to make 3.2 new plates from each old plate. After 6 d, when there were 5.3×10^5 mast cells per plate, 20 μg of 48/80 in 2 ml Dulbecco's medium were added and the cultures were incubated for an additional 3 d. Then the medium was removed, cultures washed twice and fresh 48/80-free medium was added. At intervals the medium was removed and either 1 ml of Dulbecco's medium containing 10 μg 48/80 was added or 0.4 ml of antiserum (PCA titre in mice-1000), dilution 1:5 was added and followed 3 min later by 0.4 ml of 1% horse serum. After 20 min incubation the plates were processed for histamine assay.

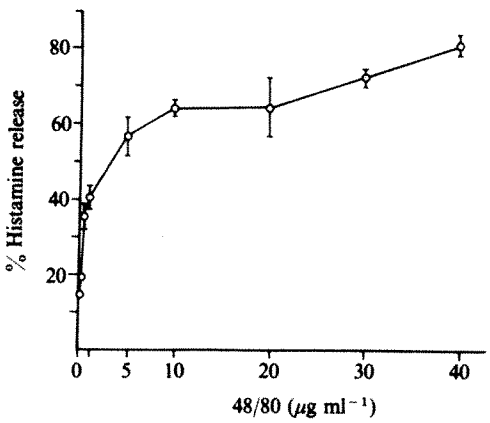


Fig. 1 Percent of histamine release as a function of dose of 48/80. A 16-d-old culture, 6 d after trypsin passage, making 3.2 new plates from each old plate. Counts of mast cells yielded an average number of 8.9×10^5 per plate. The cultures were incubated for 20 min before being processed for histamine assay.

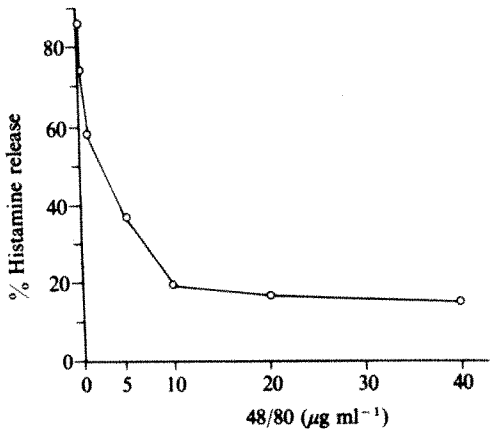


Fig. 2 Same culture as Fig. 1, but plates were incubated further for 3 d with the medium containing the various amounts of 48/80. These plates were then rechallenged with 15 μg 48/80 in 1 ml of medium. They were incubated for 20 min before processed for histamine assay.

determined immediately after plating in fresh medium and at different times thereafter. Results are shown in Fig. 3. At time zero histamine was not released, as compared to 10% in the control cultures; but the degranulation capacity gradually increased during the first 24 h, coinciding with the replenishment of the cellular histamine content until 48–72 h, when the response to 48/80 completely recovered. The study shows that without trypsinisation, restoration of the degranulation capacity was limited but virtually complete after such treatment. We presume that the refractoriness is induced by 48/80 which is bound to the cells during regeneration and detaches after trypsinisation.

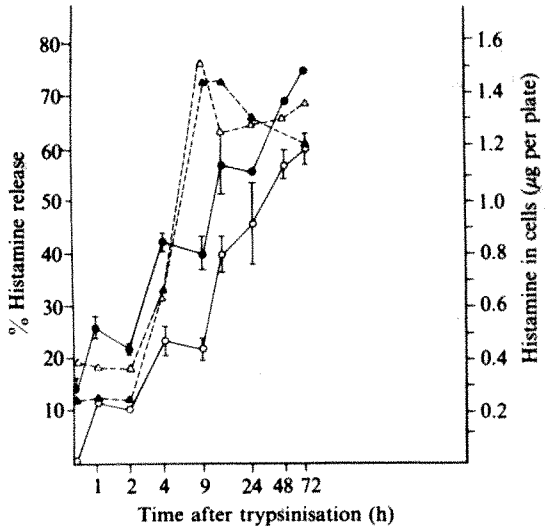


Fig. 3 Restoration of the degranulation capacity after passaging by trypsinisation and plating in 48/80-free medium. Culture was established by plating 10^7 lymph node cells prepared from 4-month-old BALB/c mice 14 d after last injection with horse serum. Culture was passaged 8 d later to make three new plates from each old plate. Seven days later, 40 μg 48/80 in 2 ml Dulbecco's medium were added to half the plates while the other half received fresh medium only. 65% of the histamine was released. The cultures were incubated further for 3 d. Then the medium was removed and trypsin solution (0.3% in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline) was added. After 20 min the cells were collected, spun down, resuspended in Dulbecco's medium and horse serum and plated in 35-mm Petri dishes to make 3.5 new plates from each old plate. At different intervals, the medium was removed and 1 ml medium containing 15 μg 48/80 was added. After 20 min the plates were processed for assay of histamine. % Histamine release: ○——○ 3 d with 48/80; ●——● 3 d without 48/80. Histamine in cells (μg): ▲----▲ 3 d with 48/80; △----△ 3 d without 48/80.

This work has revealed hitherto unknown properties of the mast cell. The new information is important in view of its role in allergy. It is clear that in the continuous presence of the histamine releaser 48/80, the cell is reversibly desensitised, an effect which possibly comes under the heading of tachyphylaxis—development of tolerance to second exposure to a drug or an agonist[6,7]. Obviously, it is still to be seen whether there is a similar response to reaginic antibodies and the corresponding allergens. Unfortunately, attempts to maintain mast-cell cultures in the continuous presence of mouse anti-horse serum plus horse serum as antigen, in dilutions optimal for total degranulation proved unsuccessful. This was probably because of the stimulation of a high metabolic rate with effects on the medium *p*H. A similar effect was obtained when such antibody–antigen complexes were added to fibroblast and macrophage cultures. This observation indicates recourse to classes and subclasses of purified immunoglobulin; thus, application of pure IgE, IgG_1, IgG_{2a} and IgG_{2b} to the different cells grown in our cultures would make it possible to assign the *in vitro* manifestation to its cause. This is important, as while both IgE and IgG_1 degranulate mast cells, they neither fix complement nor opsonise macrophages; by contrast, IgG_{2a} fixes complement and attaches to the Fc receptor on the macrophage[8].

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Orderly compression of the retinotectal projection following partial tectal ablation in the newborn hamster

MANY hypotheses have been proposed to account for the development of the exquisitely specific systems of neuronal connections in the brain. Axons might read chemical labels on their target cells, attaching only to cells with a matching label[1]; axon and target cell populations might arrange a match without cell-to-cell specificity, using such mechanisms as differential advantage in competition for terminal space[2,3]; or some feature of the development and maturation of an axon and target system might produce ordered patterns of connectivity. The principal model system used to test these hypotheses has been the retinotectal projection of animals with nervous systems which are capable of regeneration, although it is not known whether this system in fact behaves like a developing nervous system. After removal of half the tectum in goldfish or frog, for example, an entire retina will compress its representation uniformly onto the remaining half tectum[4,5]. We have repeated this experiment in the developing mammalian brain, and have found that following a partial tectal ablation in a neonatal hamster, the projection from the retina to the superior colliculus will terminate in an

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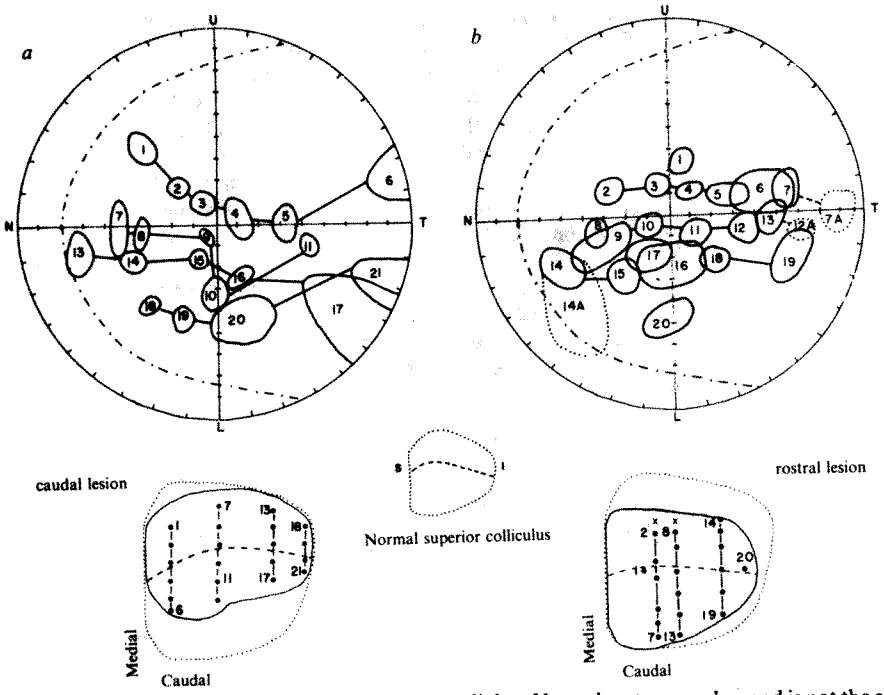


Fig. 1 Colliculi with reconstructed electrode penetrations and associated visual fields from a hamster with a neonatal lesion of the right caudal colliculus (*a*) and of the right rostral colliculus (*b*). The colliculi are shown in dorsal view; solid outlines indicate the remaining superficial gray layer in each hamster and dotted lines the normal extent of the superior colliculus. Electrode penetrations associated with receptive fields with defined borders are represented by black dots, and their corresponding numbered visual receptive fields are shown in the visual field diagram above. For visual convenience rostral to caudal series of penetrations and corresponding nasal-to-temporal visual receptive field are connected with lines. Medial colliculus, where upper visual field is represented, was not explored because of occlusion by the vein of Galen. The two penetrations marked by crosses were associated with diffuse receptive fields in the upper nasal quadrant including, but not limited to, the receptive field with the appropriate topographical position. The map of the visual field is centred about the projection of the optic disc. The broken line (- · - · -) indicates the perimeter of the visual field in normal hamsters. The nasal (N) to temporal (T) axis is determined by the projections of the attachments of the medial and lateral rectus muscles, and is not the same as the horizontal plane defined by gravity. The orthogonal upper (U) to lower (L) axis is defined by the insertions of the superior and inferior rectus muscles. This superior–inferior axis, which divides the visual field into nasal and temporal halves, is shown projected on the surface of a normal superior colliculus (centre inset) and both damaged colliculi as a dashed line (– – –). Dotted receptive fields indicate fields found deep to the surface of the superior colliculus.

orderly way in a smaller than normal tectal volume, as in goldfish and frog[1-4]. However, we have found preferential representation of nasal visual field which has not been reported in studies of non-mammalian animals in which the optic tract axons can regenerate. This may suggest some fundamental differences in developing and regenerating systems; in any case, it poses new problems for theories of neuronal specificity in developmental neurobiology.

On the day of birth, the brain of the hamster is quite immature. The first fibres from the retina have grown to the superior colliculus, entering the structure at its rostral border[6]. Although some axonal end arbors may already have begun to form near the tectal surface, major arborisation seems to begin around day 3, with the caudal, medial and lateral margins lagging behind rostral and central colliculus. An adult-like pattern and density is found by the age of 12–14 d. Partial lesions made in the colliculus on the day of birth thus intercept the initial stage of tectal innervation, and major reorganisations of retinal projection patterns do occur[7-9].

The superior colliculus is a prominent structure in the newborn hamster, and its superficial layers may be destroyed directly through the thin cranium by local application of heat[7-9]. In 15 newborn hamsters partial lesions of the colliculus were inflicted on the day of birth by this method. After surgery, the animals were returned to the mother and allowed to mature normally. When these animals had reached the age of at least 2 months, the topography of the retinotectal projection was assessed electrophysiologically[10,11], and the extent of the neonatal lesion was determined thereafter by conventional histological reconstruction[7-11]. In the 15 animals studied, the surface area of the remaining intact superior colliculus ranged from 40% to 80% normal, and tissue loss was confined principally to the superficial gray layer, as determined from sectioned material.

The receptive fields associated with a series of electrode penetrations of the surface of the superior colliculus in a hamster with a neonatal lesion of the caudal part of the superficial layer are shown in Fig. 1*a*. The entire visual field was represented at the tectal surface. Multiunit receptive field sizes reflected this compression of the visual field onto a smaller tectum. The average receptive field size in a normal hamster is $8.6 \pm 0.3°$ (s.e. mean); in animals with caudal lesions average field size was $12.4 \pm 0.4°$. The topography was generally orderly, although localised mislocations or inversions were observed in this animal, as in every other. One striking anomaly was present in this and similarly prepared cases: the representation of the nasal visual field on the collicular surface was proportionately much greater than the representation of the temporal visual field (compare the positions of the superior–inferior meridian in the normal with the caudal lesion case in Fig. 1). The rostral-to-caudal extent of the nasal 70° of visual field was close to normal (75% normal length, as measured along the naso–temporal meridian). For the temporal 90° of visual field, the representation along the same meridian was only 48% of normal. This disparity in the rostro-caudal extent allotted to the nasal as opposed to the temporal visual field was present in all eight animals with caudal lesions. In three of the animals, in addition to the general compression, there was loss of 10–30° of visual field in the temporal periphery.

Lesions of rostral colliculus were made in a further seven newborn hamsters. The consequences of this lesion for axons entering the colliculus are somewhat different than the caudal lesion: axons entering the tectum must traverse an area of damaged tissue before innervating the remaining superficial gray layer. The mechanical disruption caused by this procedure may have contributed to a pattern of disorder in retinotopy observed in regions on the rostral border of the remaining superficial gray in four of the seven cases, an anomaly which has also been observed neuroanatomically[7-9]. Receptive fields of units at the rostral border were large, encompassing whole quadrants or hemifields and were topographically disorderly, both in relation to neighbouring penetrations and throughout the depth of any particular penetration.

The compressed representation of the visual field observed after day-of-birth lesions of the rostral tectum was considerably more like normal in representation of nasal and temporal visual field than was observed in cases with lesions of caudal tectum. Nevertheless, when asymmetries occurred (three of the seven cases exceeded a 5% difference in representation of nasal visual field versus temporal visual field when compared to normal), nasal visual field was again preferentially represented. In Fig. 1*b*, the rostrocaudal extent of colliculus devoted to the remaining nasal field was 95% of normal; the extent devoted to temporal visual field was 70% of normal. The preferential representation

was never so extreme as that observed after caudal lesions. In no case, however, was there marked compression of the representation of nasal field relative to temporal field, even though the normal tectal terminal area for axons of retinal cells representing nasal visual field had been removed at birth.

An additional deviation from the normal representation of the retina in the tectum was observed in 12 of the 15 animals studied: extreme temporal visual field was represented both at the tectal surface and also deep to the tectal surface, lying directly ventral to receptive fields representing more nasal areas. In some cases, extreme temporal field could be found represented only deep below the tectal surface (Fig. 1*b*, dotted fields). This corresponds to an anatomical pattern observed previously[7–9]; in adult hamsters which had suffered caudal tectal lesions at birth, lesions of the nasal retina elicited axon terminal degeneration in deep superficial gray and in the upper part of intermediate gray in the caudal part of the remaining colliculus. These observations indicate an orderly topographic representation of the retina perpendicular to the surface of the superior colliculus which is never found in normal animals[10,11]. Factors such as inappropriate angles of electrode penetration and reorganisation of collicular lamination, investigated thoroughly, could not account for these results[7–10].

We do not know if the nonlinear compression of the visual field in the hamsters represents the stable end point of a process of compression, or a fixation of the map by maturation before a stable end point has been reached. In either case, the priority of nasal field representation in these compressed maps is an interesting clue to the mechanisms of map formation. The two most likely sources of this priority are features of development: the direction of retinal fibre entrance to the tectum from the rostrolateral quadrant where nasal field is represented, and the sequence of maturation of retinal ganglion cells in hamster retina. We are presently investigating these two possibilities.

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A rich VIP nerve supply is characteristic of sphincters

VASOACTIVE INTESTINAL PEPTIDE (VIP), isolated from extracts of porcine duodenum by Said and Mutt[1], was at first thought to be a hormone[2]. Immunohistochemical studies have since revealed that VIP has a neuronal localisation. VIP-containing nerves occur throughout the body, being particularly

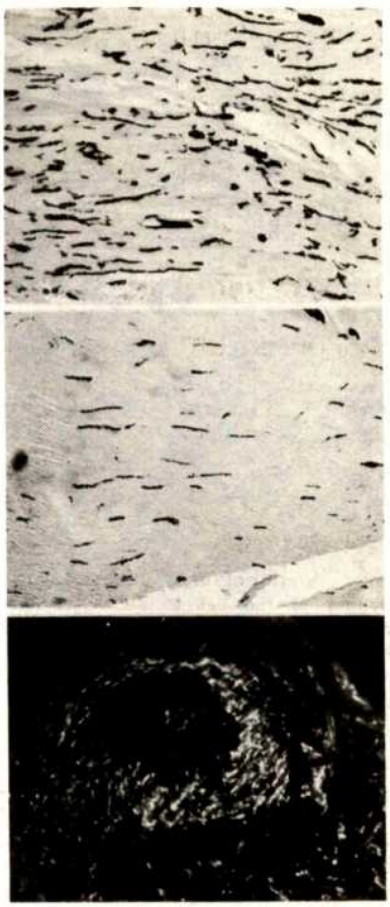


Fig. 1 VIP-immunoreactive nerves (PAP staining) in smooth muscle of the feline pyloric sphincter (top) and duodenum (middle). Note the aggregation of coarse nerves in the sphincter region compared with the much lower number of immunoreactive nerves in the duodenum (×250). Bottom, VIP nerves (immunofluorescence) around the opening of the pancreatic duct in the papilla of Vater (×150).

frequent in the digestive tract[3–7], the genitourinary tract[8–10] and the upper respiratory tract[11]. The nerves storing VIP or other neuropeptides (substance P, somatostatin and enkephalin) apparently represent additional types of autonomic nerves, distinct from the adrenergic and cholinergic ones. A great proportion or the peptidergic nerves seems to originate in nerve cell bodies located close to or within the innervated organ[5–9,11]. The physiological significance of these new types of nerves is a matter of speculation, but a knowledge of their precise anatomical distribution will assist in defining their targets. We have previously observed that structures believed to exert a sphincter function receive a particularly rich supply of such nerves. We have now examined several sphincters, recognised or anticipated, and have established the presence of VIP nerves in all of them.

For chemical analysis specimens were taken from the mid and lower portion of the oesophagus, the stomach (cardia, antrum and pylorus) and the upper duodenum of seven cats of either sex. The material was immediately frozen and stored at −20 °C until extraction in acidified ethanol and analysis by radioimmunoassay[12]. For histochemistry material was collected from the above mentioned locations and from the sphincter of Oddi and the papilla of Vater from three cats. In addition, we collected specimens from the mid and lower portion of the ureter, from the bladder–urethra connection and from the bladder wall. From three male cats specimens were taken from the prostate, urethra and urethral collicles. The specimens were frozen to the temperature of liquid nitrogen in a propane–propylene mixture and freeze-dried. They were then exposed to formaldehyde gas at +80 °C for 1 h (ref. 13) or to diethylpyrocarbonate vapour at

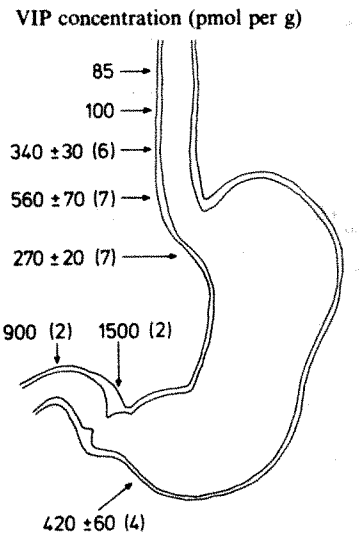


Fig. 2 Schematic drawing of the lower oesophagus, stomach and upper duodenum of the cat to show the distribution of immunoreactive VIP, measured by radioimmunoassay. Single determinations if not otherwise stated. The values for oesophagus and cardia refer to the whole wall, the values for antrum, pyloric sphincter and duodenum refer to smooth muscle only. Mean values ± s.e. (n).

+55 °C for 3 h (ref. 14) and embedded in paraffin. Sections were cut at 5 μm and subjected to the indirect immunofluorescence method[15] of the PAP staining technique of Sternberger[16] for the demonstration of VIP, substance P or Leu-enkephalin. The VIP antiserum (code no. 5603) used in the radioimmunoassay was also used for immunostaining; it has been characterised in detail elsewhere[12,17]. (For details on the use of the VIP, substance P or Leu-enkephalin antisera see refs 4, 18, 19.) The VIP antiserum was used in a dilution of 1:80 (immunofluorescence) or 1:5,120 (PAP staining), the corresponding values for the substance P antiserum dilutions were 1:40 or 1:160 and for the Leu-enkephalin antiserum 1:60 or 1:240. Controls were sections incubated with antiserum inactivated by the addition of excess antigen (pure porcine VIP, synthetic substance P or Leu-enkephalin; 10 μg per ml diluted antiserum). Adrenergic nerves were demonstrated microscopically by fluorescence in the formaldehyde-fixed material[13].

The region of the oesophago-gastric junction, pyloric sphincter, sphincter of Oddi and papilla of Vater received a very dense VIP nerve supply, more so than the smooth muscle of adjacent regions (Fig. 1). Immunochemical assay disclosed much higher VIP concentrations in the lower oesophageal and pyloric sphincter regions than in adjacent regions (Fig. 2). The openings of the ureters and of the urethra into the trigonum area of the bladder received a particularly rich supply of VIP nerves (see also refs 8, 9). By comparison, the body of the bladder and the ureters were sparsely innervated. Also, the smooth muscle surrounding the openings of the vasa deferentia and the prostatic ducts into the urethra were richly supplied with VIP nerves. In addition to VIP nerves the sphincter regions received adrenergic nerves and nerves displaying substance P (see also ref. 18) or enkephalin immunoreactivity. There were many enkephalin nerves in the lower oesophageal sphincter region and in the pyloric sphincter but not more than in adjacent regions; they were very few in the sphincter regions of the genitourinary tract. Acetylcholinesterase-positive nerves are very numerous in the sphincter of Oddi[20], whereas there are few adrenergic nerves in sphincters of the feline gut[21]. Generally, such nerves are not more numerous in sphincter regions than in adjacent regions. Subsequently, we have also found VIP-immunoreactive nerves to be comparatively numerous in sphincters of the guinea pig and rat. On the whole, however, the number of VIP nerves in smooth muscle is lower in these species than in the cat. In the rat immunoreactive-VIP nerves were poorly demonstrated by antiserum 5603, but were easily demonstrated by another VIP antiserum (code no. 98P).

Thus, in the cat a very rich supply of VIP nerves is consistently found in the smooth muscle of all recognised sphincters and in some anticipated sphincters. We propose that a rich local supply of VIP nerves is characteristic of smooth muscle sphincters to the extent that an evaluation of the density of VIP innervation may assist in anatomically defining a sphincter. From what is known of the action of VIP on smooth muscle it seems likely that VIP is involved in sphincter relaxation[22].

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Increased sensitivity to (+)amphetamine self-administered by rats following meso-cortico-limbic dopamine neurone destruction

DRUG self-administration (SA) in laboratory animals shows some of the characteristics of intracranial self-stimulation and of other natural reinforcers (for review see ref. 1). Animals will self-administer many of the same drugs abused by human subjects. Recent evidence has shown that (+)amphetamine reinforces behaviour in the same manner as conventional reinforcers[1–3] that it acts mainly on dopamine (DA) transmission, and that locomotor activation and stereotypy are mediated by the mesolimbic–mesocortical (A10 group) and nigro-striatal (A9 group) DA systems, respectively[4,5]. Using various specific noradrenergic and dopaminergic blocking agents in a (+)amphetamine SA retention paradigm, DA transmission blockade was shown to produce effects similar to reward reduction and reward termination[3,6,7]. DA transmission in the frontal-mesolimbic system has been implicated in brain stimulation

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Table 1 Effects of (+)amphetamine injections (2 mg per kg, i.p.) on the locomotor activity of sham-operated rats and rats with VMT–A10 lesions

Groups	Locomotor activity (counts)			Time for maximal activation (min)
	1st 10 min	1st h	2nd h	
Control $N=6$	131±24	1,771±225	1,178±177	43±5
VMT–A10 $N=8$	291±38*	1,283±100	496±101*	12.5±1.6*

* The locomotor activity was measured in a circular corridor with photocell beams. The results are presented for each rat as the difference between (+)amphetamine and solvent scores.

reward[8], and the link between self-administration of (+)amphetamine and reward mechanisms, both of which depend on DA transmission, may be related to the craving and early dependence that certain subjects display for addictive drugs. However, the mechanisms which lead to addiction are still unclear, and it is possible that in drug addicts the particularly strong and rapid addicting effect of the drug could be due to a pre-existing imbalance in some homeostatic mechanism. Thus, the use of healthy animals in a steady-state paradigm is not necessarily a good model for the study of the psychological or neurobiological vulnerability to drug addiction acquisition. Because the meso-cortico-limbic DA system may be an essential target for psychostimulant drugs, the lesion of this system could induce disruption of SA behaviour.

We report here that lesions of the ventral mesencephalic tegmentum (VMT) in the area of the meso-cortico-limbic DA cells (A10) induces paradoxically fast acquisition of intravenous self-administration of (+)amphetamine and also greater consumption of this drug.

Of 50 male adult Sprague–Dawley rats anaesthetised with ketamine, 30 received bilateral (±0.4 mm) radiofrequency lesions of the VMT–A10 area surrounding the interpeduncular nucleus and in the remaining 20 rats (controls) the electrode was lowered 1 mm above the VMT area, and no current was passed. One month later, a silastic cannula, filled with a polyvinylpyrollidone solution to prevent blood coagulation in the tubing, was chronically implanted in the jugular vein. The cannula was fixed with a stainless-steel connector on the calvarium. Three days later the SA experiments began. The rats were placed in an operant conditioning chamber with two levers situated at opposite sides of the cage. Depression of one lever activated a syringe pump that delivered 7.5 μg per kg of (+)amphetamine in 2.5 μl in 2 s for each press on a CRF schedule of reinforcement; depression of the other lever resulted in the same noises but led to no infusion. Each session (one every 2 d) lasted 12 h, from 1900 to 0700. The first session served as an operant control, during which the pump was disconnected, and the second and third sessions were the first and second (+)amphetamine sessions, respectively, and in the fourth session the (+)amphetamine was replaced by saline. Each rat was then killed under deep anaesthesia, the brain removed and the lesions examined histologically. Three rats with lesions considered to be unilateral were eliminated from data analysis. The lesions destroyed an area surrounding the interpeduncular nucleus and sometimes reached the medial part of the substantia nigra laterally and the ventral part of the nucleus ruber dorsally. Figure 1 inset shows a typical lesion.

The results are represented in Fig. 2. A two-factor analysis of variance was computed: lesioned against controls (A): $F=53.7$, $P<0.001$; (+)amphetamine against operant-saline (B): $F=49.05$, $P<0.001$; $A\times B$ interaction: $F=17.55$, $P<0.001$. The following remarks can be made. (1) There was no significant difference between the score of the two groups on either the operant or the saline session. (2) In the first (+)amphetamine session, the lesioned group's score was markedly greater than that of the control group ($t=6.1$, $P<0.001$), and different from that of its operant session ($t=10.8$, $P<0.001$). There was a small but significant difference between the control group's score on the first (+)amphetamine session and on their operant session ($t=2.05$, $P<0.05$). (3) The second (+)amphetamine session confirmed the preceding results for the experimental group. The control group's drug session score differed statistically from that of its operant session ($t=3.1$, $P<0.001$). (4) The mean presses on the control lever (for the four respective sessions) were: control group, 8±1, 10±4, 13±5, 9±2; VMT–A10 group, 7.5±1, 18±5, 17±7, 10±3. Typical acquisition and retention scores for a lesioned rat and a control rat are represented in Fig. 1, which shows that lever pressing for (+)amphetamine in the lesioned rat evolved dramatically, with an extreme sensitivity to the drug and enhanced acquisition of the operant response for (+)amphetamine.

The method used here was unusual because it focused on the acquisition of responding, a paradigm which may be particularly good for revealing pre-existing vulnerability to the drug. For this reason, a very small amount of the drug was available for each lever press, permitting only a low blood concentration at the beginning of the session. As the control operant behaviour scores of the two groups were identical, we can conclude that a different brain sensitivity to the drug determined the differing subsequent acquisition time courses. A lowered reactivity to (+)amphetamine in the VMT–A10 group can also be excluded as decreased sensitivity would contradict the rapid acquisition at very low blood concentration. The second clear modification of (+)amphetamine SA induced by VMT lesion is the intake of greater amounts of the drug. This may reflect a pathological need for the drug and a requirement for a higher blood concentration to obtain an identical 'rewarding' effect[3,10,11]. These results must be compared with the relatively low score of the control group for the first (+)amphetamine session. In contrast to the gradual acquisition in control rats, the lesioned group acquired the response in less than 1 h, often less than 30 min and in some cases less than 15 min. Dugthis time the total amount of the drug infused was less than 0.5 mg per kg, allowing us to eliminate motor preservation and stereotypy artefacts often observed in SA experiments[12]. Moreover, although the number of presses on the control lever during the (+)amphetamine session was slightly enhanced, the animals did not show any apparent stereotypy.

The results are paradoxical, as previous data have shown that the blockade of DA release and re-uptake by (+)amphetamine are prerequisites for (+)amphetamine SA[3,6,7]. Consequently, destruction of meso-cortico-limbic DA neurones, suspected to maintain (+)amphetamine reinforced behaviour, should have led to a disappearance of SA acquisition. To clarify this paradox we tested the reactivity and sensitivity to (+)amphetamine after

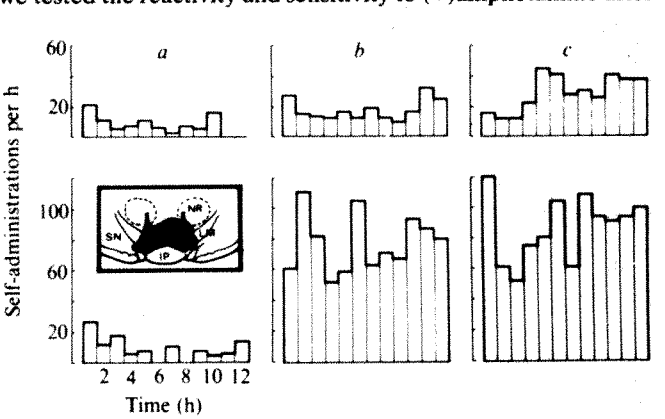


Fig. 1 Typical self-administration behaviour (0.75 mg per kg (+)amphetamine per 100 administrations) of a control rat (rat 5314, upper histograms) and a rat with bilateral VMT–A10 lesions (rat 5340, lower histograms). The lesioned area represented is typical of the lesions of the experimental group (IP, interpeduncular nucleus; SN, substantia nigra; NR, nucleus ruber; LM, lemiscus medianus). *a*, Operant session; *b*, first (+)amphetamine session; *c*, second (+)amphetamine session.

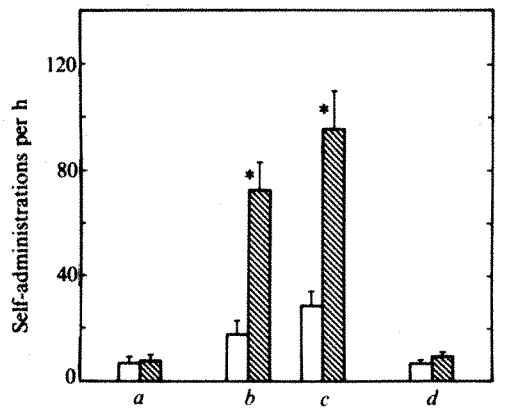


Fig. 2 (+)Amphetamine self-administration (0.75 mg per kg (+)amphetamine per 100 administrations) in rats after radiofrequency lesions of the dopaminergic meso-cortico-limbic (A10) cell group lying in the ventral mesencephalic tegmentum (VMT) surrounding the interpeduncular nucleus (lesioned against controls: * $P < 0.001$, Student's t-test). *a*, Operant session; *b*, first (+)amphetamine session; *c*, second (+)amphetamine session; *d*, saline. □, Controls; $n = 20$; ▨ VMT–A10 lesioned; $n = 27$.

VMT–A10 lesioning. Eight VMT–A10-lesioned and six sham-operated rats, with no experience of the drug, received 2 mg per kg (+)amphetamine intraperitoneally (i.p.), and behavioural activation was measured in a circular corridor. Table 1 indicates that the lesion induced a 'hyper-reactivity' to (+)amphetamine, observed immediately after the injection. The maximal effect of the drug occurred at 12.5 min and 43 min, respectively, in the lesioned and control groups. However, it is also clear that the lesioned rats were 'hyposensitive' to the drug as the behavioural activation was observed for only 1 h in the lesioned group but lasted more than 2 h in the control group.

This second experiment may explain the SA behaviour of VMT–A10-lesioned animals. The hyper-reactive phase to (+)amphetamine could explain the rapid acquisition of SA, and the decreased time course could account for the high response rate (to maintain the rewarding effects of the drug). Our results clearly indicate that (+)amphetamine-induced behavioural activation is not completely blocked by our radiofrequency lesion of the VMT–A10 area, in contrast to the blockade obtained after massive destruction of DA–A10 terminals induced by 6-hydroxydopamine (6-OHDA) administration[13]. Therefore, it is probable that some DA neurones involved in (+)amphetamine-induced behavioural activation are still intact, that they are hyperactive, and that they react more strongly to (+)amphetamine but for a much shorter time. This hypothesis could explain the discrepancy between our results and previous data, which have shown that SA of psychostimulant drugs such as (+)amphetamine and cocaine are dependent on DA mechanisms; in these studies SA is suppressed by blockade of DA activity either by injecting a specific DA-receptor blocker[3,6,7] or by total 6-OHDA destruction of DA-A10 innervation[14]. The hypothesis of incomplete destruction of DA neurones to explain (+)amphetamine SA effects could also explain the discrepancy between the behavioural syndrome induced by destruction of DA–A10 neurones after 6-OHDA injection into the terminal areas[5,13] and the syndrome induced by lesion of the DA cell bodies. Radiofrequency lesion or local infusion of small amounts of 6-OHDA into the VMT–A10 area elicit a permanent behavioural perturbation characterised by locomotor hyperactivity and disruption of complex behaviour[9,15–17]. This syndrome is correlated with depletion of DA but not of serotonin or noradrenaline[18], and it may be due to a pathological state produced by incomplete destruction of VMT–DA neurones and overactivity of the remaining DA neurones. The syndrome is antagonised by chronic (+)amphetamine treatment or by very low acute doses of apomorphine[19]. These drugs shift the activity of the lesioned rats towards lower, normal levels. The possible existence of DA receptors supersensitivity involved in reward mechanisms could explain the increased nucleus accumbens self-stimulation scores induced by radiofrequency lesion of the VMT area[20], while other studies using different lesion techniques demonstrated a decrease of this behaviour[21]. Our results may thus help in understanding the psychopathological disturbance which leads to drug addiction.

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Extracellular Ca^{2+} and excitation–contraction coupling

THE role of external Ca^{2+} in excitation–contraction coupling has been controversial since Sandow[1] put forward the idea that contraction is initiated by an influx of Ca^{2+} on depolarisation. In the very thin lamellae (1 μm) of *Branchiostoma* myotome muscle 'Ca^{2+}-free' solutions with 1 mM EGTA block twitches completely[2,3]. It was concluded that the Ca^{2+} influx during the action potential directly activates the myofilaments[2]. In frog muscle twitches remain unchanged in 1 mM $EGTA^{2-}$ (refs 4, 5). To ensure that $[Ca^{2+}]$ is sufficiently reduced in the narrow transverse tubular (T) system Barrett and Barrett[6] raised the Ca^{2+} buffer concentration to 80 mM and observed that action potentials were no longer followed by twitches. In this letter we demonstrate that some further changes in the composition of the external fluids result in the restoration of contractions in both *Branchiostoma* and frog muscle indicating that a Ca^{2+} influx is not necessary for the initiation of contraction.

Single muscle fibres from the M. semitendinosus of the frog (*Rana temporaria*) were transferred into a Perspex chamber[7] which allowed a quick exchange of solutions. Isometric contraction was measured with a force transducer[5]. To prevent a decrease in resting potential[5,6] and an increase in external surface charge density[8] at high EGTA concentrations, the solution included 5 mM free Mg^{2+}. The increase in ionic strength and osmolarity had no appreciable effect on the fibres. Figure 1 shows that in 80 mM free EGTA ($[Ca^{2+}] < 10^{-10}$ M) twitch

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height decreased to 30% after 2 min. It then declined more slowly, reaching 5% after 2 h. Addition of 15 mM NaSCN caused a considerable recovery up to 30% of the initial level. The early fall in twitch force is probably due to an accelerated onset of inactivation, as in similar conditions the reduction in free Ca by as little as 2.5 mM $EGTA^{2-}$, in the presence of Mg^{2+}, causes a shift of the steady-state inactivation curve by 20–30 mV towards more negative potentials[5]. Our present finding that twitches did not disappear completely after a few minutes[6] might be due to the presence of Mg^{2+}, which prevents an increase in external surface charge density and thus a further shift of the inactivation curve beyond the resting potential. The continuous slow decrease in twitch force to very low values after the early fall might then be explained by assuming that (1) the final concentration of $EGTA^{2-}$ in some part of the T-system is reached very slowly, (2) a continuous loss of Ca^{2+} from the muscle cell causes a depletion of internal Ca-stores, or (3) $EGTA^{2-}$ has deleterious effects on the T-system.

However, the assumption that $[Ca^{2+}]$ in the T-system decreases only very slowly below the myoplasmic Ca^{2+} concentration[11] ($\sim 10^{-7}$ M) is very unlikely considering the SCN^- effect. This anion, which should not alter tubular free [Ca], causes a remarkable recovery of the twitch amplitude. It is known to shift the activation threshold to more negative potentials[12] without causing a corresponding shift in inactivation (our unpublished observations). This strongly suggests that a passive influx of Ca^{2+} during membrane depolarisation is not a prerequisite for activation.

The possibility that loss of internal Ca^{2+} and/or secondary effects are mainly responsible for the slow decline in force under high EGTA is also unlikely, as shown by the nearly complete recovery from $EGTA^{2-}$ application and from the tetanus results. In the experiment shown in Fig. 1 tetanus force fell to 95% and 23% after 40 and 120 min, respectively, but rose again to 68% in SCN^-. The tetanus/twitch ratio increased from 2 : 1 in normal conditions to about 10 : 1 in high EGTA. In one of the 11 experiments it reached 55 : 1. The increase in tetanus/twitch ratio shows that the coupling efficiency improves during repetitive activity, probably by a 'normalisation' of the inactivation kinetics[5]. In this context it should be noted that the coupling process improves, although internal $[Ca^{2+}]$ and thus the outwardly directed driving force for calcium ions increases. Thus it is likely that a further acceleration of inactivation is mainly responsible for the slow decline in twitch force in high EGTA.

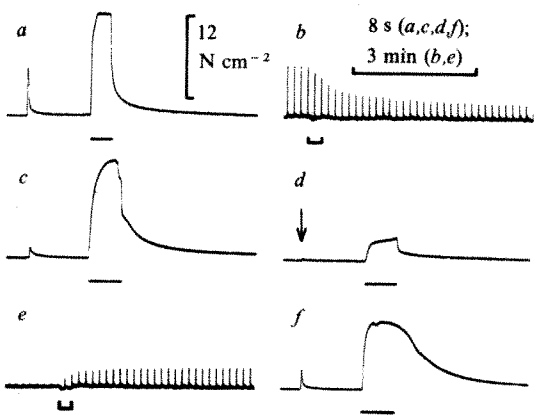
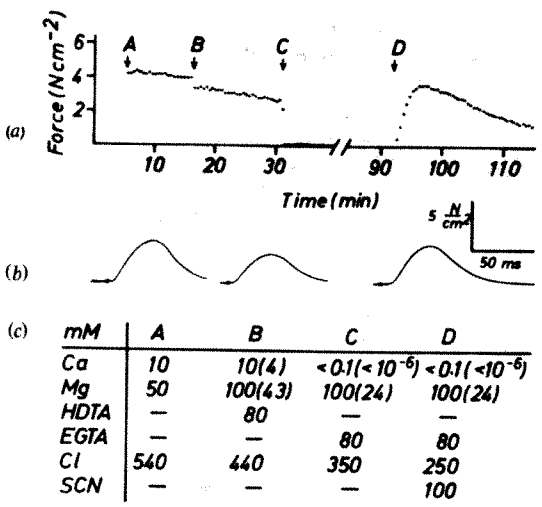


Fig. 1 Effect of high EGTA and SCN^- on twitches and tetani (50 Hz; indicated by horizontal bars) of an isolated skeletal muscle fibre (diameter 130 μm). *a*, Ringer solution with 1.8 mM Ca^{2+}; *b*, change from modified Ringer (see below) to a solution with 80 mM free EGTA ($[Ca^{2+}] \leq 10^{-10}$ M) and 5 mM free Mg (96.2 mM total EGTA and 21.2 mM total Mg). The free concentrations were calculated as described by Portzehl, Caldwell and Rüegg[9] using the absolute binding constants given by Sillén and Martell[10] and assuming a Ca^{2+} contamination of 10^{-2} mM. *c* and *d*, Twitches and tetani after 40 and 120 min in high EGTA, respectively. *e*, Change from high EGTA to high EGTA plus 15 mM SCN^-, *f*, after 6 min in the latter solution. The brackets show the time needed for the exchange of solutions. In order to avoid a transient depolarisation beyond the contraction threshold after the replacement of NaCl in Ringer by Na–EGTA we exchanged 50 mM NaCl of Ringer by 50 mM Na propionate 10 min before application of $EGTA^{2-}$. All solutions contained 2.5 mM KCl and 5 mM $MOPS^-$ as buffer, $pH = 7.0$, $T = 22$–23 °C.



(c)

mM	A	B	C	D
Ca	10	10(4)	$<0.1(<10^{-6})$	$<0.1(<10^{-6})$
Mg	50	100(43)	100(24)	100(24)
HDTA	—	80	—	—
EGTA	—	—	80	80
Cl	540	440	350	250
SCN	—	—	—	100

Fig. 2 Effect of high EGTA and SCN^- on twitch amplitude of a myotome strip from *Branchiostoma*. Diameter 170 μm. Stimulating frequency 2 per min. *a*, Twitch amplitude in different solutions. *b*, Time course of single twitches. *c*, Composition of solutions in order of application (mM) with free concentrations of Ca and Mg in brackets. All solutions contained in addition (in mM): Na^+ 400, K^+ 10 and Tris (as buffer) 10, $pH = 8.0$; $T = 10$ °C. Since 80 mM EGTA replaces a considerable amount of Cl^-, HDTA (hexamethylenediamine*N,N,N',N'*-tetra-acetic acid) was added before application of EGTA to moderate the depolarising action of Cl^- reduction. This substance has a comparable binding characteristics for Mg^{2+} but possesses a 10^6 times smaller absolute binding constant for Ca^{2+} than EGTA. Because very low concentrations of free Mg led to spontaneous twitches and contractures in Ca^{2+}-free solutions a drastic reduction in free Mg was prevented in sol. *B–D* by increasing the total concentration to 100 mM. A large part of Mg^{2+} was reversibly bound to EGTA, thus reducing free EGTA but not its capacity to buffer $[Ca^{2+}]$ below 10^{-9} M.

For experiments with myotomes of *Branchiostoma lanceolatum* muscle strips, 1.5 mm long and 0.1–0.4 mm thick, were dissected and isometric twitches were elicited by short supramaximal current pulses. Figure 2 shows twitch amplitude in solutions of different composition. The pretreatment with HDTA (see legend to Fig. 2) caused a small decrease in twitch height, which might be due to the concurrent decrease in free calcium. When, however, external $[Ca^{2+}]$ was drastically reduced by high EGTA (solution *C*) twitch amplitude fell to very low values within 1 min. After 1 h, when twitches were no longer visible, 100 mM Cl^- was replaced by SCN^- (solution *D*). Subsequently twitch height increased almost to the original value within a few minutes. Thereafter, force gradually declined again, reaching 1/3 after 30 min.

Apart from a prolongation in latency time by about 100%, the time course of twitches in $EGTA^{2-} + SCN^-$ was very similar to the control twitches (Fig. 2*b*). This again suggests that SCN^- simply strengthens the natural coupling process.

In our experiments we used 80 mM $EGTA^{2-}$ to reduce the concentration of free Ca^{2+} below 10^{-10} M. Even if we assume that the actual Ca^{2+} concentration in the T-system or extracellular clefts remains 2–3 orders of magnitude higher than in the bulk solution it still should be below that measured in normal conditions in the myoplasm. In agreement with recent conclusions drawn from experiments with the calcium indicator dye arsenazo III[13] we therefore believe that an influx of Ca^{2+} is not a prerequisite for the activation of contraction. Models[14] which directly combine the Ca^{2+} release from the sarcoplasmic

reticulum with the membrane potential of the T-tubular membrane seem more appropriate to explain excitation–contraction coupling.

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Parallel regulation of acetylcholinesterase and pseudocholinesterase in normal, denervated and dystrophic chicken skeletal muscle

IN vertebrate skeletal muscle acetylcholinesterase (AChE, EC 3.1.1.7) is usually considered to be involved in the termination of impulse transmission by hydrolysis of acetylcholine[1]. AChE is often accompanied by a second enzyme[2], pseudocholinesterase (ψChE, EC 3.1.1.8), which differs from AChE in substrate specificity and in susceptibility to anticholinesterases[3,4]. No role comparable with that of AChE in synaptic transmission is known for ψChE, despite its abundance at some sites and its occurrence (as shown histochemically) with AChE in various muscle endplates[1,5]. It has been suggested that ψChE is a precursor of AChE in the superior cervical ganglion[6,7]. Moreover, in ganglia and skeletal muscle of rats, forms of ψChE have been demonstrated[8] homologous with forms of AChE observed previously[9–11]. Elevated levels of AChE have been demonstrated in aqueous extracts of fast-twitch muscles of dystrophic as compared with normal chickens[12,13]; ψChE levels increased similarly[13], and also in such extracts of denervated muscles of normal chickens[14]. We show here that the total amounts and forms of AChE change in parallel with those of ψChE during development of skeletal muscles of both normal and dystrophic chickens, as well as on denervation of normal muscle. Our data strongly indicate that in these tissues the two enzymes are controlled by a common regulatory mechanism.

It has been reported[12,15] that embryonic chick muscle has high AChE concentrations which fall during maturation, but those studies used conditions of no or low salt or detergent, which are now known[8,11,16,17] to give low AChE extraction. We have homogenised the tissues in 1% Triton X-100/1 M NaCl, shown[17] to extract 95–100% of the total muscle AChE, and find (Table 1) that in the pectoral muscle of normal chickens AChE

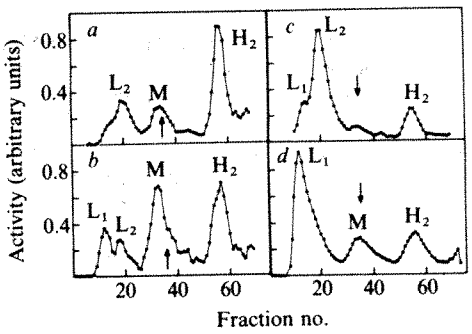


Fig. 1 Sucrose gradient centrifugation profiles of AChE and ψChE activity in extracts of pectoral muscle of normal (line 412) and dystrophic (line 413) chickens. *a*, AChE in muscle of a normal 19-d embryo; *b*, ψChE in muscle of a normal 19-d embryo; *c*, AChE in muscle from a 32-d dystrophic chicken; *d*, ψChE in muscle from a 32-d dystrophic chicken. Centrifugation was carried out on 5–20% sucrose gradients as described previously[19], using extracts obtained as described in Table 1. AChE was assayed using 0.75 mM acetylthiocholine after 30 min preincubation with 10^{-4} M *iso*OMPA. ψChE was assayed using 0.75 mM butyrylthiocholine in the presence of 10^{-5} M BW 284C51. The arrow denotes the position of a catalase marker, taken as having sedimentation coefficient 11.4S.

levels are strikingly reduced in 1-month-old, as opposed to embryonic birds, and ψChE levels follow a similar course. In dystrophic birds, an initial decrease in AChE levels in pectoral muscle is soon followed by a dramatic increase with onset of the disease, and again the ψChE levels increase in parallel. Also, in the slow tonic anterior latissimus dorsi (ALD) muscle, which is not affected by dystrophy, significant (and similar) amounts of ψChE occur with AChE in both normal and dystrophic muscle. Table 1 also shows that following denervation of another fast-twitch muscle, the posterior latissimus dorsi (PLD), a 30-fold elevation in AChE levels occurs in 3-week-old birds, accompanied by a >20-fold elevation in ψChE levels.

Sucrose gradient centrifugation of extracts of chicken skeletal muscle reveals[18,19] several molecular forms of AChE which are principally concentrated in three zones of the gradient, arbitrarily designated as heavy (H, 16–20S), medium (M, 10–13S) and light (L, 4–7S). It has recently been demonstrated that the H_2 form (~20S) predominates in normal adult pectoral and PLD muscles[17,19], that the L_2 (~6.5S) form is greatly elevated in dystrophic fast-twitch muscle[17,19–21], and that in young or embryonic pectoral muscle increased levels of M (~11S) and H_1 (~16S), as well as L forms are observed[17,19]. Figure 1*a* shows a typical sucrose gradient profile for AChE obtained with an extract of embryonic muscle, displaying the L_2, M and H_2 forms as the dominant components. Figure 1*b* shows a profile for ψChE in the same tissue extract. Activity peaks of ψChE are observed in the H, L and M regions of the gradient, as for AChE. The principal difference is that a considerable part of the activity in the L region corresponds to an L_1 (~4S) component. In the case of AChE the L_1 form is seen in large amounts only when protease inhibitors (always used here; see Table 1) are omitted from the extraction medium[19]. L_1 AChE thus seems to be produced by proteolytic degradation of L_2, which is apparently a dimer of L_1 (ref. 22). Figure 1*c* shows that in adult dystrophic pectoral muscle the L_2 component of AChE is considerably larger than the H_2 component, with the M species making only a minor contribution. As shown in Fig. 1*d*, in the dystrophic adult muscle ψChE activity is also present in all three regions of the gradient. Again, the light forms are elevated, with the L_1 form predominating, but the M and H_2 forms are also prominent. The ψChE activity in the gradients displayed in Fig. 1*b*, *d* was assayed using butyrylthiocholine in the presence of the selective AChE inhibitor[4], BW 284C51. When butyrylthiocholine and BW 284C51 were supplemented with the selective ψChE inhibitor[4], tetra-*iso*-propyl pyrophosphoramide (*iso*OMPA), no activity was observed in any part of the gradient. This clearly establishes that all peaks observed represent ψChE. It should also be noted that each ψChE peak seems to be fractionally

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lighter (by 0.5–1S) than its corresponding AChE form. Thus, L_1, L_2, M and H_2 for ψChE have on average $s_{20,w}$ = 4.0, 5.9, 10.5 and 19.1 S, respectively.

The content of ψChE in the fast-twitch muscles of normal chickens is very low at 28 d of age (Table 1) and later. Nevertheless, this enzyme was shown (ref. 17 and additional unpublished data) to be a real component of both fast-twitch and slow tonic muscles of mature normal chickens, by the use of prolonged enzymatic reaction incubations of similar sucrose gradients: their pectoral and PLD muscles (at 32–100 d, perfused to remove blood ψChE) showed significant L ψChE (mainly L_2) and less, but distinct, M and H ψChE. In their ALD muscles, L_1 and L_2 are higher, with some M (and a trace of H) ψChE also present; the pattern is as shown[19] for AChE there. On maturation of normal fast-twitch muscle, therefore, the parallel control of ψChE and AChE forms is lost, but it remains in the mature slow tonic muscle.

Vigny *et al.*[8] reported only minute amounts of an H form of ψChE in rat supercervical ganglia and could not demonstrate any H form in mature skeletal muscle. It is thus particularly interesting that, using embryonic or dystrophic birds, we can demonstrate considerable ψChE activity in the H region, as the H forms of AChE are those which are ascribed to the endplate region in skeletal muscle[9,10,18]. Consistent with this occurrence of the H forms of AChE at the endplate region is their appearance on innervation and their loss on denervation[18,21]. It was, therefore, important to see whether the H form of ψChE would also disappear on denervation. This was investigated in the tonic ALD muscle of very young (~7-d) chickens, where the H forms of both AChE and ψChE are quite prominent (Fig. 2*a*, *c*). We confirmed that the H_2 form of AChE is largely eliminated within 7 days, with a concomitant increase in amounts of the lighter forms of the enzyme (Fig. 2*b*). The H_2 form of ψChE is similarly reduced (Fig. 2*d*), and, as in the case of AChE, increased amounts of the lighter forms of the enzyme are observed. A similar denervation experiment (not shown) on the PLD muscle of a mature dystrophic bird also demonstrated disappearance of the prominent H peak of ψChE together with disappearance of the corresponding AChE peak.

The role of the motor nerve in controlling muscle differentiation is especially pertinent to attempts to understand whether a given myopathy is of neurogenic or myogenic origin. In the case of inherited muscular dystrophy in humans, as well as in chickens and other experimental animals, this issue is extremely controversial[23]. Although AChE levels increase in chicken dystrophic muscle as in normal denervated muscle, this does not imply that the control of AChE levels is strictly neurogenic, as ACh receptor levels increase after denervation but not during dystrophy[24], and limb-bud transplant evidence has suggested that dystrophy in chickens is of myogenic origin[25]. Moreover, whereas after denervation the H_2 form of AChE is virtually eliminated (see Fig. 2 and refs 18, 21), in the dystrophic condition it is elevated[17,19–21]. Whatever the primary lesion in the dystrophy, our data show that it affects the levels and forms of ψChE similarly to those of AChE.

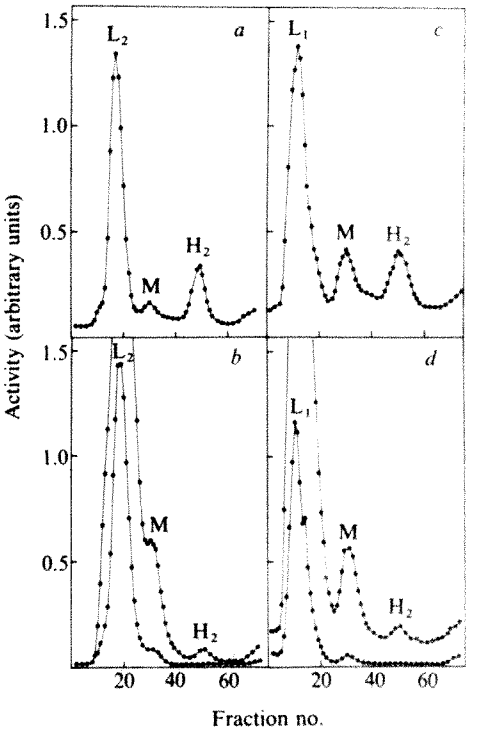


Fig. 2 Sucrose gradient profiles of AChE and ψChE activity in extracts of innervated and denervated anterior latissimus dorsi (ALD) muscles. Leghorn chickens were denervated on day 1 post-hatch as described in Table 1, and killed on day 7 together with control birds. *a*, AChE in control bird; *b*, AChE in denervated bird; *c*, ψChE in control bird; *d*, ψChE in denervated bird. Extracts were made as in Table 1, gradients were run as in Fig. 1 and assays carried out as in Fig. 1 except that BW 284C51 was omitted in the assay of ψChE. In the case of denervated muscle (*b*, *d*) the upper profile, in each case, was obtained by developing the gradient for the same time as for the corresponding control muscle (*a*, *c* respectively); the lower profile represents incubation for a much shorter time so as to reveal the relative magnitudes of the L peaks.

Table 1 Amounts of acetylcholinesterase and pseudocholinesterase in extracts of fast-twitch muscle from normal, denervated and dystrophic chickens

Muscle	Normal AChE	Normal ψChE	Normal, denervated AChE	Normal, denervated ψChE	Dystrophic AChE	Dystrophic ψChE
Pectoral, 19 d *in ovo*	8.4	0.7			5.8	0.6
Pectoral, 32 d post-hatch	0.15	<0.01			1.9	0.6
PLD, 28 d post-hatch	0.88	<0.01	26	0.24		
ALD, 32 d post-hatch	3.90	0.27			2.26	0.20

Pectoral and ALD muscle samples were dissected from freshly killed embryos or 32-d-old chickens (normal [line 412] or dystrophic [line 413] from the University of California flock). Posterior latissimus dorsi (PLD) muscles were dissected from freshly killed normal Leghorn chickens. Denervation was carried out 7 d before sectioning the main nerve trunk at the brachial plexus. Tissue extracts were prepared as described previously[19], in a medium containing protease inhibitors which had the following composition: 5 mM *N*-ethylmaleimide, 2 mM benzamidine, 10 mM EGTA, 20 μg ml^{-1} pepstatin, 40 μg ml^{-1} leupeptin, 1 mg ml^{-1} bacitracin, 1% Triton X-100, 1 M NaCl, 0.01 M phosphate, *p*H 7.0. AChE was assayed on 0.75 mM acetylthiocholine as described previously[19], after preincubation with 0.1 mM *iso*OMPA for 40 min to permit full inactivation of ψChE[4]. ψChE was assayed using either 0.75 mM acetylthiocholine in the presence of 5×10^{-5} M BW 284C51 (pectoral and ALD) or 0.75 mM butyrylthiocholine (PLD). The figures shown represent units per g tissue, where 1 unit is the amount of enzyme hydrolysing 1 μmol of substrate per min in the assay conditions used.

Because AChE and ψChE show large differences in binding properties[3,4], and no immunological cross-reactivity[8], we presume they are products of different genes. For human serum ψChE a separate locus has, in fact, been established by analysis of genetic variants, including an inherited deletion of only this enzyme[26]. Consistent with the concept of separate genes for muscle AChE and ψChE, the two enzymes do not always vary in parallel (in maturation of normal fast-twitch muscle). Thus, the present finding, that in several different states their various forms do change in parallel, is best explained by a regulatory mechanism that is common to the two genes. The close homology of the molecular forms of AChE and ψChE suggests that they are assembled into functional species by a similar mechanism. This is particularly remarkable in the case of the H form, where current evidence with respect to AChE supports the concept of a complex structure in which the catalytic subunits are attached to a collagenous tail[16,22,27] which anchors the enzyme to the basal lamina[28].

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Human embryonic haemoglobins Gower 1 and Gower 2

THE human embryonic haemoglobins (Hbs) Gower 1 and Gower 2 (ref. 1) are present in fetuses up to 60 mm crown–rump measurement[2]. However, relatively little is known about the structure and function of these haemoglobins because of the difficulty in obtaining suitable material, particularly since the introduction of the suction method for terminating pregnancy in the first trimester. Here we present the amino acid composition and sequence data for the human ε-chain, prepared by purifying Hb-Gower 2 ($\alpha_2\varepsilon_2$) from early abortion samples. In addition, separation of embryonic red cells from the majority of the maternal red cells has led to the isolation of Hb-Gower 1 and the demonstration that it has the composition $\zeta_2\varepsilon_2$.

Material was collected from vacuum abortions of 6–10 weeks gestation carried out by the standard technique for unrelated indications. The sample was initially passed through a coarse sieve to remove any tissue fragments, and then through a 20-μm pore-size filter. The red cells were packed and washed three times with isotonic saline, then lysed with water and the membranes extracted with carbon tetrachloride. These haemolysates contained only small amounts of Hb-F, and Hb-Gower 1 or Hb-Gower 2 were detected in only a few samples by starch gel electrophoresis.

The first step in the purification was the removal of Hb-A using a modification of the chromatographic method described by Huisman and Dozy[3]. The haemolysates were equilibrated against 0.05 M Tris-HCl, *p*H 8.05 ± 0.05, and loaded at room temperature on to a DEAE-Sephadex column (3 × 30 cm) equilibrated against the same buffer. The haemoglobins were eluted with *p*H 8.05 buffer and the first haem-containing peak collected; this included Hb-Gower 2, Hb-A_2, small amounts of Hb-A and some carbonic anhydrase. This fraction was concentrated and dialysed against developer A consisting of 0.2 M glycine and 0.01 g dl^{-1} potassium cyanide, *p*H 7.4–7.7, and loaded at room temperature on to a column (1.5 × 25 cm) of microgranular DEAE-cellulose DE52 equilibrated with developer A (ref. 4). Hb-Gower 2 was eluted using developer A only, all other haemoglobins remaining firmly attached to the cellulose in these conditions. The Hb-Gower 2 was checked for purity by measuring the *A* ratio 280/345 nm of each fraction, and only those uncontaminated (ratio <1.5) with non-haem proteins were concentrated and used.

The electrophoretic mobility of Hb-Gower 2 was similar to that described previously[1]. Spectral analysis between 250 and 700 nm of the oxygenated purified Hb-Gower 2 showed only minor differences when compared with a solution of oxyHb-A. Sephadex G-100 gel filtration[5] gave a value of 30,000 for the molecular weight of Hb-Gower 2 and 45,000 for Hb-A, presumably indicating that the tetramer dissociates into $\alpha\varepsilon$ dimers more readily than does Hb-A in similar conditions. SDS-polyacrylamide gel electrophoresis of Hb-Gower 2 in a concentration gradient of acrylamide[6] that just resolved α- from β-chains, gave a broad band, the fastest portion corresponding to the α-chains of Hb-A with an overlapping zone between the α- and β-chains. There was no electrophoretic resolution of the α- from the ε-chains after treatment of the protein with *p*-chloromercuribenzoate (PCMB)[7]. Attempts to separate the globin chains using chromatography on CM-cellulose in 8 M urea[8] yielded a single protein peak consisting solely of α-chains; the ε-chains could not be eluted from the column even using much stronger buffers and a higher *p*H. Separation of the globin chains was achieved by electrophoresis on cellulose acetate in 6 M urea, *p*H 6.4 (ref. 9), the ε-chains migrating anodally, close to the α-chains. However, this method was not suitable for preparative purposes. Further analysis was therefore carried out on whole globin prepared from purified Hb-Gower 2 by acid/acetone precipitation.

Tryptic peptide maps prepared by standard techniques[8] showed many ninhydrin-positive spots immediately recognisable as peptides from the α-chain, but at least 10 were thought to be peptides from the ε-chain; all peptides were eluted with 6 M HCl for amino acid analysis. From the data obtained it was concluded that the ε-chain is similar to both the β- and γ-chains, and using tables from Dayhoff[10] a sequence of the first 10 tryptic peptides (residues 1–87) of the ε-chain was constructed by comparison with known mammalian β-chain sequences. Six milligrammes of Gower 2 globin were also analysed in a Beckman model 890C protein sequencer. The data confirmed the sequence of the first 40 residues (Fig. 1).

Throughout these procedures no peptides belonging to the second half of the ε-chain were demonstrated. Presumably, these had precipitated as an insoluble core of undigested globin which was not detected in the small amount of material used. Cyanogen bromide digestion of Gower 2 globin followed by tryptic digestion and peptide mapping of the fragments separated by Sephadex chromatography showed the characteristic C-terminal peptide of β- and γ-chains: Tyr-His. There were other peptides which had compositions resembling known peptides from the C-terminal halves of the β- and γ-chains, and

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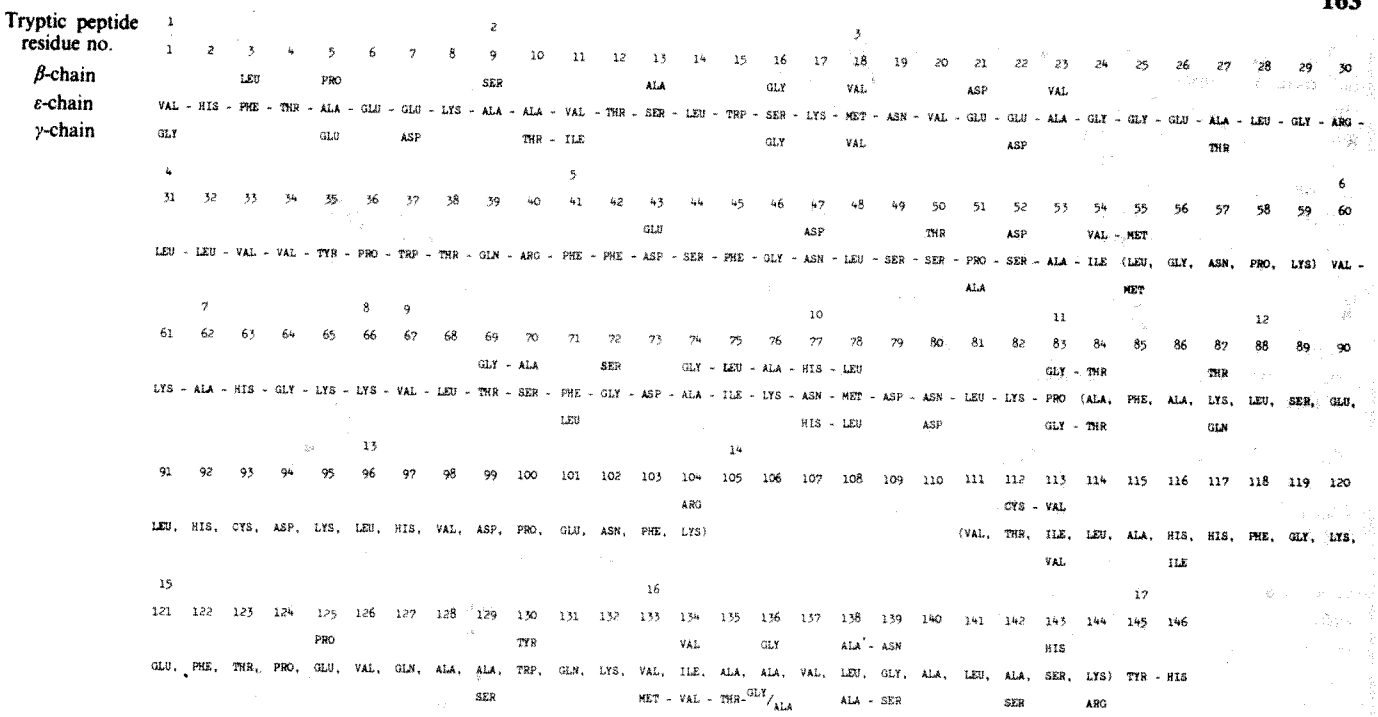


Fig. 1 Amino acid sequence of the human embryonic ε-chain. Residues 1–54, 60–83 and 145–146 are determined sequence, residues 55–59, 84–104 and 111–144 are a tentative sequence constructed by homology with known mammalian β-chains. Only differences in the human β- and γ-chains have been included.

from these a tentative sequence for the second half of the ε-chain was constructed. In addition, manual and automatic sequencing of one of the cyanogen bromide fragments and some tryptic peptides derived from it enabled a sequence for residues 41–83 to be established (Fig. 1). All the sequence data corresponded entirely to the amino acid compositions of peptides previously determined. The assignment of amide residues where sequence is not available was made using amino acid composition and electrophoretic mobility of the peptides, as well as homology with other β-like chains.

The sequence of the ε-chain established so far shows that it differs from both the β- and γ-chains. Using the definitive sequence information in Fig. 1 there are 24 differences between the ε- and β-chains and 18 between ε- and γ-chains, compared with 26 differences between β- and γ-chains. Tryptic peptides 4, 6, 7, 8, 11 and 17 maintain the invariant composition found in their equivalents in the human β-, γ- and δ-chains. Further, the ε-chain contains four isoleucine residues whereas there are none in the β-chain and three in the γ-chain. There is no cysteine at position 112, explaining the inability to separate the α- from the ε-chains after PCMB treatment. The difference in charged amino acids so far established is consistent with the differences in electrophoretic mobility between Hb-Gower 2 and Hb-A or Hb-F.

Hb-Gower 1 could not be isolated using the procedure described above, presumably because it was either not separated from Hb-A or precipitated during the purification. Therefore, as a preliminary step in the isolation of Hb-Gower 1, maternal red cells were separated from the embryonic cells. Although the latter are conspicuously large (~15–20 μm mean cell diameter) and retain their nucleus in a condensed form, they constitute less than 0.01% of the total red cells collected from an abortion sample. Separation of the two cell types was achieved using a large-scale Ørskov–Jacobs–Stewart reaction[11] which is based on the low levels of carbonic anhydrase present in fetal cells. The cells were incubated initially for 2 min with acetazolamide and NH_4HCO_3. Because of the large volumes necessary for the preparation, the mixture was then centrifuged for 5 min at 2,000*g* at 4 °C to sediment unlysed cells. The latter were resuspended in isotonic saline and spun through Ficoll[11]. Placental cells and leukocytes were removed by centrifuging the suspension through first 40% and then 60% Percoll at 2,000*g* at 4 °C for 5 min. With sufficiently early embryonic samples a preparation containing 100% large nucleated red blood cells could be obtained.

The embryonic red cells were lysed with water and the membranes pelleted by spinning at 18,000*g* for 15 min. No organic solvents were used as this denatures the haemoglobin. The haemolysates were dialysed against developer A and loaded on to a DEAE-cellulose DE52 column as described previously. After elution of Hb-Gower 2 with developer A a salt gradient was applied by mixing 150 ml developer A containing 0.005 M NaCl in a constant-volume chamber with developer A containing 0.03 M NaCl. Tryptic peptide maps of one haemoglobin fraction which migrated electrophoretically close to Hb-A_2 showed peptides which were characteristic of the ε-chain. Of the peptides in Fig. 1, 1, 3, 4, 5, 6, 7, 7–8 and 9 were identified and confirmed by amino acid analyses. Other ninhydrin-positive spots were clearly peptides from the ζ-chain[12], with identical compositions for residues 17–31 (ζ4), 32–40 (ζ5), 57–61 (ζ7–8), 90–92 (ζ10), 93–99 (ζ11) and the C-terminal peptide, residues 140–141 (ζ14).

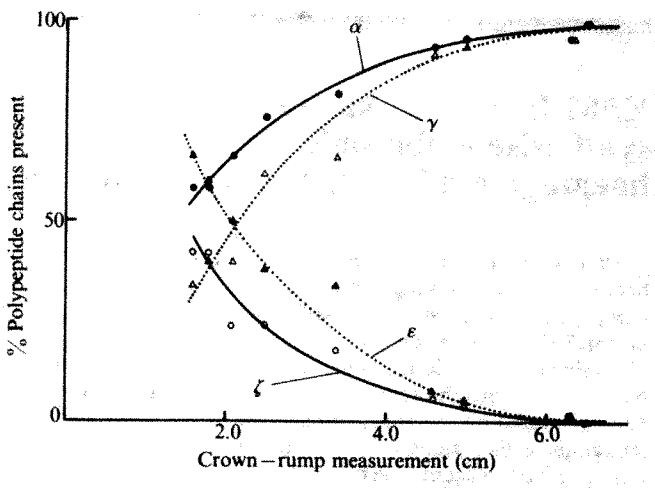


Fig. 2 Developmental changes in human globin chains. The curves have been constructed using the data in refs 1, 2 and 15 and the composition for Hb-Gower 1 $\zeta_2\varepsilon_2$.

These results confirm previous suggestions[13] that the ζ-chain is the human embryonic α-type-chain and that Hb-Gower 1 has the structure $\zeta_2\varepsilon_2$. This structure explains the finding that oxygen dissociation studies of embryonic red cells show a full cooperative effect[14]. Furthermore, the globin chain composition of the human embryonic haemoglobins corresponds to that found in other mammals. From the available data on the haemoglobins in embryos at various stages of development it can be concluded that the disappearance of the ζ- and ε-chains occurs in parallel (Fig. 2), suggesting that there is a coordinated change-over from the production of the embryonic ζ- and ε-chains to the fetal α- and γ-chains which occur at about the same time as the liver replaces the yolk sac as the main site of erythropoiesis.

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Note added in proof: Proudfoot and Baralle[16] have recently cloned a human ε-globin gene. The DNA sequence of a fragment of this gene gives an amino acid sequence which corresponds exactly with residues 61–100 in Fig. 1.

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K562 human leukaemic cells synthesise embryonic haemoglobin in response to haemin

STUDIES of the regulation of human erythropoiesis and haemoglobin synthesis at different phases of development are hampered by the lack of a self-sustaining culture in which erythroid differentiation can be examined, and by difficulties in obtaining erythropoietic tissue in embryonic and early fetal life[1]. New insights into the regulation of murine erythropoiesis have been obtained by the study of established cultures of erythroleukaemic cells (Friend cells) which can be induced to differentiate *in vitro*[2]. Recently, Andersson *et al.*[3,4] have reported that the K562 human cell line, obtained from a patient with an acute transformation of chronic myeloid leukaemia (CML) has properties in common with erythroid cells. In particular, cells of this line contain glycophorin and spectrin in their membranes and synthesise minute amounts of haemoglobin, detectable by radioimmunoassay. We have investigated the capacity of these cells to differentiate *in vitro* and report here that they are induced by haemin to synthesise large amounts of haemoglobin; preliminary analysis indicates that this is predominantly of the embryonic type.

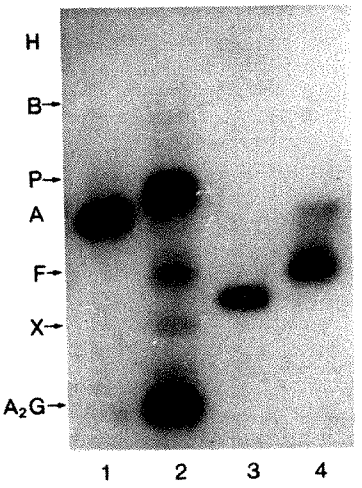


Fig. 1 Cells were grown for 6 days in the presence of 0.1 mM haemin. They were then spun down, washed three times in Hank's balanced salt solution and the final packed cell pellet resuspended in an equal volume of distilled water and lysed by three cycles of freeze/thawing. Stroma was spun out at 2,000 r.p.m. for 10 min and 40,000 r.p.m. for 45 min. Haemoglobins were separated by starch gel electrophoresis at *p*H 8.6 (ref. 6). Bands were visible with the naked eye and stained for photography with benzidine[6]. Bands in the sample (channel 2) were identified as Hb-Bart's (B), Hb-Portland (P), Hb-F, Hb-X and Hb-Gower 1 (G) by reference to the following standards: channel 1, Hb-A + Hb-A_2; channel 3, Hb-Q and Hb-H; channel 4, Hb-A + Hb-F.

Various potential inducers, including dimethyl sulphoxide, *n*-butyric acid, *N*-methylacetamide and hexamethylenebisacetamide, were tested for their effect on K562 cells and proved ineffective at concentrations that induce Friend cells to synthesise haemoglobin. However, K562 cells grown in the presence of haemin synthesised large amounts of haemoglobin. The cells were subcultured twice weekly in RPMI-1640 medium containing 10% heat-inactivated fetal calf serum. Haemin was added to cultures in logarithmic growth to a concentration 0.1 mM. The presence of haemoglobin was confirmed by absorption bands at 540, 576 and 415 nm and quantitated at 415 nm (ref. 5). Cells cultured with haemin accumulated 3 pg haemoglobin compared with 0.3 pg without haemin. The haemoglobin seemed to be relatively evenly distributed within the cells and we did not observe the benzidine-positive particles reported by Andersson *et al.*[4]. Shedding of cytoplasmic blebs was observed but was rare and was not greater than seen in control cells.

Cells were grown for 6 d with 0.1 mM haemin, lysed with 1 volume of water, the membranes removed by centrifugation and the haemoglobin analysed by starch gel electrophoresis[6] (Fig. 1). Two major haemoglobin components were found, one migrating slightly faster than a Hb-A marker in the position of the embryonic haemoglobin, Hb-Portland ($\zeta_2\gamma_2$)[7] and the other slightly more slowly then Hb-A_2 in the position of another embryonic haemoglobin, Hb-Gower 1 ($\zeta_2\varepsilon_2$)[8,9]. In addition, there were trace amounts of fractions moving in the positions of Hb-F ($\alpha_2\gamma_2$) and Hb-Bart's (γ_4), and an as yet unidentified haemoglobin (fraction X) found in variable amounts in early embryos[10]. The 'Hb-Gower 1' fraction was isolated from 6-day induced K562 cells by a combination of gel filtration and DEAE-cellulose chromatography. Following digestion with trypsin, fingerprinting and staining with ninhydrin, peptides

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were found which corresponded to ζ and ε peptides of Hb-Gower 1. The identity of peptides εI and ζV were confirmed by amino acid analysis.

Further evidence that the induced K562 cells synthesis γ-globin chains and small amounts of α-chains was obtained by incubating the cells with ^{3}H-leucine, separating the globin chains by CM-cellulose chromatography and determining the amount of radioactivity incorporated into each chain[11-12]. It was found that γ- and α-chains were labelled and that the α/γ-globin chain synthesis ratio was 0.08; no β-chain synthesis was detected. Presumably, the small numbers of α-chains synthesised in these cells combine mainly with γ-chains to produce Hb-F and the remainder of the γ-chains are associated with ζ-chains in Hb-Portland, the excess forming the γ_4-tetramers of Hb-Bart's[1]. It has previously been shown that ζ and ε chains are not recoverable in this chromatographic system[9,13].

The reason for the induction of embryonic haemoglobin synthesis in this cell line is not clear. Neither embryonic nor fetal haemoglobins have been found in association with adult CML[14]. The electrophoretic pattern of K562 cell haemoglobin corresponds closely with that observed in haemoglobins from purified embryonic erythroblasts[7-10]. The subunit compositions of the human embryonic haemoglobins are now known[7-9,13,15] although the amino acid sequences of their component chains are still incomplete. The present observations on the haemoglobins of K562 cells suggest that these cells may offer a source of embryonic haemoglobins for the further elucidation of their structure and function, and that they may provide a useful model for studying the developmental biology of human embryonic erythroblasts and for examining the mechanism of the switch from embryonic to fetal haemoglobin synthesis.

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Induction of sporulation in *Bacillus brevis* by peptide antibiotics

THE regulatory mechanisms of the process of bacterial sporulation are unknown, although some authors have speculated that sporulation and the synthesis of peptide antibiotics are somehow connected[1-4]. Paulus and coworkers proposed that tyrocidine and gramicidin D, both synthesised by *Bacillus brevis*, may interact with the transcriptional process during sporogenesis[5].

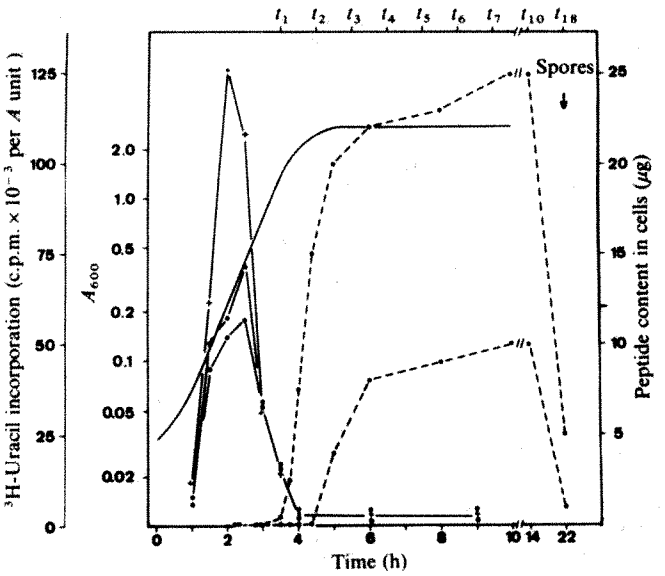


Fig. 1 *Bacillus brevis* (ATCC 8185) cells were cultured in Hanson's medium[7] at 37 °C with aeration in a water bath shaker. Growth was followed as a function of time by measuring the absorbance at 600 nm (———). One *A* unit corresponds to 7×10^6 cells ml^{-1}. At indicated times 1-ml samples were incubated with 20 μg of tyrocidine (●——●) (Serva), gramicidin D (Serva) (○——○) or without antibiotics (+——+) for 10 min and then supplemented with 0.2 μCi of ^{3}H-uracil (Amersham Buchler, specific activity 48 Ci mmol^{-1}). After 5 min incubation at 37 °C, samples were treated with trichloroacetic acid (final concentration 5%) at 0 °C. The acid-precipitable material was collected on glass fibre filters and the radioactivity measured in a scintillation counter. Radioactivity as c.p.m. per *A* unit was plotted against time of incubation. In parallel, the tyrocidine and gramicidin D content of cells was estimated by Sephadex L-20 column chromatography according to the method of Bartley *et al.*[8]. The tyrocidine (●---●) and gramicidin D content (○---○) is plotted as μg peptide in cells in 1 ml of the culture.

They have isolated a mutant of *B. brevis* which is unable to synthesise gramicidin D and which produces defective spores. The defects could be cured by exogenous gramicidin D, suggesting that this peptide is involved in sporogenesis[6]. In this report we demonstrate that sporulation of early vegetative growing *B. brevis* cells can be induced by exogenous tyrothricin, a mixture of tyrocidine and gramicidin D, provided that the cells are exposed to a culture medium lacking the nitrogen source. The induction of sporulation occurs concomitantly with a significant increase in RNA synthesis. The results suggest that sporulation and the action of these two peptides are causally connected. Both peptides may play a positive regulatory role in cell differentiation.

Figure 1 shows some characteristics of a *B. brevis* culture grown in Hanson's medium[7]. The beginning of spore formation can be detected microscopically at t_4, which corresponds to stage III of the sporulation as defined for *Bacillus subtilis*[2]. Spores are released at t_{18}. The production of tyrocidine begins at t_0, and that of gramicidin D between t_1 and t_5. From t_3 the peptide concentration in the cells increases only slightly. Free spores, however, contain only 20% of the tyrocidine and 10% of the gramicidin D found in sporulating cells. We investigated the RNA synthesis of cells at different times during growth by testing their ability to incorporate tritiated uracil. Figure 1 shows the uracil incorporation into acid-precipitable material within 5 min. The uptake increases up to the early logarithmic growing phase, decreases in the mid and late logarithmic phase and becomes low in sporulating cells. This decrease could reflect a lowered permeability of the cells for uracil. The uracil incorporation was also studied in the presence of 20 μg ml^{-1} of tyrocidine or gramicidin D (Fig. 1). At the beginning of growth the cells were relatively insensitive to

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the peptides, but became sensitive in the early logarithmic phase; their RNA synthesis was dose-dependently inhibited by the peptides (not shown). This sensitivity decreased when the cells were in the mid logarithmic phase and, finally, the cells became completely insensitive.

Based on the observation depicted in Fig. 1 we tried to induce sporulation in cells at different stages of growth by the addition of tyrothricin, the natural mixture of tyrocidine and gramicidin D (about 2:1). The addition of tyrothricin to mid and late logarithmic cells had no effect on sporulation: treated and untreated cells started to sporulate within the same time. Addition of tyrothricin to early logarithmic cells inhibited RNA synthesis and at higher concentrations led to cell death.

In another approach cells were grown in Hanson's medium up to 0.2–0.3 *A* units, at which their uracil incorporation was sensitive to exogenous peptides, and were then transferred to a modified medium defined by Mach[9] containing glycerol as the carbon source but no nitrogen (C^+N^- medium). On addition of tyrothricin these cells began to sporulate and 5 h later had the microscopical appearance of prespores of stage III. The number of sporulating cells was dependent on the tyrothricin concentration (not shown).

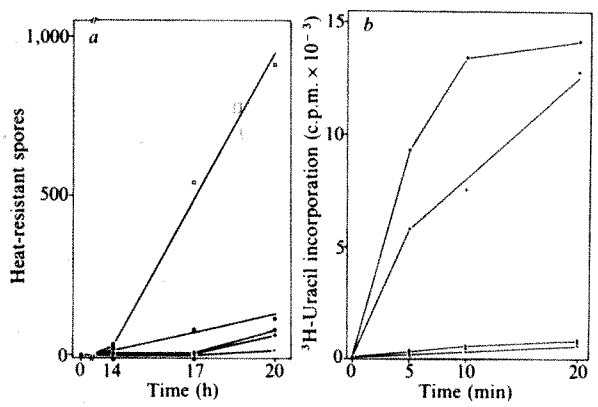


Fig. 2 The effect of tyrothricin on the formation of heat-resistant spores and on RNA synthesis of *B. brevis*. Cells were grown in Hanson's medium to an absorbance of 0.2, then filtered, washed and suspended in C^+N^- medium in the presence of 0 (+——+), 5 (△——△), 10 (○——○), 20 (●——●) and 40 (□——□) μg ml^{-1} of tyrothricin. *a*, After incubation for the indicated times at 37 °C, samples were heated for 20 min at 80 °C. Aliquots were plated on agar plates containing the components of Hanson's medium. The number of colonies was plotted against incubation time. The total number of viable cells was estimated by plating as 10^4. *b*, After 14 h incubation in C^+N^- medium 1-ml samples of the culture were incubated with 0.2 μCi of ^{3}H-uracil (Amersham Buchler, specific activity 48 Ci mmol^{-1}). At the indicated times the uracil uptake was stopped by the addition of trichloroacetic acid and the precipitates then treated as described in Fig. 1. The ^{3}H-uracil incorporation was plotted as c.p.m. against incubation time.

Figure 2*a* shows the onset of the formation of heat-resistant spores and indicates that at low tyrothricin concentrations only a few spores become heat resistant. At concentrations comparable with that extracted from sporulating cells (Fig. 1) there is a dramatic increase in heat-resistant spores, whereas in the absence of tyrothricin only a few heat-resistant spores are found after 20 h. We conclude from these results that exogenous tyrothricin induces the sporulation process of early vegetative cells. A specific metabolic situation due to the absence of nitrogen might be a prerequisite for the sporulation-inducing activity of the two peptides. In a parallel experiment, cells were incubated in C^+N^- medium in the presence of equivalent amounts of either gramicidin D or tyrocidine. With gramicidin D no prespores were detectable; with tyrocidine less than 10% of the cells formed prespores after 14 h. These results clearly indicate that the induction of sporulation must result from specific actions of both peptides.

If early vegetative cells incubated in C^+N^- medium in the presence of tyrothricin undergo sporulation they may exhibit rates of RNA synthesis different from those of cells starved in the same medium but in the absence of tyrothricin. This was tested by transferring early vegetative cells into C^+N^- medium with or without tyrothricin. After incubation for 14 h the cells were tested for their RNA synthesis. As shown in Fig. 2*b*, the rate of uracil incorporation is largely increased by tyrothricin at concentrations necessary for the induction of sporulation (Fig. 2*a*).

How might tyrocidine and gramicidin D act at the molecular level? Earlier studies indicated that tyrocidine inhibits RNA synthesis *in vivo*[5,10] and *in vitro*[5,11], in the latter by forming a complex with the DNA[11] and interacting with the initiation process of transcription[12]. Also, gramicidin D inhibited RNA synthesis[13,14] by destabilising the open initiation complex[15]. However, when gramicidin D was added to a tyrocidine-inhibited transcription system reactivation of transcription was observed[13]. This reactivation was only partial and was specifically dependent on the RNA polymerase from *B. brevis* and on a well defined equilibrium between tyrocidine and gramicidin D[16]. Studies on the molecular mechanism of this reactivation suggested that gramicidin D interacts with the DNA–tyrocidine complex, thereby weakening the DNA–tyrocidine interaction, and thus allows the RNA polymerase to transcribe[12,17]. As both peptides affect the transcriptional process they could be involved in gene regulation during differentiation of *B. brevis*. Tyrocidine could act as a nonspecific repressor, turning off transcription, whereas gramicidin D could de-repress part of the genome to allow transcription of those genes necessary for the spore-forming process. Different RNA polymerases[18] or additional protein factors[19] might be essential for the selection of these DNA sequences. Much more work is required to verify this hypothesis. The results presented, however, suggest that the two peptides are, in fact, involved in the control of sporulation.

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Bacillus megaterium resistance to cloxacillin accompanied by a compensatory change in penicillin binding proteins

BACTERIA have been shown to become resistant to β-Lactam antibiotics (penicillin and cephalosporins) by the following methods: decreased permeability[1,2], increased production of an enzyme (β-lactamase) which degrades the antibiotics[1], acquisition of a plasmid that produces a β-lactamase[3,4], and a decreased affinity of the lethal target for the antibiotic[5]. β-Lactam antibiotics are thought to kill bacteria by interfering with the terminal stages of cell wall biosynthesis[6]. The cross-linking of the cell wall by a transpeptidase enzyme has been implicated as the probable target site[7,8], although inhibition of a carboxypeptidase[9] and stimulation of autolytic activity[10] have also been proposed. It has been suggested that penicillin is a transition state analogue of the D-alanyl-D-alanine moiety of the donor molecule in the transpeptidation reaction[11] and that penicillin binds covalently to the transpeptidase[7]. Investigations therefore concentrated on enzymes which could fix penicillin in bacterial membranes[12,13]. Bacteria contain multiple penicillin binding proteins (PBPs) and it is important to identify which one is the lethal target. One approach used in this laboratory has been to purify the individual PBPs and investigate their enzymatic activity, although it was realised that solubilisation might lead to inactivation. In *Bacillus megaterium* the only PBP shown to possess enzymatic activity was PBP 5 (ref. 14) (the one of lowest molecular weight), and this was the D,D-Ala carboxypeptidase, which is much less sensitive to β-lactams than is growth of the organism[15]. Despite the lack of enzymatic activity the affinity of the remaining PBPs 1–4 for benzylpenicillin could be measured in whole cells. PBP 1 was the only protein that interacted covalently with benzylpenicillin at the lowest concentration of the antibiotic that would inhibit growth, and it was therefore proposed to be the lethal target[16]. An alternative approach is to determine which PBPs become altered as an organism develops resistance to an antibiotic. This method has shown that resistance can be due to a PBP having a decreased affinity for a β-lactam antibiotic. Using this technique PBP 2 has been identified as the lethal target for mecillinam in *Escherichia coli*[5] and for cloxacillin in *Bacillus subtilis*[17]. However, in this report a new mode of resistance is described in which there is a purely quantitative change in the ratio of PBP 1 to PBP 3 as resistance to cloxacillin develops in *B. megaterium*. Thus, resistance results from a decrease in the level of the lethal target which is compensated by an increase in a less sensitive target.

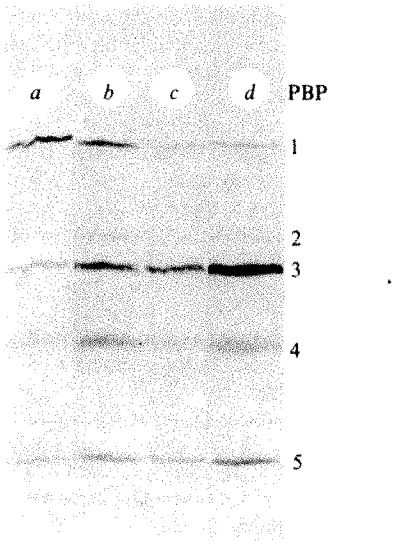


Fig. 1 Penicillin binding proteins (PBPs) of the parent and cloxacillin-resistant mutants of *B. megaterium*. The PBPs were examined as described in ref. 14. Protoplast membranes were prepared from exponentially growing cells and treated with ^{14}C-benzylpenicillin (10 μg ml^{-1}) for 10 min at 37 °C. The proteins were then fractionated by SDS-polyacrylamide slab-gel electrophoresis and the PBPs detected by fluorography: *a*, parent; *b*, XR2r; *c*, XR2; *d*, XR3.

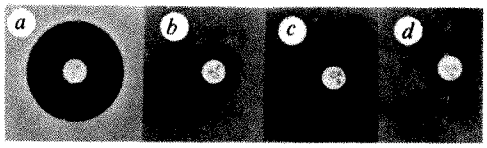


Fig. 2 Cloxacillin sensitivity of the parent and mutant organisms. Bacteria were grown in Penassay broth to a density of 0.4 mg dry wt per ml; 0.2 ml cells were added to 20 ml Penassay agar at 46 °C and overlaid onto a 9-cm plate containing 30 ml Pennassay agar. Cloxacillin sensitivity disks (Mast) were applied to the centre of each plate and diffusion allowed to occur at 4 °C for 2 h. Plates were incubated overnight at 37 °C: *a*, parent; *b*, XR2r; *c*, XR2; *d*, XR3.

Spontaneously resistant bacteria were isolated in a stepwise fashion[17]. *Bacillus megaterium* was grown on plates of Penassay agar containing cloxacillin at 37 °C. Cloxacillin was chosen to avoid selection of β-lactamase-producing organisms as it is not degraded by the β-lactamase from *B. megaterium*. A resistant mutant, designated XR2, was then purified by three rounds of single colony isolation. XR2 was filamentous and grew more slowly (mean generation time 40 min) than the rod-shaped parent (mean generation time 25 min). Membranes were prepared from XR2, treated with ^{14}C-benzylpenicillin, the proteins fractionated by SDS-polyacrylamide slab-gel electrophoresis and the PBPs detected by fluorography[18,19]. The results indicated an increase in the amount of PBP 3 and a decrease in PBP 1 (Fig. 1*c*) compared with the parent (Fig. 1*a*).

If the ratio of PBP 3 to PBP 1 is important in the development of cloxacillin resistance, one would expect a further increase in this ratio if the PBPs are examined in a mutant with an increased resistance to cloxacillin. Such a mutant (XR3) was derived from XR2, and was again filamentous and grew more slowly (mean generation time 60 min) than XR2. The PBPs of mutant XR3 were examined and the ratio of PBP 3 to PBP 1 had increased (Fig. 1*d*) relative to XR2. The fact that the cloxacillin-resistant mutants grew more slowly than the wild type suggests that there would be a selective pressure to revert to the wild type when the resistant mutants were grown in the absence of cloxacillin. In an attempt to obtain such revertants mutant XR2 was grown overnight in glucose-limited batch cultures until a morphological change was detected (eight successive subcultures at 24-h intervals). This change involved a more obvious and frequent septation of the filaments. An organism was purified through two rounds of single colony isolation on solid media and designated XR2r (a partial revertant). When the PBPs of XR2r were examined the ratio of PBP 3 to PBP 1 was found to have decreased (Fig. 1*b*) relative to XR2. The growth rate of XR2r had increased (mean generation time 35 min) and the cloxacillin resistance had decreased relative to XR2.

Thus, we have a series of three mutants in which increasing resistance to cloxacillin (Fig. 2) is related to an increase in the ratio of PBP 3 to PBP 1 (Fig. 1), a decreasing growth rate and filamentation of the organism. To ascertain whether any change in the affinity of the PBPs for cloxacillin had a role in resistance, the sensitivity of the five PBPs was measured for all the mutants (see Fig. 3 for results with the parent and XR3). No change in the affinity of PBPs for cloxacillin in the parent and mutants was detected. However, a difference was observed in the sensitivity profiles of PBP 1 and PBP 3. Cloxacillin bound to PBP 1 of all strains at low concentration (without saturating the protein completely) but required a high concentration (>100 μg ml^{-1})

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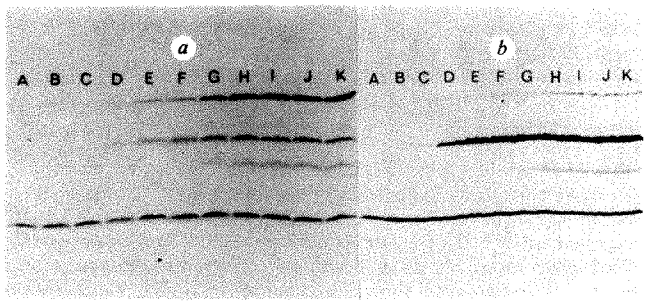


Fig. 3 Sensitivity of penicillin binding proteins (PBPs) to cloxacillin in the parent (*a*) and XR3 (*b*). As radioactive cloxacillin was not available the sensitivity of PBPs to cloxacillin was measured by prelabelling the PBPs with unlabelled cloxacillin followed by treatment of the membranes with an excess of ^{14}C-benzylpenicillin which will bind to PBPs unoccupied by cloxacillin (as described for cephalosporins in ref. 14). Experimental details were as for Fig. 1 except that membranes wkincubated with cloxacillin for 10 min at 37 °C before addition of ^{14}C-benzylpenicillin. The concentrations of cloxacillin were (μg ml^{-1}): (A) 100, (B) 30, (C) 10, (D) 3.0, (E) 1.0, (F) 0.3, (G) 0.1, (H) 0.03, (I) 0.01, (J) 0.003, (K) 0.001.

to inhibit completely the subsequent binding of ^{14}C-benzylpenicillin. In contrast, PBP 3 was saturated by cloxacillin at approximately 30 μg ml^{-1} but did not apparently interact with the antibiotic at concentrations lower than 3 μg ml^{-1} (Fig. 3). It is the sensitivity to low concentrations of cloxacillin which is important as the minimum growth inhibitory concentration for the parent is 0.08 μg ml^{-1} (ref. 15).

If the role of PBP 1 in cellular growth can be taken over by PBP 3 then the organism would become more resistant to cloxacillin if PBP 3 was less sensitive to the antibiotic than PBP 1. This situation is apparently fulfilled in the cloxacillin-resistant mutants reported here. There is a 1.5-fold increase in PBP 3 and a 10-fold decrease in PBP 1 in mutant XR2 compared with the parent strain (calculated by standardisation with PBP 5 which is assumed to be unaltered in quantity in all the organisms as it can be ruled out as the lethal target[14,15]). This may well be sufficient to alter the cloxacillin sensitivity of the organism. Compensatory changes in PBPs have also been observed in *E. coli* where supersensitive mutants lacking PBP 1Bs partially reverted to normal sensitivity to β-lactam antibiotics and this change was accompanied by an increase in PBP 1A and PBP 2 (ref. 20). The results also indicate that PBP 1 is the lethal target for cloxacillin in the wild-type *B. megaterium* and this agrees with the results obtained by a different method with benzylpenicillin[16].

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Influence of leaves on sporophore production by fungi forming sheathing mycorrhizas with *Betula* spp.

MOST of the intimate and often beneficial associations formed between plant roots and some soil fungi can be classified as either endomycorrhizas of various types or sheathing (ecto-) mycorrhizas[1,2]. The latter, in which colonised roots are shorter and thicker than their uncolonised counterparts, are mainiy attributed to basidiomycetous fungi that produce sporophores (toadstools) typical of the Agaricales. Sometimes fungi forming sheathing mycorrhizas produce large numbers of sporophores. P. Larsen, quoted by Romell[3], estimated sporophore production by the mycorrhizal *Suillus bovinus* (Fries) O. Kuntze to be about 180 kg (dry weight) per hectare per yr. Although their evidence is questionable, experiments done by Romell[4] and Laiho[5] suggest that the mycorrhizal *Boletus subtomentosus* Fries and *Paxillus involutus* (Batsch ex Fr.) Fr. usually form sporophores only if they are organically linked to living trees. Hacskaylo[6] found that shading foliage of *Pinus virginiana* Mill decreased sporophore formation by *Thelephora terrestris* Fr. The results reported here extend the understanding of this relation by showing that sporophore production by some mycorrhizal associates of birch (*Betula* spp.) is strongly dependent, in the field, on the presence of green foliage.

Two experiments, done at the Bush Estate (lat. 55°52′ N) 10 km south of Edinburgh, are described here. In one, sporophore production was related to the different rates of natural autumnal leaf yellowing found in saplings grown from seeds of *Betula pendula* collected by Roth from three widely spaced locations, whereas in the other, the effects of experimental defoliation were investigated.

Seeds of *B. pendula*, collected from natural stands at lat. 50°40′ N (southern England), 57°20′ N (Latvia) and 66°30′ N (northern Sweden), were treated with Captan, a 75% fungicidal dust of *N*-trichloro-methyl-mercapto-4-cyclohexene-1,2-dio-carboximide, before being sown on 7 January 1971, germinated and subsequently transplanted to pots (approximately 100-mm diameter) of partially sterilised John Innes type compost. After being grown in a glasshouse for 10 months and then hardened-off, three replicate saplings of each seedlot (provenance) were planted in November 1971. They were spaced 3 m apart, within and between rows, in three randomized blocks on an adequately drained medium silty loam soil where their roots were colonised by naturally occurring mycorrhizal fungi. During each succeeding autumn and at intervals of about 2 weeks the position of every sporophore was recorded for each tree. At the same time a note was made of 'percentage leaf yellowing', an integrated measure of yellowing and defoliation.

In 1975, 4 yr after planting, it was apparent that the origin of the *B. pendula* seeds (provenance) greatly affected the production of sporophores, mostly basidiomycetous, but also a few of the ascomycete *Peziza badia*, Persoon ex Mérat, a pattern confirmed in 1976 and 1977. Whereas 81, 150 and 760 sporophores were associated with each tree of southern origin (lat. 50°40′ N) in 1975, 1976 and 1977, respectively, only 29, 53 and 370 were found around trees of more northerly origin (lat. 57°20′ N), with virtually none, zero, zero and three, associated with those from the most northerly location (lat. 66°30′ N) (Fig. 1). In Autumn 1977 the number of species of sheathing mycorrhizal fungi producing sporophores increased from three associated with the 'Swedish' trees (*Hebeloma crustuliniforme* (Bull. ex Saint-Amans) Quélet, *Hebeloma* sp. and *Inocybe lanuginella* (Schroet.) Lange) to 10 with the southern England provenance which had many specimens of the types already mentioned and also *Cortinarius* sp., *H. fragilipes* Romagnési, *H. mesophaeum* (Pers. ex Fr.) Quélet *H. sacchariolens* Quélet, *Laccaria laccata* (Scop. ex Fr.) Cooke, *L. proxima* (Boud.) Pat.,

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Lactarius pubescens (Fr. ex Krombh.) Fr. and *Leccinum scabrum* (Fries) S. F. Gray.

Total numbers of sporophores, irrespective of species, were inversely related to rates of foliar yellowing. Whereas virtually none was associated with trees grown from seed from lat. 66°30′ N, which were completely defoliated in mid-August, many occurred where yellowing was delayed until late September (57°20′ N) and October (50°40′ N) (Fig. 1).

Because numbers of sporophores associated with *B. pendula* grown from seeds from different latitudes were systematically and inversely related to the rates of natural foliar yellowing, the effects of experimental defoliation (with scissors) were tested during 1978 on 192 saplings of *B. pendula* and *B. pubescens* Ehrh., which were aseptically and vegetatively propagated and planted in 1975. They were arranged in eight randomised blocks each testing $24(2\times3\times4)$ factorially arranged treatments including complete defoliation on either 25/26 July or 22/23 August compared with controls subject to natural defoliation which started at the end of September and reached 39% and 86% on 5 October and 3 November, respectively.

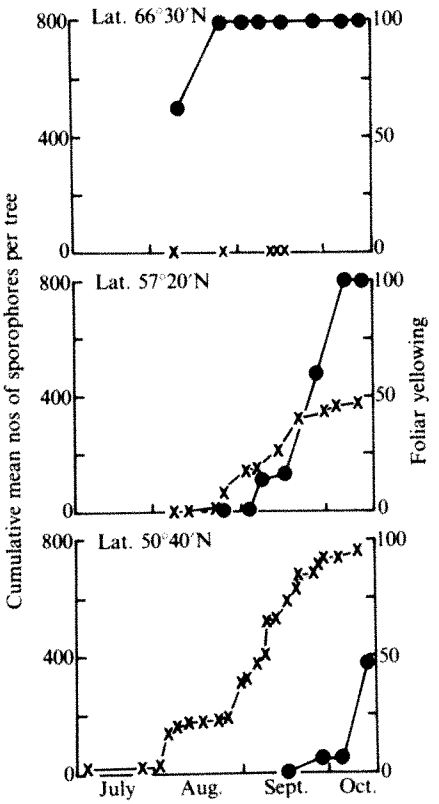


Fig. 1 Patterns during 1977 (6 yr after planting) of seasonal leaf yellowing (●), and the occurrence of sporophores (×) of sheathing mycorrhizal fungi associated with *Betula pendula*, when saplings from seed collected at three different latitudes were grown at lat. 55°52′ N (Bush Estate, near Edinburgh).

During 1978 sporophores, which appeared unusually early, were recorded at least twice during each of the six observation periods (A–F) from July to November (Fig. 2). By the end of July sporophores were associated with 25 of the 64 replicate control trees. A peak of 56 was reached in September/October before sporophore production ceased in November. From July to October numbers of newly produced sporophores ranged from 7.8 to 12.6 per tree in the different 3–4 week periods of observation (Fig. 2), contributing to a seasonal total, based on geometric means, of about 45.

During July and early August sporophore production around trees to be defoliated on 22/23 August was similar to that in the control series. As observations made on 25 August indicated, sporophore production ceased immediately after defoliation, with only one tree producing two sporophores during September, contrasting with 55 control trees averaging 10 each. This result duplicates the effects of defoliation in July but, in this instance, axillary and terminal buds started to 'break' in late August with a resultant new flush of leaves. From two trees producing a total of three sporophores in August, numbers of trees with sporophores increased to 16 and 43 in September and October as amounts of foliage increased; this trend was paralleled by increased mean numbers of sporophores per tree which reached 3.8 during October.

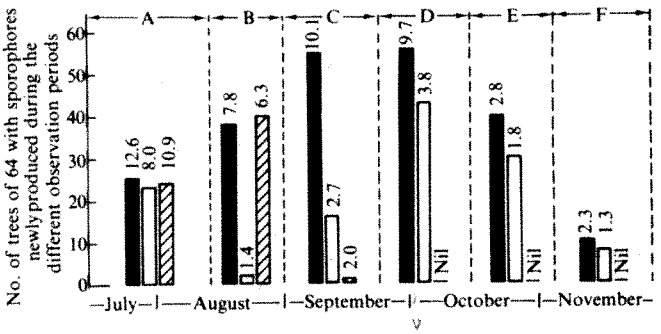


Fig. 2 Mean effects of defoliating *Betula* spp. on either 25 July (□) or 22 August 1978 (▨) on numbers of replicate trees with sporophores of associated mycorrhizal fungi newly produced within the different periods of observation. ■, Naturally defoliating set of control saplings. (Figures at tops of columns are mean numbers of sporophores per tree produced during each period; they were calculated from replicates producing at least one new sporophore during the relevant period.)

The effects of defoliation confirm that the production of sporophores by sheathing mycorrhizal fungi is disrupted when leaf function is impaired. Our observations support Lewis' contention[7] that many mycorrhizal fungi are 'ecologically obligately symbiotic biotrophs'. But, although they may be entirely dependent on their hosts for carbon[8], which they sequester, the rapidity of the responses, within 2 d of defoliation, suggest that the involvement of other substances produced by leaves, such as auxins, which can hasten the enlargement of sporophore primordia[9], should not be overlooked.

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matters arising

Upper limits on production rate of NO per ion pair

RECENTLY, Fabian *et al.*[1] have suggested that the rate of production of NO per ion pair during a solar proton event should be 2–2.5 instead of the 1–1.5 used by other authors. They justify the larger rate because of rocket measurements of Arnold[2] taken during an auroral precipitation event. While Arnold's[2] measurements may be correct, we question the application of these rates to energetic solar protons as these particles mainly deposit their energy below 80 km. Here we place realistic upper bounds on the NO per ion pair production rate.

First, at altitudes between 80 and 120 km, each N or N^+ produced can potentially form one NO molecule and each N_2^+ can potentially form two NO molecules by well known ion chemistry[3–5]. Thus, by bounding the N, N^+ and N_2^+ production rates, we bound the NO production rate.

To calculate the maximum NO production rate, we follow the method of Porter *et al.*[6]. However, in this computation we assume that 20% of the $N_2(a^1\Pi_g)$ excitations form two NO molecules and that all excitations of N_2 and N_2^+ with thresholds above 9.76 eV, the dissociation energy, also form two NO molecules. This calculation yields an upper limit of 2.68 NO per ion pair for 10 keV electrons, which is larger and thus consistent with rates implied by the measurements of Arnold[2] as quoted by Fabian *et al.*[1]. Although there are some uncertainties in our calculations due to the cross-sections, we feel that our results are accurate to ±15%, in view of the use of self-consistent energy degradation methods by Porter *et al.*[6].

Second, we note that at altitudes below 80 km the maximum rate of NO production should be bounded by the production rate of N and N^+ formation because at these altitudes N_2^+ reacts almost exclusively to form water clusters and is removed[3]. In a second calculation we subtract the N_2^+-produced NO, leaving only the direct N and N^+ sources of NO and find a maximum production rate of 1.46 and 1.53 NO per ion pair for protons with energies of 20 and 200 MeV, respectively.

We thus conclude that in the altitude range below 80 km it is difficult to justify NO/ion pair rates greater than about 1.5. In fact, the NO per ion pair production rates for altitudes <80 km are probably close to the 1.2–1.3 calculated independently by Frederick[3] and Porter *et al.*[6]. However, in the thermosphere between 80 and 120 km, numbers as high as those quoted by Arnold[2] are clearly possible.

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Amount is not supply rate in energy intake control

ON the theory that rate of energy supply determines intake[1], it is not as Rothwell and Stock state, "logical to assume that isoenergetic stomach loads will have similar effects on voluntary energy intake" (ref. 2), or that—when energy intake does not compensate for changes in energy expenditure—the animals may be "regulating food intake to meet their requirements for some dietary component other than energy" (ref. 3). The amount of energy in the stomach (or anywhere else) does not necessarily have a one-to-one relationship to the instantaneous delivery rate of that energy. The relationship depends on the physiological characteristics of the energy store's turnover.

We have emphasised[4–7] the relevance of others' findings that the rate of passage of energy from the stomach (and onwards to the tissues) depends to a considerable extent on the form that the energy is in, for example, the energy density of the diet[8]. We have calculated the long-term effects of some dissociations between amount and supply rate[5,6] and in the same context pointed out further such mechanistic as well as thermodynamic calculations which are required by an unmodified energy-rate or power-measuring theory of energy intake control. The report by Rothwell and Stock[2] clearly indicates the importance for theory and practice of extending the simulation work with experimental work to determine the as yet poorly analysed short- and long-term implications of satiation by energy metabolism.

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ROTHWELL AND STOCK REPLY—If, as Booth claims, "the rate of energy supply determines intake", why do rats force-fed a balanced diet adjust voluntary intake such that their total energy intake is the same[1] as that of free-feeding controls? The rate of energy supply is radically different in these two situations and should therefore produce marked differences in intake; it certainly causes differences in energy retention because the force-fed rats become obese. This latter finding indicates that a greater fraction of absorbed energy is available to metabolism and should, also according to Booth[2], result in compensatory adjustments to energy intake. In our original paper[1] we suggested that the theory of intake control proposed by Booth and Toates[3,4] was unlikely to provide an explanation of our results. Given that neither the rate of energy supply nor the metabolic fate of that supply influences intake, we see no reason for modifying that statement and, as a comment on Booth's final sentence, suggest that it is

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obviously necessary to extend the 'simulation work' if it is to keep pace with experimental developments.

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ROTHWELL AND STOCK[1] demonstrated that partially tube-fed rats gained significantly more weight than their free-feeding controls even though both groups ate very nearly the same amount. We suggest the possibility that the weight differences result from changed levels of activity. This approach obviates the paradoxical problems suggested by Rothwell and Stock.

A single energy reservoir model is used, as the rats are mature adult males unlikely to change their fat-free weight on over-feeding. We can describe the rate of change of fat[2] quantities by the following differential equation

$$\alpha\, \mathrm{d}f/\mathrm{d}t = \varepsilon P - \mathrm{BMR} - \delta(l+f) \quad (1)$$

where α is the energy equivalent of fat, f, t is time, ε is the efficiency of food utilisation, P is the average daily food energy intake, BMR is the basal metabolic rate, l is the fat-free weight assumed to be constant or slowly varying, and δ is a constant independent of the total weight and indicates the average activity level of the rats. We have made no direct distinction between feeding and non-feeding activity of the rats. MacMillan *et al.*[3] have demonstrated densimetrically that in man the BMR is independent of the amount of fat present; we assume this to be valid in the case of rats also. Because the BMR is independent of f we can solve equation (1) directly obtaining

$$w = [(\varepsilon P - \mathrm{BMR})/\delta][1 - \exp(-\delta t/\alpha)] + w_0 \exp(-\delta t/\alpha) \quad (2)$$

where we have replaced $(l+f)$ by the total body weight w and $(l+f_0)$ by the initial total body weight w_0.

We used Kleiber's 3/4-power law[4] to deduce the BMR. The BMR of a 'standard' 65-kg man is 6.27 MJ per day (1,500 kcal d^{-1})[5]; thus, the average rat will have a BMR of 128 kJ per day. Within the rat groupings we scale the BMR by a 2/3-power law as Kleiber's rule only relates to average interspecies quantities.

To use equation (2) we take α to be 39.2 MJ per kg (9,370 kcal per kg)[5]. The value of ε is unknown but should reflect losses due to the thermic effect and the passing of small energy amounts in the faeces. Antonetti[6] used a value of 0.9 in man. We take ε to be unity, because this must be the limiting case, and apply it to both tube-fed and free-feeding rats. Table 1 shows the data of Rothwell and Stock[1] and includes the results of our calculations. There is enough information to solve equation (2) for the activity coefficient, δ (Table 1).

In each case δ was less for the tube-fed rats than for the control groups, suggesting that the observed weight gain difference can be explained by the decreased activity of the test animals. The possibility that the tube-fed rats might increase their non-feeding activity is not reflected in the decreased values of δ. The average value of the activity coefficient of the controls was (423 ± 73) kJ per kg per day; the daily activity energy expenditure was 153 ± 26 kJ.

We calculate that the average control rat expended about 54% of the total energy on all forms of activity. Rothwell and Stock[1] consider activity energy to be negligible; Miller and Mumford[7] deduce that about 3% of the total energy is expended on activity by severely exercised rats by applying a fixed energy expenditure factor of 0.5 kcal per kg per km to rat, man and elephant. Morrison[8] has measured spontaneous activity calorimetrically and reports it to be 25% of the total energy expenditure. Our results agree slightly better with those of Morrison[8] by assuming ε to be 0.9 (ref. 6). Repeating the numerical calculation in this case we find δ to be 333 ± 56 kJ per kg per day, giving a 48% value for activity.

We can deduce the level of activity for rats completely tube-fed. We have plotted in Fig. 1 the ratio of the activity coefficient of the tube-fed rats to that of the control group as a function of the fraction of tube-fed energy. We have included the limiting point for no tube-feeding. The linear fit extrapolated to the limit of complete tube-feeding gives a value of 0.25. We conclude that 75% of caged rat activity is food related. This value cannot be compared with Morrison's results[8] because our definition of food-related activity is broader.

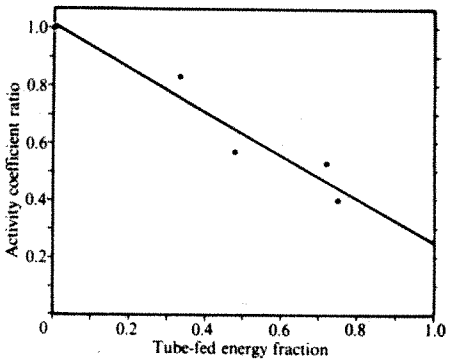


Fig. 1 The ratio of the activity coefficient of the tube-fed rats to that of the appropriate control group plotted as a function of the fraction of tube-fed energy. The point on the ordinate is the limiting value of the ratio for no tube-feeding. The other points are experimental. The linear least-squares fit is displayed and interpolates to a value of 0.25 for complete tube-feeding.

Equation (2) essentially describes the exponential increase of fat and allows an interesting speculation. The equilibrium value is approached to within $1/e$ of its final value in a time given by α/δ which is 93 ± 16 days. If a parameter proportional to the fat store size is sensed as an 'error signal' for a regenerative control process, and if the sensing time is similar to the rise time of the fat store, we can conclude that experimental studies dealing with problems of control must be carried out for times of the order of or longer than 93 days.

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Table 1 Data and calculated quantities

Expt	No. of animals	Av. initial weight, w_0 (g)	Av. final weight, w (g)	Energy intake, P (kJ d^{-1})	Duration of expt (d)	Fraction of tube-fed energy (%)	BMR, calculated (kJ d^{-1})	Activity coefficient, δ, calculated (kJ kg^{-1} d^{-1})
1 Control	6	436	480	378	30		145	383
Tube-fed	5	440	511	388	30	33.4	146	314
2 Control	12	321	388	405	21		118	454
Tube-fed	8	320	420	400	21	48.1	118	258
3 Control	6	300	358	385	23		113	525
Tube-fed	6	290	385	366	23	71.8	110	278
4 Control	12	402	442	367	21		137	367
Tube-fed	9	400	489	367	21	74.7	136	147

ROTHWELL AND STOCK REPLY—We need not go beyond Alpert's first paragraph to see that the paradox in the control of energy intake revealed in our paper and further discussed below (see reply to Mrovosky) has been confused with the question of why tube-fed rats gain more weight than control rats on identical energy intakes.

Our paper was not intended to explain this effect of 'meal-feeding' on energy metabolism; we simply took advantage of this well known effect so as to induce excessive weight gains and observe the effect on residual voluntary intake. We were concerned with how intake is related to the regulation of energy balance whereas Alpert is concerned with mechanisms responsible for the greater efficiency of energy utilisation in tube-fed rats.

Although Alpert's theoretical considerations are not pertinent to the paradox discussed in our paper we would, nevertheless, like to make the following points.

First, differences in body energy gain in animals on identical metabolisable energy intakes must be due to differences in energy expenditure. Apart from the 'basal' contribution, expenditure can vary due to changes in activity and/or the efficiency of energetic transformations within the body (that is, diet-induced thermogenesis or the heat increment of feeding). Alpert assumes that the efficiency of energy utilisation within the body is constant and therefore ascribes differences in total energy expenditure entirely to changes in activity. He states that this is logical and mathematically consistent but ignores the fact that it is just as logical and mathematically correct to fix activity at a constant level and ascribe the changes in total energy expenditure to differences in the efficiency of utilisation.

Second, there is evidence that metabolic efficiency is greater in meal-fed animals (see ref. 1 for review). Activity may also be affected but observations have not revealed any noticeable differences. Furthermore, we have calculated the difference in activity that would be required to explain the greater energy retention of our tube-fed rats. Parkes[2] has determined the energy cost of walking in the rat to be 0.105 kJ per km per g body weight. Applying this value to the rats which were tube-fed 74% of control intake, we estimate that control rats would have to walk 2,000 m per day more than tube-fed rats to explain the differences in energy retention. Note that these were adult rats housed in pairs in cages with floor dimensions of 40 cm × 30 cm, so that it is extremely unlikely, if not physically impossible, for two rats to each walk 2 km per day in these conditions—and even this assumes that tube-fed rats were completely inactive.

In conclusion, we are not convinced by Alpert's deductions concerning the cause of the greater energy retention in our tube-fed rats and he has not attempted to explain why these rats failed to depress voluntary intake to compensate for their excessive weight gain.

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Rothwell and Stock's paradox and multiple controls of feeding

ROTHWELL AND STOCK[1] reported recently that rats tube-fed a balanced diet intragastrically ingest overall (the tubed plus the voluntary intake) the same amount of energy as do control rats. However, the tube-fed rats gained more weight, presumably because of increased efficiency of energy utilisation associated with receiving intragastric loads in a few discrete meals. They considered it paradoxical that the total energy intake was not affected by the fate of the ingested energy.

These are interesting results, but how paradoxical they appear may depend on how strongly one considers energy content of the body to be the fundamental determinant of food intake. Most people, however, would probably accept that intake is under multiple controls, including such factors as palatability, previous conditioning and cost in obtaining food. Energy content of the body could be one factor in the control of food intake but is not necessarily the most important factor in every instance[2].

To determine if energy content of the body is one factor that affects food intake, or utilisation, in the intragastric tube-feeding situation, further analyses of the data might be useful. For instance, intake during the last few days of Rothwell and Stock's experiment, when the tube-fed rats were somewhat heavier than controls, could be compared with that during the first few days when the animals were similar in weight; if there were differences, energy content of the body would be implicated. If, on the other hand, it could be shown that intake and utilisation in the intragastric tube-feeding situation are independent of body weight, this would be much more surprising than the fact that tube-fed animals fail to control their intake in a way that maintains constant body weight. However, to show conclusively that intake is independent of weight might require longer experiments involving greater weight gains than those occurring in Rothwell and Stock's study.

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ROTHWELL AND STOCK REPLY—Mrosovsky suggests further analyses of the data to see if intake and utilisation are independent of the changes in body weight occurring during the experiment. A comparison of the energy intake of tube-fed rats during the first 5 days of each experiment with the intake during the last 5 days shows that they are identical, in spite of weight gains equivalent to 20% of starting weight. Furthermore, the rate of weight gain was remarkably constant throughout each experiment, therefore suggesting that both intake and utilisation were independent of body weight. By his own admission, Mrosovsky will be surprised by these results but we would agree that a conclusive demonstration of this independence of intake and body weight probably requires longer experiments with greater weight gains.

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Is the benzodiazepine receptor coupled to a chloride anion channel?

FROM their observations on the ability of several anions to facilitate the binding of ^{3}H-diazepam to membrane fragments from rat cortical tissue, Costa, Rodbard and Pert[1] have inferred that this binding site is closely associated with a chloride ion channel. Their data are qualitatively similar to ours with regard to the rank order of potency of the anions and we can confirm that the bromide ion also causes a significant increase in the affinity of the benzodiazepine receptor for the ligand diazepam without significantly affecting the total number of sites available.

Costa *et al.*[1] have drawn a correlation between the ability of various anions to enhance ^{3}H-diazepam binding and their ability to penetrate the activated inhibitory postsynaptic membrane of cat motoneurones as reported by Eccles[2]. As the binding experiments were carried out in cortical tissue membrane fragments it is probably advisable to attempt correlations with electrophysiological data on chloride channels obtained in the same region of the central nervous system. Such data are available in the literature[3] and comparison of the electrophysiological data from Eccles[2] and Kelly *et al.*[3] immediately shows that the anion-size specificity in cortical tissue is considerably less than that in the spinal cord; this may

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be explained by the fact that the chloride channel in the spinal cord is probably glycine linked whereas that in the cortex is linked to γ-aminobutyric acid.

We wish to refine the bulk correlations attempted by Costa *et al.*[1] by ranking the ability of the various anions to facilitate specific ^{3}H-diazepam binding and attempting correlations between the rank order so obtained and the ability of these ions to reverse the cortical inhibitory postsynaptic potential (i.p.s.p.) as reported by Kelly *et al.*[3]. It is difficult to ascertain which parameter from the anion facilitation data should be selected for such a correlation: percentage increase of ^{3}H-diazepam binding at maximal facilitation, percentage increase at an arbitrarily selected ion concentration, or the lowest ion concentration at which facilitation is seen. One parameter which encompasses these various possibilities is obtained by simply measuring the area under the curves for the facilitation of specific ^{3}H-diazepam binding against ion concentration (0–200 nM) (Table 1). None of the above correlations reached statistical significance, in the latter case the correlation coefficient with the area under the curve and the percentage probability of reversal of the i.p.s.p. was 0.61 whereas with the mean time to equilibrium it was 0.28. Using the non-parametric Spearman rank test we again found no significant correlation. The major criticism which can be raised against the use of these electrophysiological data is that the technical complexity of such experiments leads to quite large standard errors. It is interesting that Araki *et al.*[4] also observed differences in the ability of various anions to penetrate the cell membrane and in this case also we have been unable to find a significant correlation with the ability of the ions to facilitate ^{3}H-diazepam binding.

An alternative explanation is that the effects of these anions are due to their chaotropic properties. This possibility is not favoured by Costa *et al.*[1] because they feel that the concentration ranges over which chaotropic properties are normally observed are generally higher than the ED_{50} values for the anions in facilitating ^{3}H-diazepam binding, and also that perchlorate, while exhibiting powerful chaotropic properties, is relatively inactive in facilitating binding. In our study F^- caused a significant though small facilitation of the binding throughout the concentration range studied (5–200 mM), and SO_4^{2-} also caused a significant facilitation at 100 mM. It should be noted, however, that the rank orders of potency of the chaotropic properties are modified by several factors and are not invariant[5]. As an example of such a situation we may cite the work of Collins and Salton[6] in which 100 mM $Ba(SCN)_2$ is nearly as effective as 1% Triton X-100 in solubilising mannan from *Micrococcus lysodeikticus* membranes while being almost completely ineffective in the case of cytochrome *c*. Until we are more familiar with the characteristics of this membrane preparation it would be unwise to draw any conclusions about the effects of chaotropic ions upon it.

Table 1 Correlation between the facilitation of ^{3}H-diazepam binding and ion concentration

Ion	Area under specifically bound ^{3}H-diazepam curve (0–200 mM arbitrary units)	Weighted half time to equilibrium[3] (T_{Cl}/T_A)	% Reversals[3]
Br^-	704	1.04	100
I^-	650	2.24	93
NO_3^-	403	0.79	75
Cl^-	399	1.0	100
F^-	258	—	33
IO_3^-	173	—	0
SCN^-	165	1.98	67
ClO_3^-	126	0.41	80

Diazepam binding assays were carried out with well washed whole brain (less medulla-pons). Membrane fragments were prepared as described previously[7]. Membrane fragments equivalent to 125 μg protein were incubated on ice with 3 nM ^{3}H-diazepam in a total volume of 1 ml 100 mM Tris-citrate buffer, *p*H 7.1, and nonspecific binding was determined by displacement of ^{3}H-diazepam with 3 μM clonazepam. Sodium salts were used throughout with the exception of NH_4SCN, and compared with the maximal level of facilitation, inhibition was found with I^- at 200 mM and SCN^- at and above 50 mM. Areas under the curves were estimated by triangulation and even if SCN^- is omitted, because of its marked inhibitory activity, correlation coefficients do not increase markedly. Attempts to draw correlations between the electrophysiological data and the lowest anion concentrations required to produce a significant facilitation of ^{3}H-diazepam binding produced even weaker correlation coefficients.

We feel that the data so far available are insufficient to distinguish adequately between the possible explanations available for the facilitation of ^{3}H-diazepam binding caused by certain anions, and that it is premature to infer a direct linkage from benzodiazepine receptor to a chloride ion channel.

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RODBARD, COSTA AND PERT REPLY—In our recent study[1], only five anions (I^-, Br^-, Cl^-, NO_3^- and SCN^-) of a series of 18 tested increased the affinity of diazepam binding to rat brain membranes. Confirming the report of Tallman *et al.*[2], Martin and Candy[3] have reported the enhancement of diazepam binding by γ-aminobutyric acid (GABA) and have further noted the facilitatory effect of NaCl and KCl on the GABA-enhanced binding. However, we believe that their attempt to correlate the binding data with the electrophysiological data of Kelly *et al.*[4] is spurious for the following reasons. (1) The area under those biphasic binding curves has no apparent physicochemical meaning; (2) the anions have variable inhibitiory effects; (3) Kelly *et al.*[4] pointed out that the relationship between percentage reversal and anioninic mobility "is clearly not a simple one", and that the T_{Cl}/T_A equilibrium ratios 'only give an approximate estimate of the relative mobilities'.

It seems reasonable to view the facilitatory ion effect of anions on binding as an all or none phenomenon, as Eccles[5] described anion channel permeability. Viewed this way, it is striking that the same five anions which detectably enhance diazepam binding are the five most active anions according to Kelly *et al.*[4]. In fact, the probability of this being coincidental is about 1 in 1,000.

The possibility that these anions are effective by virtue of a nonspecific chaotropic effect is extremely unlikely in our view: (1) the active anions displayed an ED_{50} in the range of 2.8–20 mM, concentrations far below those usually necessary to elicit chaotropic effects; (2) strong chaotropic agents (guanidinium) are inactive or even decrease binding of diazepam to its receptor (unpublished data); (3) anti-chaotropic agents (fluoride, sulphate, phosphate) do not reverse the iodide enhancement of diazepam binding.

In conclusion, we agree with Martin and Candy's contention that differential linkage of neurotransmitter receptors with the same anion channel is a possibility. In fact, we have previously interpreted our finding[6] of large regional differences in the ability of iodide and GABA to enhance diazepam receptor binding as indicative of differences in the extent to which GABA and diazepam receptors share the same ionophore.

Finally, we report new data which we are preparing for publication. The compound 4,4′-diisothiocyano-2,2′stilbene disulphonic acid (DIDS) is a specific anion channel blocker in the erythrocyte membrane[7], which has an anion specificity similar to that of cortical neurones. In competition experiments, DIDS can completely reverse the enhancement of diazepam binding by active anions at concentrations (ED_{50} = 100 μM) which have no effect on control binding.

Although definitive proof of the linkage of benzodiazepine receptors with anion channels will require further electrophysiological substantiation and structural biochemical evidence, we believe that the benzodiazepine binding data

strongly support the notion[6] that benzodiazepine, GABA and perhaps glycine receptors interact with the same chloride ion channels.

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Another interpretation of communal breeding in green woodhoopoes

LIGON AND LIGON[1] presented information which they stated "suggests helping is a strategy for personal gain", and concluded that their observations "do not support an interpretation of helping behaviour relying on kin-selected altruism." Obtaining critical evidence on these two points is difficult, perhaps justifying presentation of preliminary data. Nevertheless, it should be pointed out that their results are open to other interpretations and, in my view, they are entirely consistent with my proposed theory[2] incorporating kin selection and individual selection, which they rejected.

Ligon and Ligon apparently invoked the personal-gain theory because the helpers showed no evidence of discrimination in their feeding of nestlings according to genetic relatedness. They did not actually demonstrate any personal gain; they merely inferred that it must exist because they saw that some helpers were not related to the young they fed. I consider this to be insufficient evidence on which to base the interpretation of personal gain. The few data presented are equally compatible with a zero-gain or even a loss (that is, altruism) hypothesis, as long as, on average, helping causes a gain in inclusive fitness to the helper. The apparent eagerness of helpers to feed young could be similarly explained.

Although personal gain to helpers by helping has been invoked by many authors and seems highly likely (for review see ref. 3), I know of no case in which a personal gain in terms of direct (individual) fitness has been convincingly demonstrated. Although there is some evidence for this[4], firm empirical data based on a strong-inference hypothesis are lacking. Apparently, Ligon and Ligon considered that their observations of a few helpers feeding unrelated young was inconsistent with kin-selection theory. They did not give their reasoning, but it seems to be based on two points: lack of discrimination of genetic relatedness in feeding efforts, and presence of presumed personal gain.

Discrimination was not evident in the few cases described, but the sample is unfortunately quite small (three cases). It may be of interest that a similar, though insignificant, lack of discrimination has been shown in jays (Fig. 1 of ref. 5). Even some parents in communal birds sometimes fail to discriminate between their own young and that of others (p. 375 of ref. 6), but one would not interpret this as evidence that parents in general have no genetic interest in their young. In any case, discrimination is not a necessary feature of kin selection[7–12], even though some authors have written as if it were[13].

Personal gain, although inconsistent with altruism, is entirely consistent with kin selection. This is not always evident in writings on kin selection[13,14], but, as clearly shown by Hamilton[11], the concept of inclusive fitness specifies that genetic costs and benefits be accounted across all relatives (not just offspring), regardless of the sign of the effect. My theory[2] for the evolution of helping was based on a combination of kin selection and individual selection. Although I called helpers 'altruists', altruism or sacrifice by the helper was not a necessary or even desirable part of the theory, which is equally applicable when personal gain to the helper is involved.

As kin selection requires neither discrimination by relatedness nor individual sacrifice, it is difficult to see how the data of Ligon and Ligon are inconsistent with kin selection. The important condition is that the indirect fitness (inclusive fitness minus direct or individual fitness[12]) be raised by helping, and the Ligons did not consider this point.

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LIGON AND LIGON REPLY—Brown's major criticism of our report[1] is that we rejected his theory[2] incorporating both individual and kin selection in the evolution of avian helper systems. Actually, we questioned only kin-selected or phenotypic altruism[3,4] in one kind of bird. Because green woodhoopoes can gain breeding status and helpers by first being helpers, whether or not they are related to the nestlings aided, we suggested that helping is a strategy for personal gain. For example, older birds gain and hold new breeding positions as a result of the support provided by younger individuals[5]. The system we describe corresponds to Hamilton's[4] selected category and can also be referred to as cooperation[6], reciprocal altruism[7], reciprocity[3] or mutualism[8]. Kinship ties may or may not be present but are not required for this kind of behavioural interaction.

Brown proposes alternative explanations for our observations. We feel that because of the many unknown factors related to kin-selection theory[9] the most parsimonious explanation for avian helping behaviour is personal or direct gain[10], when this can be ascertained. Very recently, Brown[11] has acknowledged that kin selection may not always be involved in communal helper systems, and Brown and Brown[12] have urged abandonment of the term 'altruism' to refer to avian helping behaviour. Both points are addressed in our report and we support both suggestions.

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reviews

Scientific R&D in France

R.W. Cahn

Le Pouvoir et la Science en France. By Pierre Papon. Pp. 315. (Editions Centurion: Paris, 1979.) Paperback Fr.65.

THIS book is one of the few detailed studies of the politics of scientific research and development in France. The author is professor of physics in one of the Grandes Ecoles, has been involved in the central planning machinery of French government, and is active in advising the French Socialist Party on matters of scientific research.

Professor Papon is extremely informative — the book is full of historical facts and figures; he is critical of French administrative procedures and again backs his criticism with much historical evidence; and in the last third of the book he steps back from the here and now to analyse, with an impressive grasp, the problems of method and objective involved in attempting to plan scientific research at all. Starting with the ideas of Bernal (from whose *Social Functions of Science* he quotes a long passage) he analyses very fairly the temptations, pitfalls and possible benefits of planning applied to science and development, and faces the inescapable conflicts between scientific research perceived as an autonomous liberal profession and science as a servant of the Commissariat au Plan.

An initial chapter reviews the history of the scientific profession in France; there is a tantalising glimpse of 'le grand patron' (he says of Pasteur that "il ne put faire triompher ses idées qu'une fois qu'il eût vaincu le scepticisme d'un patron important"), but that crucial species is not further analysed. Professor Papon then goes on to recount in detail the development and repeated changes in central scientific administration. As in Britain, the changes were many. A wilderness of advisory committees, both general and specialised, interministerial committees and delegations waxed and waned (though some, particularly the Committee of the Twelve Wise Men of which Professor Papon was a member, have shown great staying power). The CNRS, which sets up and finances research teams, and the DGRST, which administers studentships, fellowships and grants, together fulfil the functions of the SRC in Britain. Last year, the various bodies and committees were brought together under the aegis of a specialised Minister of Science — a condition which, for better or worse, Britain has not yet reached.

The author goes on to analyse in some depth the position of scientific research in universities and the Grandes Ecoles. Although recognising the major stimulus CNRS has given French science, he criticises it for having sapped the scientific independence of academic institutions: many CNRS laboratories are off-campus. The engineering Ecoles come in for special condemnation for having neglected research, and this is partly laid at the door of CNRS for being very fundamentally oriented, and partly attributed to the mathematical and analytical bent of the Ecoles, beginning with the nature of their competitive entrance examinations.

A substantial part of the book is devoted to research in industry and in the large quasi-industrial state institutions, especially the Commission à l'Energie Atomique, whose history is recounted in detail. This topic leads Professor Papon on to analyse de Gaulle's hostility to supranational (as opposed to bilateral) scientific arrangements, the cause of his unrelenting persecution of Euratom. A preference for bilateral or trilateral accords survives today: sometimes it works very well, as with the Airbus Consortium; sometimes badly, as with French attempts to foster the computer industry (again the story is told fully). Perhaps the crowning irony in French technological history, as Professor Papon describes, was the CEA's decision in 1969 to replace the home-grown graphite/gas reactors with American-style light-water reactors; Britain, which is more open to American suasions in general, has not yet been persuaded.

For a British reader, perhaps the most surprising claim made by Professor Papon is that it is virtually impossible for a research scientist or engineer to penetrate the top echolons of industrial management. He tells us that the R&D laboratory is a place of exile eschewed by ambitious would-be industrialists, and many Polytechniciens and other graduates of Grandes Ecoles, though trained formally as engineers, go straight into administration. We are often told in Britain that the French approach to engineering education as an elite enterprise ensures proper recognition of engineering skills at the highest level. Professor Papon's arguments suggest that this is only so if the engineers contrive to forget their professional origins. It would be instructive to have chapter and verse, and perhaps the Finniston Committee in the UK will provide it as part of their international comparisons.

Only part of one sentence in the book is devoted to the influence of research workers' trade unions, of which there are several in France. Could this have happened if the book had been written by an English socialist?

Professor Papon is often hard on his fellow-countrymen and on his country's institutions, but he generally documents his assertions, his reading has been catholic, and he freely makes international comparisons. This modestly priced book will be of interest and value to all those concerned with science politics, whatever their nationality. □

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Marine communities

Biogeography and Adaptation: Patterns of Marine Life. By G. J. Vermeij. Pp. 332. (Harvard University Press: Cambridge, Massachusetts, and London, UK, 1978.) £17.50.

THIS thought-provoking book is mainly concerned with the influence of biological interactions, especially predation, on the architecture of hard-shelled marine organisms and on the stability and biotic history of marine communities. It is divided into three main parts and is followed by an appendix, comprehensive reference list and index. The first part points out the occurrence of gradients in shell form of gastropods, bivalves and other organisms along latitudinal gradients and with depth below the high water mark. The author suggests that these primarily reflect anti-predatory defences which are developed to a greater degree and are found in an increasing proportion of species along a gradient from high to low

latitudes. The second part is concerned with inter-oceanic differences in the degree to which predation-related adaptations in shell structure are developed, and the third part reviews inter-oceanic differences in patterns of extinction and speciation along latitudinal gradients.

One has the impression that the author has drawn on a considerable personal experience of marine communities in many parts of the world and although most of the examples are taken from the Mollusca, particularly from the shelled gastropods, this does not seriously detract from the fundamentally interesting ideas which emerge from the text. The main problem with the book is that in many ways the intuitive ideas expressed in the text are ahead of the data necessary to support them. This is due in part to the difficulty of quantifying architectural differences in shell type and partly to the global comparisons ranging through geological time. Nevertheless the trends are illustrated as far as possible by profuse photographs, although the uninitiated (including myself) may find some of these difficult to interpret.

The chapter on vulnerability to extinction in stress-tolerant, opportunistic and "biologically competent" species and its relevance to biogeography throughout an evolutionary time scale will be of particular value to scientists and senior students with interests in palaeobiogeography. Nevertheless, I personally would have felt the work as a whole to have been more satisfying if there had been some more serious attempt to quantify the trends which are illustrated in the photographs. As the author himself admits frequently in the text, a great deal more data need to be obtained to verify some of the ideas raised in the book. However, informed speculation, particularly when expressed in a thoroughly readable and lucid style as it is in this book, can play an important part in promoting new work on the nature of animal adaptation. In this respect the book is a valuable addition to the literature and represents a significant contribution to our understanding of the ways in which biological interactions influence the structural design of living organisms.

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Radiation damage in semiconductor materials

Radiation Effects in Semiconductors and Semiconductor Apparatuses. By V.S. Vavilov and N.A. Ukhin. Pp. 280. (Consultants Bureau/Plenum: New York and London, 1978.) £30.

AN English translation of a scientific book by an authoritative Russian author is always of interest as an insight into Russian thinking. When the subject has strong connections with technological fields such as semiconductors and nuclear science, the interest is even greater. It is thus a disappointment to find that the original of this book was finished over ten years ago — much too long ago in a fast-developing subject such as this. Viktor Vavilov has done distinguished work in the field of radiation damage in silicon, especially in the fields of photoconductivity and photovoltaic effects, and such work requires an extensive background knowledge of radiation damage in bulk silicon and germanium. The surveys of these latter topics are useful, especially as the classic work in this field was done before 1967. Moreover, the accounts given of the carrier removal rates and other defect phenomena in these semiconductors include summary tables which can be used by research workers for a long time. On the other hand, the fact that the semiconductor technology discussed is over 12 years old lends to other parts of the book an overwhelming impression of ancient history. In addition, the crediting of work to various authors is vaguely done, so that many sections do not even serve as historical guide. No specifically Russian insights are revealed, as much of the best work discussed is American. Evidence that the present anonymous translator was not an expert in the field comes from the mangled versions of US workers' names (Prines for Pines; Byub for Bube, and so on — products of translation into Russian and then back to English). "Apparatus" in the title should, of course, read "Device".

The surveys of radiation damage and transient radiation effects seem fairly complete, except for a curious (even political?) omission of the work of Rappaport, Wysocki and co-workers on lithium doping in silicon. The discovery by this group of the action of lithium in the 'healing' of radiation damage in solar cells was an astonishing technological application of basic radiation damage physics. Could it be that Vavilov does not wish to admit that, although engrossed in basic studies of lithium in damaged silicon, his group missed this particular trick; or is there some defence application of the principle which puts the work outside the realm of discussion in the USSR?

Due to lack of other good summaries of the field, this book will find a place on the shelves of research workers, but its main rôle is that of an historical record.

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Mechanical properties of ceramics

Mechanical Behaviour of Ceramics. By R.W. Davidge. Pp. 165. (Cambridge University Press: Cambridge, 1979.) £15.

THIS book fulfils a need that has existed for some time now. The mechanical properties of ceramics have been a subject of comparatively intense research for some two decades or more — in fact, ever since the 1950s, when the hope was expressed that these brittle materials might be made tougher by more research, the optimism centring initially at any rate on the prospects for improvements in the ductility of polycrystalline ceramics by analogy, I suspect, with the history of improvements to cast iron. As becomes apparent from reading this book these hopes were not well founded. It would be hard to find a better author for this book than Dr Davidge who has been at the forefront of research in this subject for numberous years.

The layout of the book is essentially classical, mapping out our knowledge of the factors affecting the intrinsic elastic and strength properties of ceramics, the treatment of flaws through fracture mechanics and finally reviewing the essential details of plasticity and the reasons for many ceramics being inherently strong. These areas are reviewed in the first 5 chapters of the book.

Chapters 6-9 deal with the fracture strength,impact resistance, toughness, thermal shock, and last but not least, engineering design data. The scientific arguments are well presented and the accent is inevitably on the so-called engineering ceramics which are comparatively simple ones, usually single-phase polycrystalline and low in porosity. I suspect that the industrial ceramist who is often dealing with multi-phase, inhomogeneously textured ceramics often containing appreciable porosity, may view the book critically. For example, he will not find any comment on the well established strength/porosity relationships. Neither is there any attempt to demonstrate how the knowledge developed can be used to design a ceramic. No doubt Dr Davidge takes the view that our understanding has not developed sufficiently to take such issues on board.

However, the above remarks are not meant to detract from the excellence and usefulness of this book which I am sure will be welcomed by the scientist researching into the subject.

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This book gives a general account of the biology and ecology of the fall webworm *(Hyphantria cunea)*, an insect pest introduced to Japan from North America at the end of World War II.
The main topics covered include larval behaviour, life cycle programming, the biological clock, mating behaviour, population dynamics, past and present adaptation and speciation. Although the emphasis is given to the present status of the species in Japan, both the point of origin of the Japanese population on the American continent and the status of the larval types found there are discussed.

The Silkworm: an important laboratory tool

Ed. Y. Tazima
320PP. ¥9,000

The economic importance of silk, especially in Japan, has prompted exhaustive research on the domesticated silkworm, *Bombyx mori*. Tremendous progress has been made with this insect during the past 50 years in several sectors of pure and applied entomology, such as developmental physiology, nutrition, endocrinology, mutation studies, and toxicological testing. This progress derives not only from the ingenuity of the researchers concerned, but also owes much to the characteristics of the silkworm as an experimental animal. That is to say, it is easy to raise, easy to deal with, of moderate body size, shows uniform growth, and is readily available in large numbers at a single developmental stage.

Molecular Basis of Enzyme Function —Lysozyme—

By. K. Hamaguchi and K. Hayashi
216PP. ¥6,000

A considerable amount of modern biochemical research is directed toward elucidating the relationship between structure and function of proteins, and of enzymes in particular. Lysozyme is one of the simplest and best-studied enzymes; its structure and that of the enzymesubstrate complex have been analyzed in detail by X-ray crystallography, and the physicochemical properties of the lysozyme molecule in solution have been studied extensively. The aim of the present book is therefore to describe the physicochemical properties and enzymic action of lysozyme in relation to its structure, with special emphasis on the authors' own work, in order to develop a better understanding of the molecular basis of enzyme function.

Practical Mass Spectrometry for the Medical and Pharmaceutical Sciences

By. A. Tatematsu, H. Miyazaki, M. Suzuki and Y. Maruyama
296PP. ¥8,000

While numerous books are available that introduce the principles and basic applications of mass spectrometry, no single book has ever covered the theme of, 'how to use mass spectrometry practically and more effectively.' In the present volume, the mass spectrometer is discussed first, followed by the interpretation of mass spectra, fragmentation patterns, and a description of computerized high resolution techniques. Three full chapters are devoted to gas chromatography-mass spectrometry, covering topics from the column packing, glass capillary-column handling and sample derivatization to mass fragmentography and mass chromatography. The final four chapters provide a novel feature in that they deal specifically with advanced techniques, including the utilization of stable isotopes in drug metabolism, the principles and applications of never ionization techniques (viz. chemical ionization and field desorption mass spectrometry), and discussion of liquid chromatography-mass spectrometry combination analysis.

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The first nanosecond

Chlorophyll Organization and Energy Transfer in Photosynthesis. Ciba Foundation Symposium (new series). Edited by G. Wolstenholme and D. W. Fitzsimons. (Excerpta Medica: Amsterdam, Oxford and New York, 1979.) $38.25; Dfl86.

THIS book presents the proceedings of a meeting held in February 1978, on the primary processes of photosynthesis. The events in question span the time from the moment when light is absorbed, to the first separation of electric charges. All this happens in less than a nanosecond (10^{-9}s). The subsequent processes of biochemical electron transfer were deliberately excluded from consideration in this book. Those readers who find this too restrictive might consult *Primary Processes of Photosynthesis* (*Topics in Photosynthesis*, Vol. 2, edited by J. Barber; Elsevier: Amsterdam, New York and Oxford, 1977) which takes a broader view of the primary processes, at least as far as the millisecond time scale.

The present book is principally concerned with that remarkable molecule, chlorophyll and the way in which it is arranged in the chloroplast membranes to give the properties that are required for photosynthesis. In fact chlorophyll seems to exist in several types of proteolipid complex, each having a different function. The bulk of the chlorophyll serves as antenna to capture light energy and rapidly transfer it to reaction centres. The spectroscopic work of Katz and his group has convincingly shown that each reaction centre contains a special pair of chlorophyll molecules. The next step is presumed, by analogy with the simpler systems of photosynthetic bacteria, to be the formation of a radical pair in the reaction centre. From then onwards, the rest is chemistry. The details of these processes are being investigated from various directions. The structure of the system can be investigated by electron microscopy, and by biochemical investigation of the chlorophyll-protein complexes. The dynamics of energy transfer is investigated by spectroscopic techniques; at present only optical methods are fast enough. The most spectacular is laser-pulse spectroscopy which makes it posssible to study fluorescence lifetimes of the order of 10^{-11}-10^{-10}s. 'Picosecond' has become something of a buzz-word in this field. By this means the initial processes of energy transfer are now accessible, if the results are compared with suitable chemical and/or theoretical models.

The papers are interspersed with long discussions, (which even have their own reference lists and index). These are one of the most valuable features of the book, for here the experts on these disparate methods are trying to reconcile their results to give a coherent picture. How can the chlorophyll-protein complexes extracted by Thornber, Anderson and others, be identified with the various lumps seen in the chloroplast membranes in the freeze-etch electron micrographs of Staehelin and Arntzen? How does the theory of exciton transfer allow energy to be transferred from them to the reaction centres? Perhaps there is more chlorophyll in between, and so on.

The view of the photosynthetic unit which this book takes as its starting-point is considerably more advanced than it was a few years ago. It is possible, as Sir George Porter suggests in his Chairman's introduction, that a fairly complete understanding may emerge in the next few years. We are not there yet, but the book gives an indication of the techniques by which this understanding may be achieved.

Richard Cammack

Richard Cammack is Lecturer in the Department of Plant Sciences, King's College, University of London, UK.

Electrical phenomena in polymeric materials

Electrical Properties of Polymers. By A. R. Blythe. Pp.191. (Cambridge University Press: Cambridge, 1979.) £17.50.

THIS book provides an excellent basic introduction to a wide range of electrical phenomena in polymeric materials in terms of the molecular and electronic processes involved. It brings together the well-established area of dielectric relaxation, which has contributed greatly to our understanding of molecular conformations and correlated motions in polymers, with the less well understood phenomena of conduction and electrostatic charging, each of which are assuming increasing importance because of their relevance to polymer applications.

The introductory chapter includes a brief survey of the chemical and physical structure of polymers, providing a useful background to those with limited experience of polymer science. In chapter 2 the derivations of standard relationships between the equilibrium dielectric constant and molecular dipole moment are clearly outlined. The third chapter introduces and defines the frequency-dependent dielectric constants and discusses the associated relaxation parameters and underlying molecular reorientational processes. Chapter 4 presents a comprehensive account of dielectric measurement techniques. An illuminating discussion of current knowledge and ideas regarding ionic and electronic conduction mechanisms is given in chapter 5 together with relevant sections on conducting composites and resistivity measurements and some interesting suggestions on the feasibility of obtaining materials with novel conduction properties. The two final chapters are concerned with the practically important topics of dielectric breakdown and static charge phenomena, respectively.

The book is written with care and authority and is particularly recommended to students of polymer science as an educative text on electrical behaviour. Polymer research workers, industrial manufacturers and users of plastics should also find the book a useful acquisition, bearing in mind the detailed accounts of measurement techniques, the references to further reading at the end of each chapter and relevance of electrical properties to polymer applications.

B.E. Read

Bryan E. Read is in the Division of Materials Applications of the National Physical Laboratory, Teddington, UK.

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Raman effect

The Scientific Papers of C. V. Raman. Vol. 1: The Scattering of Light. Pp. 699. (Indian Academy of Sciences: P.O. Box 621, Bangalore 560 006, 1978.) £8

THE Raman effect is one of the most significant discoveries ever made in spectroscopy. Professor Sir C. V. Raman (1880–1970) was awarded the Nobel prize for physics in 1930 for his work on the scattering of light which resulted in this discovery.



Sir C. V. Raman

This clearly produced volume contains a selection of 94 of the scientific papers published by Professor Raman, either by himself or along with his students, on the subject of light scattering. It contains an historical introduction to the life and work of this "grand old man" of Indian Science by S. Ramaseshan who was responsible for selecting and editing the papers on behalf of the Indian Academy of Sciences. The collection contains papers on colloid scattering, molecular scattering, surface scattering and Brillouin scattering and also his pioneering publications on molecular anisotropy.

The monograph he wrote in 1922 on the *Molecular Diffraction of Light* and also his investigations on X-ray scattering and Compton scattering have been included, as these played a significant role in his discovery of the Raman effect in 1928. The intense interest he developed in the scattering of light was due to the visual impact the blue of the Mediterranean sea made on him on his first voyage to Europe in 1921. Even on this journey he was well prepared for experimental observations, for he carried in his pocket Nicol prisms, a small telescope to which polarisers and analysers could be attached, a slit, and even a diffraction grating. In later years he studied the scattering from pure organic materials which S.. Venkateswaran, a part-time worker in his laboratory, had succeeded in purifying by slow distillation *in vacuo*. Using filtered sunlight to irradiate these liquids in his work with K. S. Krishnan, Raman observed that all pure organic liquid showed this "feeble fluorescence" and he became convinced that this was the modified scattering of altered wavelength corresponding to the "milder fluctuations" in the state of the scattering molecule. Pointing a direct vision spectroscope on to the scattered track he saw that the scattered light contained not only the incident colour but at least another separated in wavelength from it by a dark space. Then replacing the filtered sunlight by a mercury arc he recorded sharp modified Raman lines for which the frequency shifts were identified with some of the characteristic infrared active vibrational frequencies of the molecule.

He was a man of wide interests in physics and published many papers on sound, ultrasonics, the solid and liquid state as well as optical phenomena. His physical intuitive abilities enabled him to jump several steps in mathematics and his capacity to expound complicated concepts in physics in a simple and appealing manner always made him a popular speaker. The lecture in which he announced his discovery of the Raman Effect and his Nobel lecture are included in the volume.

Sir C. V. Raman lived long enough to see the whole field of Raman spectroscopy rejuvenated and increased in importance by the use of lasers, which form ideal sources for the observation of the effect. This has enabled much of the earlier work to be repeated under conditions of greater resolution and signal to noise ratio. However, this volume reminds us of the way that Raman blazed new trails by discovering new phenomena such as shear waves in liquids and the 'soft mode' process in crystals. This book will be of value to all interested in light scattering and the Raman effect. At £8 it represents outstanding value for money as it is a bound volume of nearly 700 pages. Along with the International Raman conference held last year in Bangalore this record of his work forms a fine tribute to the achievements of this outstanding Indian scientist.

G. R. Wilkinson

G. R. Wilkinson is Professor of Physics at King's College, University of London, UK.

Marine productivity

Marine Production Mechanisms. International Biological Programme, Vol. 20. (Edited by M. J. Dunbar. Pp. 338. (Cambridge University Press: Cambridge, 1979.) £25.

LONG before the International Biological Programme (IBP) was thought of, international collaboration was a feature of marine science and it is probably because of this that this synthesis volume from the marine productivity section does not survey the whole field but presents a number of largely unrelated chapters summarising work carried out specifically under IBP auspices. The contributions fall into two groups, regional studies of productivity and ecosystem structure, with an *entr'acte* in the form of a short chapter by Velasquez on seaweed utilisation in the Phillipines.

The regional studies span seas from the sub-Arctic — primary production in Frobisher Bay, Arctic Canada, is described by Grainger — to the tropics — primary productivity in Indian waters being reviewed by Qasim. Detailed studies of food webs in the coastal waters of Japan are described by Hogetsu, the vertical distribution of phytoplankton in the open Pacific and Atlantic oceans by Semina, and production cycles in the Dutch Wadden Sea by van der Eijk. Parsons touches on mathematical modelling in his account of the Strait of Georgia, British Columbia, but there is no extensive discussion of the potentialities of this approach. Grindley discusses productivity and upwelling in the Benguela region and Dunbar relates production in the Gulf of St Lawrence to the pattern of water circulation, but the currently fashionable topic of the relationship of production to fronts in the sea has emerged since the IBP was concluded.

The contributions on ecosystem structure are of particular interest in being all by Russian workers. Pepita gives a quantitative account of energy flow in food webs in southern seas of the USSR and the tropical Pacific. A major role in energy supply is attributed to bacterial utilization of dissolved organic matter in tropical waters both by Pepita and by Shushkina and Vinogradov in the following chapter; the contributors to the earlier chapters largely assume that the classical radiocarbon technique of Steemann Nielsen gives an adequate measure of primary production. Neyman and Yablonskaya in the two next chapters employ the useful concept of trophic groups in studies of benthic faunas, and finally Golikov and Scarlato describe the pattern of distribution of benthic faunas in the shelf seas of the USSR. All this is most interesting but few details of methods are given so that, the references being almost exclusively to papers in Russian journals, assessment of the results will be difficult for most of us.

This book will be of great value to the specialist but cannot be recommended as an introduction to the study of marine productivity.

G. E. Fogg

G. E. Fogg is Head of the Department of Marine Biology at the University College of North Wales, Bangor.

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Contents of first issue:

Biological activity of anti-brain antibodies. The effect of anti-synaptic membrane antibodies on slow and action potentials of the rat sympathetic ganglion.
by V.L. Savić, B.D. Janković and J. Horvat (Belgrade, Yugoslavia).

Ontogeny of the immuno-neuro-endocrine relationship. Early thymectomy of the chick embryo.
by B.D. Janković, K. Isaković and Z. Knežević (Belgrade, Yugoslavia).

Rapid immunofluorescent detection of a viral infection in tissue culture.
by H.H. Weetall, A.A. Luderer, H.C. Orr and P.C. Probst (Corning, N.Y. and Bethesda, Md., USA).

Anti-BSA antibodies in the repertoire of the quasi-silent ($a3^-$) variants of the rabbit V_H allotype of the a-locus,
by P. De Baetselier, R. Hamers, C. Hamers-Casterman and W. van der Loo (St-Genesivs-Rode, Belgium).

A new kind of immunosuppression associated with erythropoiesis,
by E. Conway de Macario and A.J.L. Macario (Albany, N.Y., USA).

Quantitation of neutrophil infiltration in vivo,
by A.C. Issekutz and H.Z. Movat (Toronto, Ont., Canada).

The use of polyvinyl alcohols as tolerogenic carriers for suppression of anti-hapten IgE antibody responses,
by W.Y. Lee and A.H. Sehon (Winnipeg, Canada).

Cytotoxic effect of anti-lymphocytic globulin on leukaemic myeloblasts,
by M.F. Heyworth and S.C. Truelove (Oxford, U.K.).

The effect of antibodies to mononucleotides on RNA synthesis in vitro,
by O.V. Matarova and D.M. Goldfarb (Moscow, USSR).

Cytotoxicity of an antiserum to an acidic protein present in mesodermal macrophages.
by L.I. Persson, C. Wikkelsø and L. Rönnbäck (Göteborg, Sweden).

Immunosuppressive and contraceptive effects of testosterone and cyproterone acetate in guinea pigs,
by M. Vojtíšková, M. Poláčková, V. Vicklický and Z. Pokorná (Prague, Czechoslovakia).

Natural killing of human fibroblast cell lines differing in interferon-sensitivity,
by K. Ohmon, M. Kawata, K. Okumura, T. Kuwata and T. Tada (Chiba, Japan).

Publication Schedule and Subscription Information: Volume I in 6 issues (First issue scheduled for July 1979)
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CATALOGUE OF THE OCEAN BOTTOM DEPOSITS COLLECTION IN THE BRITISH MUSEUM (NATURAL HISTORY) Part 1: Atlantic and Arctic oceans, including the European seas. (The first of two volumes).

H. A. Buckley, C. J. Elliott, Nancy M. Graham, L. R. Johnson, D. R. C. Kempe and D. B. Williams.
1979 On microfiche with introductory booklet £12.00

The Catalogue lists on microfiche the holdings of some 30,000 samples of Ocean Bottom Deposits in the Department of Mineralogy, British Museum (Natural History), consisting of surface samples, cores, manganese nodules, and dredged rocks. The Collection was founded on that of H.M.S. Challenger and contains many other interesting items. Full collection details are given for each sample: position, depth, ship, station, date, collection method, amount, and description, together with notes and references. 'Splits' of most samples are available for research purposes.

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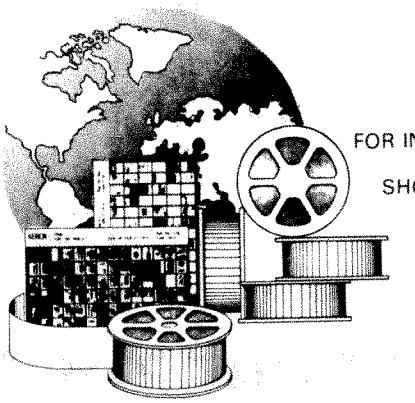
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announcements

Meetings

25-27 July, **Food Control in Action,** Guildford (Institute of Food Science & Technology, 105-111 Euston Road, London NW1, UK).

26-28 August, **Prospecting in Areas of Glaciated Terrain,** Dublin (J.A. Clifford, Murcliff, The Berries, Athlone, Co. Roscommon, Ireland).

7 September, **Solar Cell Degradation,** Southampton (P.t. Landsberg, Faculty of Mathematical Studies, University of Southampton, UK).

17-19 September, **Growth Hormone and other Biologically Active Peptides,** Milan (American Express Company, S.A.I., Piazza Mignanelli, 4, 00187 Rome, Italy).

9-14 September, **The Delivery and Targetting of Therapeutic Agents with Particular Reference to Liposomes,** Wye (The Biochemical Society, 7 Warwick Court, High Holborn, London WC1, UK).

16-21 September, **Sequence Organisation and Transcription in Eukaryotes,** Wye (The Biochemical Society, 7 Warwick Court, High Holborn, London WC1, UK).

2-4 October, **Grain Dust,** Manhattan (KSU Conference Office, Wareham Building, 1623 Anderson Ave, Manhattan, Kansas 66502).

3-5 October, **Biotechnology in Energy Production and Conservation,** Gatlinburg (Charles D. Scott, Oak Ridge National Laboratory, PO Box X, Oak Ridge, Tennessee 37830).

11-12 October, **Pathophysiology of Puberty,** Bologna (Serono Symposia, Via Casilina 125, Rome, Italy).

15-18 October, **Video Tradex International 1979,** Wembley (Sylvia Macdonald, Link House, Dingwall Ave, Croydon, Surrey, UK).

23-26 October, **1st International "Colloquy" of Angiology,** Florence (Spettabile, O.I. C., Via de' Bardi, 52, 50125 Firenze, Italy).

8-9 January 1980, **Diatomic Metals and Metallic Clusters,** Manchester (Mrs Fish, Faraday Division, The Chemical Society, Burlington House, London W1. UK).

4-8 February 1980, **IDOE International Symposium on Coastal Upwelling,** Los Angeles (International Upwelling Meeting, American Geophysical Union, 1909 K St, N.W., Washington, D.C. 20006).

6-8 February 1980, **Industrial Waste Water and Wastes,** Stockholm (3rd International Congress on Industrial Waste Water and Wastes, Box 21060, S-100 31 Stockholm, Sweden).

7-8 February 1980, **European Conference on Building Materials,** Copenhagen (Alena Enterprises of Canada, PO Box 1779, Cornwall, Ontario, Canada K6H 5V7).

7-9 February 1980, **Histocompatibility workshop and conference,** Lost Angeles (Dr Paul I. Terasaki, Dept of Surgery, UCLA School of Medicine, 100 Veteran Ave, Los Angeles, California 90024).

5-7 March, **Dredging Technology,** Bordeaux (Dorothy Thompson, British Hydromechanics Research Association, Cranfield, Bedford, UK).

10-14 March 1980, **Analytical Chemistry and Applied Spectroscopy,** Atlantic City (Dan P. Manka, 1109 Lancaster Ave, Pittsburgh, Pennsylvania 15218).

10-14 March 1980. **Natural Fibres,** London (Tropical Products Institute, 56-62 Grays Inn Rd, London WC1, UK).

20-21 March 1980, **International Chromatography Conference,** Florida (Alena Enterprises of Canada, PO Box 1779, Cornwall, Ontario, Canada K6H 5V7).

24-28 March 1980, **Eurocon '80,** Stuttgart (Prof. Dr W.E. Proebster, IBM Deutschland GmbH, Postfach 80 08 80, Germany).

24-25 March 1980, **Radio Transmitters and Modulation Techniques,** London (The Institution of Electrical Engineers, Savoy Place, London WC2, UK).

24-28 March 1980, **Vacuum Devices,** Cambridge (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

25-27 March 1980, **Combined Municipal-Industrial Wastewater Treatment,** Dallas (Prof. A. Netzer, University of Texas at Dallas, PO Box 688, Richardson, Texas 75080).

26-28 March 1980, **Phase Transitions in Molecular Solids,** Exeter (Prof. A.J. Leadbetter, Dept of Chemistry, University of Exeter, Stocker Rd, Exeter, UK).

27-28 March 1980, **Coatings-80,** Atlantic City (Alena Enterprises of Canada, PO Box 1779, Cornwall, Ontario, Canada K6H 5V7).

Awards

Dr Donald S. Fredrickson, Director of the United States National Institutes of Health has been the awarded Gerónimo Forteza Gold Medal.

The Institute of Physics, London, and the German Physical Society have awarded the 1979 Max Born Medal and Prize to **Dr J.B. Taylor** of the Culham Laboratory, UKAEA.

The American Geophysical Union have made the following awards: **Frank Press** received the William Bowie Medal; **Syun-Ichi Akasofu** received the John Adam Fleming medal; **Edward E. Irving** received the Walter H. Bucher Medal; **Wallace S. Broecher,** received the Maurice Ewing Medal presented jointly by the United States Navy and the AGU; Three James B Macelwane Awards were conferred to **Ralph J. Circerone, Michael C. Kelly, and R. Keith O'Nions.**

The 1979 Hewlett-Packard Europhysics Prize has been awarded to a group of five physicists from several European countries: **Eric A. Ash** (University College, London); **Jeffrey H. Collins** (University of Edinburgh); **Yuri V. Gulaev** (Institute of Radio Engineering, Moscow); **Kjell A. Ingebrigtsen** (Norwegian Institute of Technology, Trondheim); **Edward G.S. Paige** (Department of Engineering Science, Oxford University).

Professor A.G. Everson Pearse (Royal Postgraduate Medical School, London), has been awarded the Ernst-Jung-Prize for Medicine 1979. Two other scientists have been awarded the Ernst-Jung-Prize, **Professor Karl Lennert** (Kiel) and **Professor Ake Senning** (Zurich).

The Council of The Institute of Physics has awarded the 1979 Holweck Medal and Prize to **Professor A. Blandin** of the Universite Paris-Sud, Orsay, for his contributions to the theory of metals.

Reports & publications

UK & Ireland — May

Modern Organoselenium Chemistry. By Derrick L. J. Clive. (Tetrahedron Report No. 50.) Pp. 84. (Oxford and New York: Pergamon Press, 1979.) £4.95. [25

The Cotton Silk and Man-Made Fibres Research Association. Shirley Institute — Report and Accounts, 1977-78. Pp. 26. (Didsbury, Manchester: Shirley Institute, 1979.) [25

The Podrabinek Trial: Punitive Medicine or Fabrications Known to be False . . . ? (Translated from *Information Bulletin* No. 11 by the Working Commission for the Investigation of the Use of Psychiatry for Political Purposes, Moscow, 1978.) Pp. 39. (London: Amnesty International, British Section, 8/14 Southampton Street, WC2, 1979.) 35p; 80c. [35

The Ciba Foundation for the Promotion of International Co-operation in Medical and Chemical Research. 1978 Report. Pp. 53. (London: The Ciba Foundation, 41 Portland Place, W1, 1979.) [45

Ministry of Agriculture, Fisheries and Food. Report on Research and Development, 1977. Pp. iii + 79. (London: HMSO, 1979.) £2 net. [45

University of Cambridge. Annual Report of the Institute of Astronomy, 1977-1978. Pp. 36. (Cambridge: Institute of Astronomy, Madingley Road, 1979. [85

Education Systems: Approaches and Methods in their Evaluation. By J. N. Johnstone. (*Evaluation in Education: International Progress,* Vol. 2, No. 3, 1978.) Pp. 197-280. (Oxford and New York: Pergamon Press, 1978.) £6. [85

The Association of Public Analysts. Annual Report of the Council for 1978. Pp. 7. (London: Academic Press, 1979. Published on behalf of The Association of Public Analysts.) [85

Countryside Commission. New Agricultural Landscapes: Issues, Objectives and Action. Second edition. Pp. 14. (Cheltenham: Countryside Commission, John Dower House, Crescent Place, 1979.) *grats.* [95

Employment and Technology (A TUC Interim Report.) Pp. 50. (London: Trades Union Congress, Great Russell Street, WC1, 1979.) 75p. [95

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Applications are invited for a post of Temporary Lecturer in Engineering Geology. This post is tenable for one year from 1 September 1979 or as soon as possible thereafter.

Salary within the range £4,232 to £5,048 per annum (£4,333 to £5,199 per annum from 1 October 1979).

Further particulars from Miss Doreen Watson, Northcote House, The Queen's Drive, Exeter, EX4 4QJ, to whom applications (7 copies, candidates overseas 1 copy) should be sent by 30th July 1979. Please quote reference number 3217.
2527(A)

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

PHYSIOLOGY DEPARTMENT

A person is required for a new technical support post. Duties will include organising and carrying out determination of hormones by radioimmunoassay and other procedures and of metabolities by various analytical and autoanalytical methods; the developing of new assay procedures; and participating and co-operating in other departmental research.

Appointment will be as Scientific Officer (£2,839 to £4,415) or Higher Scientific Officer (£4,101 to £5,448) according to experience. Applicants should have a degree in a relevant subject, for appointment as SO. Degree or HNC in a relevant subject with at least five years' post qualifying relevant practical experience for HSO.

Application forms are obtainable from the Secretary, NIRD, Shinfield, Reading RG2 9AT. Quote reference 79/13.
2475(A)

UNIVERSITY OF STRATHCLYDE

Applications are invited for a

POSTDOCTORAL RESEARCH ASSISTANT

funded by the University, in the DEPARTMENT OF FIBRE SCIENCE AND TEXTILE TECHNOLOGY

to work with Dr. J. Ferguson on the production of regenerated cellulose fibres using novel techniques. Applicants should have experience in chemistry, physics or textiles.

Appointment will be for one year in the first instance on Range 1A of the national salary structure for research and analogous staff with a commencing salary up to £5,048 per annum rising to £5,199 per annum from 1 October 1979. Placing according to qualifications and experience. Superannuation benefit. The appointment will commence on 1 September 1979, or as soon as possible thereafter.

Written applications naming two referees (quoting R27/79) should be forwarded to Dr. H. M. Elder, Department of Fibre Science and Textile Technology, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW, from whom further information may be obtained.
2523(A)

The Gambia

Assistant Director
(Research and Specialist Services)
Department of Agriculture

Up to £9,975 (plus allowances)

Candidates, aged between 30 and 50, should hold a degree in Agriculture or related subject and have at least ten years' experience of agricultural research work on sub-tropical crops. They should also have some knowledge of administrative procedure in a Government Department of a developing country.

Duties will involve co-ordinating the planning and implementation of research and specialist services programmes.

Salary includes a substantial tax-free allowance paid under Britain's overseas aid programme. Basic salary attracts 25% tax-free gratuity.

Benefits include free passages, generous paid leave, children's holiday visit passages and education allowances, outfit allowance, appointment grant and interest-free car loan.

The terms on which civil and public servants may be released if selected for appointment will be subject to agreement with their present employers.

For full details and application form write quoting MD/214/NA.

Crown Agents

The Crown Agents for Oversea Governments and Administrations, Recruitment Division, 4 Millbank, London SW1P 3JD
2516(A)

UNIVERSITY OF EDINBURGH

DEPARTMENT OF GEOPHYSICS

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the post of Postdoctoral Research Fellow to work on the analysis of regional gravity data in Britain. The project is collaborative with the Applied Geophysics Unit of the Institute of Geological Sciences and other University groups, and has the objective of preparing and interpreting a regional gravity map of Britain.

Applicants, who will need to be numerate with computing experience, should have a good background knowledge of British geology. Although based in Edinburgh, they should be prepared to spend part of their time working in collaborating institutions.

The appointment is for 2½ years with a salary of £4,776 to £5,321 pa beginning on October 1st, 1979. Applications, including a full curriculum vitae and two referees, should be made by August 13th 1979 to: Dr R. G. Hipkin, Department of Geophysics, JCMB, Mayfield Road, Edinburgh, EH9 3JZ. Please quote reference 5032.
2505(A)

THE UNIVERSITY OF LEEDS
DEPARTMENT OF PHYSICS

POST-DOCTORAL RESEARCH FELLOW IN RAMAN SPECTROSCOPY

Applications are invited for a post of

RESEARCH FELLOW

in the above Department to work on a project funded by the Science Research Council. The successful applicant will study the anisotropy and polarization-dependence of Raman scattering from single crystals of organic compounds whose structures are related to that of poly(ethylene terephthalate). The work will be closely linked with that of a large research group studying the properties and microstructure of polymers. The starting date for the project is 1 October 1979 and the appointment will be made for a fixed period of two years.

Starting salary in the range £4,333 to £4,910 (under review) on the 1A scale for Research and Analogous Staff (£4,333 to £7,521). The candidate will work under the direction of Dr. D. I. Bower, who will welcome preliminary enquiries about the post (Telephone: (0532) 31751 ext. 6530 or 6485).

Applications, accompanied by a curriculum vitae and the names and addresses of two referees, should be sent as soon as possible to the Registrar, The University, Leeds LS2 9JT, quoting reference number 52/9/D.
2514(A)

RESEARCH OFFICER

LABORATORY ANIMALS CENTRE

The Director's research programme is primarily concerned with an evaluation of the advantages which accrue from the use in research of specific pathogen free animals, in particular the cat. A person in the Research Officer category is required to assist the Director in this programme. Opportunities will occur for collaborative work with other departments in the Centre, which cover the whole spectrum of Laboratory animal science.
The successful applicant will have a degree or equivalent in a biological science, and at least two years relevant research experience. Salary will be related to age, qualification and experience and is supplemented by £354 London Weighting. Contributory pension scheme.
Applications, together with a copy of curriculum vitae and the names of two professional referees, should be addressed to The Director, MRC Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey, SM5 4EF.

MRC
Medical Research Council
2417(A)

THE DEPARTMENT
OF
CLINICAL IMMUNOLOGY
has a vacancy for a
NEWLY QUALIFIED
TECHNICIAN

with relevant HNC and a sound knowledge of immunological/biochemical techniques, to work with the Senior Lecturer on problems related in particular to allergy to experimental animals.
Salary M.L.S.O. scale starting at £3,366 + £354 L.W.
Apply to the Secretary, Cardiothoracic Institute, Fulham Road, London, SW3 6HP, stating age, experience and qualifications and giving the names of two referees. 2472(A)

ENGLISH CHINA
CLAYS LTD

HEAD OF TECHNICAL
SERVICE (PRP)

English Clays Lovering Pochin & Co Ltd, the major operating Company of the English China Clays Group has a vacancy for a lively self-motivated person to head the Paint, Rubber and Plastics Section of Technical Service within Research & Development Department. The job involves managing a group of 12 people providing both a customer visiting service and the laboratory back up necessary for such a service.
Candidates should be graduates or possess an equivalent professional qualification and will have several years experience of a relevant customer technology, preferably with an emphasis on the rubber industry. Knowledge of a foreign language would be an advantage.
The position is based at the Company's well equipped laboratories at St Austell in Cornwall where facilities provided are excellent.
Competitive salary is negotiable and fringe benefits include Profit participation, pension and free life assurance; full assistance will be given with re-location expenses.
Applications, together with a curriculum vitae, quoting reference 276C to: The Company Secretary, John Keay House, St. Austell, Cornwall. 2474(A)

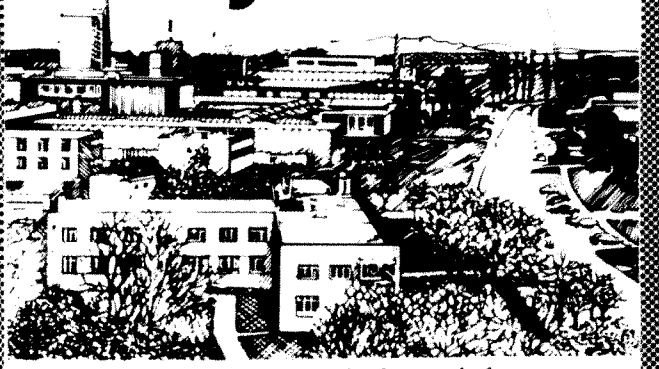
UNIVERSITY
OF EXETER
DEPARTMENT OF GEOGRAPHY
TECHNICIAN GRADE 3

Applications are invited for the post of:
GEOGRAPHY
TECHNICIAN
to work in the physical geography laboratories. The work involves the analysis of river water samples. Applicants should possess the Science Laboratory Technician's Part I Certificate or equivalent. Salary scale £2,931 to £3,336 pa with initial placement according to age, qualifications and experience.
Further particulars from Miss Doreen Watson, Northcote House, Queens Drive, Exeter, to whom applications should be sent by 27 July 1979. Please quote reference No. 5121. 2513(A)

UNIVERSITY
OF LIVERPOOL
BIOENGINEERING AND
MEDICAL PHYSICS UNIT
RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant/Senior Research Assistant/Research Associate to work on a research project concerned with the development of an artificial blood vessel.
The project has been running for three years and has led to a prototype vessel which is at present undergoing thorough evaluation, leading eventually to commercial exploitation.
The research assistant will be involved in the application and development of small scale production and testing apparatus, as well as being innovative in making improvements necessary for future generations of prosthesis. He/she will be expected to utilize His/her own specialities and interests in the research and development of the improved product.
The post is most suitable for someone with a degree in a related physical science, an interest in bioengineering and possibly research experience. Any queries regarding the post should be made to Mr. R. Clarke, 051-709 6022, ext. 2292.
The initial salary will be on the scale £3,689 to £5,321 pa, or £4,232 to £5,850 pa, for candidates with a PhD.
Applications, together with the names of three referees, should be received not later than 31st July, 1979 by the Registrar, The University, PO Box 147, Liverpool L69 3BX. Quote Ref: RV/695/N. 2528(A)

Health Physicists



Science graduates are invited to apply for professional operational Health Physicist posts in the Environmental and Medical Sciences Division of the Atomic Energy Research Establishment at Harwell. The Division is responsible for provision of health protection services for the establishment, and for a broadly based research programme in this field.
Harwell is the largest research laboratory of the United Kingdom Atomic Energy Authority, and one of Europe's premier contract research laboratories. It is situated in pleasant countryside mid-way between Oxford and Newbury, approximately 10 miles from the M4 motorway.

Duties
To be responsible to a Senior Health Physicist for running an effective radiological protection and occupational hygiene service for a major area of the establishment. The job is a challenging one involving advice to staff of all grades on protection standards and procedures, and supervision of a small team. Health Physicists are required to assist in training programmes for professional and other staff.

Qualifications and experience
Applicants should possess a degree in a scientific subject. Experience of work with radioactive materials or toxic hazards would be an advantage. Necessary specialist training will be provided.

Salary
Appointments will be made in the grade of Professional and Technology Officer Grade II. Salaries, which are currently under review, are in the range £3947-£6140 a year, depending on qualifications and experience. Benefits include four weeks annual holiday, plus public and privilege holidays, superannuation and sick pay schemes.

Accommodation
Rented housing may be available for married candidates living beyond daily travelling distance, and hostel accommodation can be provided for single officers.
For an application form and further details please write to: Mr. F. G. Northeast, Personnel Department 'E' (Ref: E3981), Room 204, Building 77, Harwell, Oxon. OX11 0RA, or telephone Abingdon (0235) 24141 Ext. 2408.
2550(A)

HARWELL

Technical Officer Plant Physiologist/ Agronomist

Plant Protection Division is the focal point in the ICI Group for the research, development and marketing of crop protection chemicals on a world-wide basis.

We are looking for an experienced post-graduate to lead a small research team engaged in the discovery of products to regulate the development of crop plants. Initially the work will be concerned with monocotyledonous crops and in particular temperate cereals. This is an important position within an expanding and exciting branch of the pesticide industry.

The job holder will be responsible for organising and technically supervising glasshouse and growth room experiments to test growth regulator chemicals, and for advising on field programmes. The person we recruit will also be a member of multidisciplinary work groups within the Plant Growth Regulation project team, and integration of the work programme with that of other disciplines is an important aspect of the job.

Applicants must have a PhD or equivalent experience in a biological discipline, preferably in the field of agricultural botany or whole plant physiology, and ideally will have gained further research or industrial experience. Specialist knowledge of the physiology and agronomy of cereals, and familiarity with the crop in the field are essential. Experience of working with plant growth substances and some background knowledge of chemistry are desirable.

Jealott's Hill Research Station is situated on an attractive farm estate between Bracknell and Maidenhead. We offer a very good remuneration, and we will help with relocation expenses where appropriate.

If you are interested in this vacancy, please write or telephone for an application form to: Mr D Spence, Personnel Officer, ICI Plant Protection Division, Jealott's Hill Research Station, Bracknell, Berks. Tel: Bracknell 24701 EXT 3350. Please quote Ref 79/PGR.

2488(A)

ICI Plant Protection Division

university college of swansea

Research Assistant

Applications are invited from candidates with a good honours degree in Biochemistry or a related discipline for the vacancy of **Research Assistant** in the **Department of Biochemistry**. The successful applicant will work on an SRC sponsored research programme concerned with the carbamoyl phosphate synthesis in the blue-green alga *Gloeocapsa*. This is part of a wider programme involving studies of nitrogen-fixation in blue-green algae.

The appointment will be for one year from October 1, 1979, the salary will be £3,775 per annum.

Further particulars and application forms (2 copies) may be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea, SA2 8PP, to whom they should be returned by *Friday, August 3, 1979.*

2503(A)

UNIVERSITY OF EDINBURGH

CENTRE FOR TROPICAL VETERINARY MEDICINE

LECTURER IN TROPICAL ANIMAL PRODUCTION

Applications are invited for the post of Lecturer in Tropical Animal Production. The appointment will be for two years.

The successful candidate will contribute to the M.Sc./Diploma course in Tropical Animal Production and Health run jointly by the Centre for Tropical Veterinary Medicine and the School of Agriculture.

The person appointed would become a member of staff of the Department of Tropical Animal Health. Preference will be given to graduates with experience of animal production in the tropics and specialist knowledge of an aspect of cattle production would be an advantage.

The salary offered would be in the range £3,689 to £8,452 (£3,775 to £8,992 from 1st October 1979).

Applications should be sent to the Director, Centre for Tropical Veterinary Medicine, Easter Bush, Roslin, from whom further particulars can be obtained. Closing date 16th July. Please quote reference 1065.

2405(A)

INSTITUTE OF CANCER RESEARCH: Technician required at the Sutton, Surrey, laboratories to assist research (supported by MRC) into the effect of drugs on the response of mouse tumours to X-rays. Experience not essential, but BSc, HNC or equivalent qualification a requisite. Salary in range £3,261 to £3,582 pa plus London Allowance of £354 pa (scales under review). Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref 301/B/79.

2535(A)

INSTITUTE OF CANCER RESEARCH: Technician required to assist in a project on human colon cancer at Pollards Wood Research Station, Buckinghamshire. Experience in microbiology essential. Salary in scale £3,261 to £3,471 pa (scales under review). Please write with full curriculum vitae in duplicate and the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 301/B/80.

2534(A)

UNIVERSITY OF GLASGOW

POSTGRADUATE RESEARCH ASSISTANT

A biochemist is required to carry out studies on neurochemical and behavioural aspects of lead exposure as part of a Scottish Home and Health Department funded project. The project, which will last for a minimum of three years, will be studying the influence of chronic environmental lead exposure on neuro-transmitter release and behaviour in animals together with aspects of brain morphology and function.

Salary will be on Range 1B of the salary scale for Research and Analogous Staff (£3775 - £5488) depending on age and experience. The post is superannuated.

The successful applicant will have the opportunity to register for the degree of Doctor of Philosophy in the University of Glasgow.

Applications should be made to Professor A. Goldberg, or Dr. M.R. Moore, Department of Medicine, Gardiner Institute, Western Infirmary, Glasgow, G11 6NT and accompanied by a Curriculum Vitae and the names and addresses of two referees.

In reply please quote Ref. No. 4484M.

2494(A)

FRENCH PHARMACEUTICAL GROUP

of International reputation seeks for its

RESEARCH CENTRE IN BRUSSELS A TRAINED SPECIALIST IN PHARMACOKINETICS

to lead a team engaged in the study of the kinetics and metabolism of new molecules in animals and man.

The suitable candidate should be a Doctor of Medicine, a Veterinary Surgeon, a Pharmacist or a Doctor of Chemistry. He or she must have had at least 5 years post-graduate experience in pharmacokinetics and preferably have worked in the pharmaceutical industry.

A good spoken and written knowledge of the French language is necessary.

All applications will be dealt with in the strictest confidence. They should be sent to Box W215(A) c/o Nature, 3 Dyers Buildings, London EC1

W215(A)

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF ASTRONOMY

Applications are invited for a post of Postdoctoral Research Assistant to carry out line profile studies of hot stars using photographic spectra and data from the Anglo-Australian telescope and I.U.E. satellite. Applicants should have some experience in the analysis of steller spectra and line profile computation. Some knowledge of the FORTH language and the use of mini-computers would be an advantage.

The appointment will be for one year from October 1st 1979 and the salary will be in the range £4,333 to £4,910 per annum (under review), plus FSSU/USS.

Applications together with the names of two referees should be sent as soon as possible to the Director, University Observatory, Buchanan Gardens, St. Andrews, Fife, Scotland KY16 9LZ.

2530(A)

The International Atomic Energy Agency

seeks

SENIOR GEOCHEMIST

FOR ITS LABORATORY IN MONACO

To work with biologists and other chemists on problems of the transfer of pollutants to and from marine sediments. PhD in Marine geology, geochemistry or chemistry and 10 years of appropriate experience. Initial contract two years, emoluments depending on experience US$36,922.30 tax-free. Additional allowances for dependants. Travel and removal expenses paid. Six weeks annual leave. Send curriculum vitae under Vacancy Notice No. 55/79 to IAEA, Division of Personnel, PO Box 590, A1011 Vienna, Austria. W220(A)

EUROPEAN MOLECULAR BIOLOGY LABORATORY

Scientific Programmer

Applications are invited for two posts in the Instrumentation Division to develop new algorithms and large portable computer programmes for mathematical and statistical analysis. Applicants should have extensive experience in writing and documenting user-oriented programmes. A background in handling large amounts of data and in areas like image processing, X-ray diffraction, system identification or large-scale linear and nonlinear programming would be helpful. A degree in numerical analysis or computer science is preferred, but a physicist or engineer with the above qualifications would also be considered.

The salary offered will be between DM3,500 and DM4,500 monthly, depending on qualifications and experience. Certain allowances are payable in addition, depending on personal circumstances.

The closing date for applications is 15 September 1979.

Please write briefly, quoting reference 79/14, with a curriculum vitae and the names of referees to:

Sir John Kendrew, Director General, EMBL, Postfach 10.2209, D-6900 Heidelberg, Germany. W214(A)

WELSH NATIONAL SCHOOL OF MEDICINE

(University of Wales)

DEPARTMENT OF HAEMATOLOGY

pplications are invited from science aduates for the post of RESEARCH FFICER in the above Department at eath Park, Cardiff. The person ppointed will take part in the investigation iron chelating agents of potential eraputic use and will be actively involved iron balance studies using animal odels. The appointment is for a three year riod. Salary on the scales for University esearch and Analogous Staff, starting lary dependent upon qualifications and perience but could be up to £4505 per num.

Applications in the form of a brief rriculum vitae with the names and dresses of two referees and quoting ref. o. H24/2/34 should be sent to the gister and Secretary, Welsh National hool of Medicine, Heath Park, Cardiff, 4 4XN, three weeks from the date of pearance of this advertisement. Further ormation available from Professor A. obs, Department of Haematology 22/755944 ext. 2375). 2492(A)

THE UNIVERSITY OF LANCASTER

CHAIR IN ENVIRONMENTAL SCIENCES

Applications are invited from scientists for appointment to a Chair in Environmental Sciences. The department currently has 19 full time academic staff. Its interests include a wide range of scientific subjects grouped into the four sections: Geological and Surface Water Processes, Atmospheric Processes, Solid Earth Sciences and Planetary Sciences. The department offers honours degrees in Environmental Sciences and contributes half of the content in each of the honours degrees in Ecology and in Geophysical Sciences. There are good workshop facilities and well equipped laboratories for research and teaching. The appointee will replace Professor A N Hunter as Head of the Department for a minimum period of five years.

Further particulars may be obtained (quoting reference L152/D) from the Establishment Officer, University House, Lancaster, LA1 4YW to whom applications (9 copies) naming three referees, should be sent not later than 31 August 1979.

2540(A)

CSIRO AUSTRALIA

Impending vacancies for Divisional Chiefs

Institute of Biological Resources

CSIRO, Australia's largest research organization with some 7,000 staff has recently grouped its research divisions into five institutes.

The objectives of the Institute of Biological Resources are to expand knowledge and understanding of Australia's biological resources and to discover and develop better methods for the management and use of its crops and pastures, forests and wildlife of both land sea with the aims of improving the efficiency and reliability of primary production while preserving and enhancing the desirable features of the Australian environments.

The Institute consists of eight divisions with a total staff of about 1,640 of whome some 600 are professional.

During 1980 and 1981, the position of Chief of the Divisions of Fisheries and Oceanography, Forest Research, Entomology and Wildlife Research within the Institute will become vacant owing to retirements. The positions will be advertised some months hence. But in the meantime, preliminary expressions of interest are invited, and **CSIRO** would be pleased to provide information on the work of the Divisions.

A Chief of the Division is responsible for the leadership, development, scientific direction and integration of the research programs of the Division, and is encouraged to promote active collaboration with other divisions, industry and research bodies working in associated areas.

Appointment to the organization is for an indefinite period and carries commonwealth superannuation privileges subject to normal conditions. The position of Chief is offered for a negotiable term of the order of seven years, with subsequent options for a further term, if mutually desired, or a senior position in the organization. Salaries are subject to negotiation, and currently are not less than $ A 34,374 p.a. (Chief, level 2).

As a brief introduction, the Division of Fisheries and Oceanography is based at Cronulla (Sydney, NSW) with other centres in Queensland and Western Australia. The Division of Forest Research is based at Canberra (ACT) with stations in Victoria, Queensland, South Australia, Western Australia and Tasmania. The Division of Entomology, based in Canberra, has centres in NSW, Queensland, Western Australia and Tasmania, and several overseas research groups. The Division of Wildlife Research based in Canberra has centres in the northern territory and Western Australia. Copies of the annual reports of the divisions are available from CSIRO Head Office and from the Australian Scientific Liaison Offices in London and Washington.

Mr. M.V. Tracy, Director of the Institute, would be pleased to discuss the position with potential applicants. He would also be pleased to receive advice concerning these appointments from people with a particular interest in them but who do not wish to be considered as applicants. He can be contacted at:

CSIRO, Head Office, P.O. Box 225, Dickson ACT 2602, Australia.

Mr. Tracy expects to visit the United Kingdom and USA in the next 12 months, and would be pleased to have discussions concerning the appointments with potential applicants and other interested parties.

2545(A)

EUROPEAN JOURNAL OF BIOCHEMISTRY

Applications are invited from graduates in biochemistry, PhD preferably, for the post of

EDITORIAL SECRETARY

at the journal's office in Liège.

Salary will be around £5,800 net per annum, or more according to special experience. Applicants should preferably be nationals of the member states of the European Economic Community.

Applications, which should include the name of at least one referee and complete curriculum vitae, should be sent as soon as possible to Professor Claude Liébecq, 69/054 Boulevard de la Constitution, B-4020 Liège, Belgium.

2533(A)

PROBLEM SOLVING MARKETEER
Physiological Measurements
(Excellent Salary + Car + Expenses)

Nicolet Instruments Limited is the United Kingdom subsidiary of the Nicolet Instrument Corporation of Madison, Wisconsin, who are manufacturers of Physiological Measurement Systems for clinical and research applications. The enormous success of these products in the United States has led to plans to expand this business into the United Kingdom.

The job of the problem solver will be to increase the sales of our medical division products by visiting medical establishments throughout the United Kingdom discussing physiological measurement problems and proposing solutions. The problem solver will also organise demonstrations, meetings and exhibitions, write editorials, advertise and generally ensure the success and growth of this business.

The person we are looking for will be a creative physiological measurements practitioner with a broad knowledge of measurement techniques including evoked responses, histogramming, signal averaging, EEG analysis, have the ability to relate equipment features to problem solutions, have a pleasant personality and a creative approach.

We believe these qualities are likely to be found in a keen physiological measurements expert working in neurology, audiology, opthalmology, medical physics or a related field, who wish to develop their career in a commercial environment. Product training will be given to the successful candidate in the United Kingdom and the United States of America.

This senior position will carry an excellent salary, company car and expenses.

FOR FURTHER DETAILS PLEASE CONTACT:
The Managing Director, Nicolet Instruments Ltd., Budbrooke Road, WARWICK. CV34 5XH or telephone: WARWICK (0926) 44111.

NICOLET INSTRUMENTS LIMITED
BUDBROOKE ROAD . WARWICK CV34 5XH TELEPHONE (0926) 44111

2525A

PHARMACOLOGISTS IN BASIC RESEARCH

We are seeking for our department of Dermatology a senior and a junior pharmacologist to join our group engaged in the study of skin pharmacology.

The successful candidates will have working knowledge of the analytical technics used in molecular pharmacology as well as experience in the detection and isolation of pharmacological mediators.

In addition, the candidates will be familiar with and have an interest in developing *in vitro* and *in vivo* animal model systems.

The senior appointment requires 8 years of post doctoral research experience and ability to motivate and direct research team, and a knowledge in skin pharmacology would be advantageous.

The junior appointee will have a minimum of 3 years of post doctoral research experience and ability to work independently.

The newly created Institute, based near Nice in the South of France, is engaged in basic research into the structure and function of the skin and its disorders.

For further information, please send curriculum vitae to:
Box W222(A), C/o Nature
MacMillan Journals,
3 Dyers Buildings,
London EC1N 2NR W222(A)

UNIVERSITY OF OXFORD
REPRODUCTIVE BIOLOGY

Postdoctoral research assistant required to work with Dr John Clarke on a three year Nuffield Foundation grant to investigate, under laboratory conditions, prenatal mortality and the endocrine control of implantation in the vole, bank vole and wood mouse. Salary on Research Grade 1A (£4232 to £7145). Applications including curriculum vitae and names and addresses of two referees should be sent to the Administrator, Department of Agricultural Science, Parks Road, Oxford OX1 3PF by 1 September. 2546(A)

UNIVERSITY COLLEGE HOSPITAL MEDICAL SCHOOL
TECHNICIAN

required to work in the
DEPARTMENT OF CLINICAL HAEMATOLOGY

on killing of microorganisms by white blood cells at a biochemical and cellular level in normal and immune deficient subjects. Whitley Council MLSO Scale.

Submit applications giving two referees to The Secretary, University College Hospital Medical School, University Street, London WC1E 6JJ Reference: ERH/Tech. 2537(A)

BEATSON INSTITUTE FOR CANCER RESEARCH
SCIENTIST

A vacancy exists for a post-doctoral scientist with experience in immunology to work on a new Leukemia Research Fund sponsored project to isolate monoclonal antibodies to human leukemia cells. Experience in hybridoma technology or cell culture would be an advantage. The appointment will be for 3 years in the first instance on the University Research Grade 1A scale (MRC Grade II) in the range £5048-6108. MRC conditions of service will apply. Applications including the names of 3 referees should be sent to Dr. P. Goldfarb and Dr. G. Birnie, Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow, G61 1BD. 2471(A)

INSTITUTE OF CANCER RESEARCH: Post Doctoral Scientist required for a three year Fellowship in the Department of Cytogenetics and Immunogenetics, at Fulham Road, London SW3, to work with human premalignant and malignant cells *in vitro*; the study will involve the genetics of cytogenetically marked cell populations maintained and manipulated in tissue culture and xenograft systems. Experience in tissue culture and cytogenetics essential. Salary in scale £4,261 to £6,627 p.a. (MRC Scientific Non-Clinical) plus London Allowance of £502 p.a. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 300/G/3. 2477(A)

COMMONWEALTH AGRICULTURAL BUREAUX
Vacancy for SCIENTIFIC INFORMATION OFFICER

at the
COMMONWEALTH BUREAU OF ANIMAL BREEDING AND GENETICS, EDINBURGH

Duties: Preparation and editin of abstracts for the journ *Animal Breeding Abstracts* indexin information retrieval and dealing wit enquiries.

Qualifications: Applicants must have degree in the biological sciences, preferab experience of an animal science field, and reading knowledge of one or more foreig languages (or be willing to acquire suc knowledge).

Salary: In the scale £2839 to £5448 (und review), plus a compensatory allowanc (taxable but not superannuable) to offs superannuation contribution. Startin point according to qualifications an experience. Promotion to higher scales o merit.

Application forms: and full particula from the Executive Director Commonwealth Agricultural Bureau Farnham House, Farnham Royal, Sloug SL2 3BN.

Closing date for applications: 20 Jul 1979. 2493(A)

UNIVERSITY OF EXETER
DEPARTMENT OF BIOLOGICAL SCIENCES
ELECTRONS MICROSCOPIST

Applications are invited for the post c Experimental Officer in Electro Microscopy. The successful candidate wh should have considerable experience i electron microscopy will be responsible fc the day to day running and maintenance c the E.M. laboratory and equipment, an will also be expected to collaborate fully i the various ultrastructural studies bein carried out within the Department. Salar will be on the scale £4232 to £7145 pe annum replacement according to ag qualifications and experience.

Further particulars obtainable fro Miss Doreen Watson, Northcote Hous Queens Drive Exeter, to who applications (five copies) with the names c three referees, should be sent by 24 August 1979. Shortlisted candidates will b invited for interview on 18th Septemb 1979. Please quote reference number 321 2539(A)

UNIVERSITY COLLEGE HOSPITAL MEDICAL SCHOOL
BIOCHEMIST/ CELL BIOLOGIST

required to work in the
DEPARTMENT OF CLINICAL HAEMATOLOGY

on killing of microorganisms by wh blood cells at a biochemical and cellu level in normal and immune deficie subjects. Whitley Council biochem scales.

Submit applications giving two refere to The Secretary, University Colle Hospital Medical School, Universi Street, London WC1E 6JJ. Referen ERH/Biochem. 2536(A)

CHAIRMAN
of the
DEPARTMENT OF
PHYSIOLOGY
WAYNE STATE UNIVERSITY
SCHOOL OF MEDICINE

Creative and energetic leader to assume the chair of this large and diverse department July 1, 1980. More than 20 faculty members, large and modern research and teaching facilities. Applicants should have an established and productive research record, experience in teaching and administration, to be able to assume responsibility for the medical, allied medical and graduate teaching programmes of the department. Sex, race, religion or national origin will not be factors in the selection process.

Interested persons should reply by August 1st 1979 to:

Dr Bernard H. Marks,
Department of Pharmacology,
Wayne State University,
School of Medicine,
Detroit, Michigan 48201.

W227(A)

UNIVERSITY COLLEGE OF WALES
Aberystwyth

POSTDOCTORAL RESEARCH IN CHEMISTRY

Two posts are now available, with commencing salaries on the 1A scale for Research Staff (£4,333 to £4,910 per annum), for work on SRC supported projects:

1. for 2 years, with Dr S Evans, on application of electron spectroscopy in inorganic and surface chemistry, and
2. for 1 year, with Dr D E Parry, on theoretical studies of chemisorption using MO calculations.

Applications including full curriculum vitae and the names of two referees to be sent to the appropriate above-named member of staff at the Department of Chemistry, Edward Davies Chemical Laboratories, The University College of Wales, Aberystwyth SY23 1NE, as soon as possible. 2531(A)

MATHEMATICS AND PHYSICS DESK EDITOR

ne International Schools Division of acmillan Education Limited, singstoke, requires a Desk Editor to rk on a rapidly expanding list of athematics and Physics textbooks for ird World countries at both primary and ondary level.

e candidate should be a young graduate th Mathematics and Physics alifications and some teaching perience, preferably in developing untries. Editorial experience would be vantageous but not essential.

ter a period of training and assessment, candidate will be expected to deal with ir own projects through all stages of ing and production so a responsible, lligent and highly meticulous attitude he work is essential.

ase apply in writing to Sheilagh Browne, sonnel Manager, 4 Little Essex Street. ndon WC2R 3LF. 2519(A)

MACMILLAN EDUCATION

PROGRAMME DIRECTOR

SYNCHROTRON RADIATION

A dedicated source of synchrotron radiation based on a 2GeV electron storage ring is under construction at Daresbury Laboratory and is scheduled to come into operation during 1980. The design allows for up to twelve beam ports which could provide for up to about fifty experimental stations. A wide ranging scientific programme in physics, chemistry, biology and materials science is envisaged involving universities and other research institutes from all over the United Kingdom and Europe.

Applications are invited for the post of Programme Director of this new facility.

The Programme Director will be responsible to the Laboratory Director for the implementation of the scientific programme on the Synchrotron Radiation Source in accordance with the decisions of the Facility Committee which advises the Council's Science Board on the usage of the new Source and gives approval to experiment proposals. The work will cover the organisation and administration of the experimental programme and the provision of equipment and facilities required to support it in collaboration with the user groups. There is an associated programme of theoretical research, and close liaison with the Theory Group is essential.

The existing group of scientific and supporting staff for this activity will be expanded to cover a wider range of science and the experimental programme will also be supported by staff from other divisions of the Laboratory. The appointee will be expected to be an active collaborator in the research being undertaken on the facility.

The successful candidate will be a scientist with an established reputation in any area of research relevant to the use of synchrotron radiation, the administrative ability to co-ordinate a complex operation and the personality to secure the effective co-operation of the users and the staff of the Laboratory.

The post will be permanent. Alternatively, the Council will consider secondment from, or a joint appointment with, the candidate's own establishment.

The salary will be within the range of Deputy Chief Scientific Officer £13,359 – £15,116 (with a further increase in April 1980).

Applicants of either sex, should write no later than 31st July 1979 giving details of qualifications and experience to:

The Director
DARESBURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD

2500(A)

UNIVERSITY COLLEGE CARDIFF

Applications are invited for the post of LECTURER IN PLANT ECOLOGY in the DEPARTMENT OF PLANT SCIENCE. Salary range: £4232 - £8452 p.a. Appointment to be made at lower end of scale. Duties to commence 1st October 1979.

Application (2 copies) together with the names and addresses of two referees should be forwarded to the Vice-Principal (Administration) and Registrar, University College P.O. Box 78, Cardiff CF1 1XL, from whome further particulars will be available. Closing date 31st August 1979. Reference 1848. 2491(A)

THE SALMON RESEARCH TRUST OF IRELAND INC.
BIOLOGIST

Applications are invited from persons with at least a good 2nd Class Honours in Zoology or equivalent qualifications for posts of Biologists at the Trust's installations near Newport, Co. Mayo. Starting salary not less than £4,000 rising to £6,450. Accommodation provided. Full details from The Secretary, St. James's Gate, Dublin 8. (Tel. Dublin 753645). Closing date for applications, 15 August. 2544(A)

POSTDOCTORAL POSITION

available to study transcription of cloned *drosophila* tRNA genes in *e. coli* and in vitro using RNA polymerase III. Project includes extensive collaboration with other laboratories at University of British Columbia working on gene expression, *drosophila* genetics and chromosome structure. Experience with nucleic acids and nucleic acid enzymes desirable. Apply with curriculum vitae to: Dr G.B. Spiegelman, Department of Microbiology, University of British Columbia, 2075 Wesbrook Mall, Vancouver, British Columbia, Canada V6T 1W5 W228(A)

COMPUTATIONAL SCIENTIST IN PROTEIN CRYSTALLOGRAPHY

The Daresbury Laboratory has a vacancy for a computational scientist in protein crystallography to develop a comprehensive suite of protein crystallography programs and to investigate new computational techniques. The post will be based in the Computational Science Group at Daresbury but will involve close collaboration with the university groups.

Applicants should possess a PhD or equivalent experience, in the field of protein crystallography and will be expected to spend some of their time on a research project.

An IBM 370/165 and a Cray 1 computer are installed at the laboratory and access is available to the IBM 360/195s at the SRC Rutherford Laboratory. The laboratory also provides a purpose-built synchrotron radiation source for the universities which will be used by protein crystallographers.

Appointments will be made at salaries (under review) between £3695 and £6216 depending on age, ability and experience. Posts are normally available for a fixed term of 3 years and are superannuable.

Closing date: 3rd August 1979

For further information please write to or telephone Pella Machin Tel No. (0925) 65000 Ext. 528.

Applications should be sent together with curriculum vitae and the addresses of two referees, quoting reference number DL/690/T to

The Personnel Officer
DARESBURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD

2541/A)

The International Atomic Energy Agency

seeks

ORGANIC CHEMIST
FOR ITS LABORATORY IN MONACO

With a strong background in gas chromatographic analyses of natural samples, willing to work with biologists and other chemists on the problems of marine pollution. At least six years of experience required. Training in bio-organic analytical chemistry and chemistry useful. Initial contract two years, emoluments depending on experience US$31,359.05 tax-free. Additional allowances for dependants. Travel and removal expenses paid. Six weeks annual leave. Send curriculum vitae under Vacancy Notice No. 50/79 to IAEA, Division of Personnel, P.O. Box 590, A-1011 Vienna, Austria. W219(A)

UNIVERSITY OF LONDON
KING'S COLLEGE
DEPARTMENT OF ANATOMY

A POSTDOCTORAL RESEARCH ASSISTANT is required to work on pattern regulation and pattern formulation in regenerating amphibian limbs. The appointment would be for three years starting from October 1st, 1979. Applicants should be interested in cell biology and tissue level controls in developing systems. Salary would be between £4,333 to £4,910, plus £502 London Weighting, depending on age and experience.

Applications, together with names of two referees and full curriculum vitae to Dr. Nigel Holder, Anatomy Department, King's College, Strand, London, WC2R 2LS. 2551(A)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
(University of London)
Post-Doctoral Research Associate

Applications are invited for a 3-year appointment supported by the Science Research Council, to work on adhesion and locomotion of tissue cells. Candidates should possess a PhD degree, or expect to obtain one this year. Salary £4,857 per annum, rising to £5,340 per annum, (inc London Allowance).

Applications, including curriculum vitae and the names of two referees to Dr. David Gingell, Department of Biology as Applied to Medicine, The Middlesex Hospital Medical School, London W1P 6DB. 2482(A)

INSTITUTE OF CANCER RESEARCH in association with the Royal Marsden Hospital, Sutton, Surrey, require Junior Technician in the Department of Histopathology. Applicants should have a minimum of 5 'O' levels including Maths, English and two science subjects or (preferably) two science 'A' levels or ONC in Medical Laboratory Sciences. Training will be given in all aspects of histological technique and Day-release will be granted. Salary in scale £2,037 to £3,015 p.a. depending on age and qualifications (scales under review), plus London Allowance of £354 p.a. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 301/B/75. 2498(A)

COMMONWEALTH AGRICULTURAL BUREAUX

POST OF DIRECTOR

COMMONWEALTH BUREAU OF PLANT BREEDING AND GENETICS

Cambridge, UK

This vacancy arises from the retirement o Dr. R.H. Richens, Director of the Burea since 1964.

The main work of the Bureau is to collec and disseminate to research worker throughout the world information on th genetics, cytology and breeding o agricultural crops. This is done principall through the publication of *Plant Breedin Abstracts*, which comprises some twelv thousand abstracts per year, summarizin papers in over thirty languages. Like a others in the CAB series, *Plant Breedin Abstracts* is replicated in machine-readabl form and is accessible online through th DIALOG system.

The Director is responsible fo maintaining the high scientific standard c the journal and for the day-to-da management of the Bureau (nine scientif staff with clerical support).

Applicants should have appropriat scientific qualifications and detaile knowledge of plant breeding in bot temperate and tropical countries Experience in scientific editing is essenti and administrative competence is als required. Some knowledge of foreig languages and the linguistic problem involved in scientific information work desirable.

The scale for the post is £6609 to £846 with merit awards to £9450 (subject t revision). The starting salary will relate t qualifications and experience. The post pensionable.

The normal retiring age is 60, but th may be extended to 65.

Application forms and full particular can be obtained from the Executiv Director, Commonwealth Agricultur Bureaux, Farnham House, Farnha Royal, Slough SL2 3BN. Closing date: 3 August 1979. 2511(A)

UNIVERSITY OF NAIROBI KENYA

Applications are invited for the post of

RESEARCH FELLOW

in the

DEPARTMENT OF ZOOLOGY
Gegaga Institute of Tropical Endocrinology.

Appointee should have a PhD in Anim Biology (preferably fish) and is expected work on a variety of aspects of Biolo Endocrinology, Physiology a Hybridization of cichlid fish Tilapia wit view to improving tropical pond cultu Salary Scale: Research Fellow K£2,016 3,312 pa (K£1 = £1.24 sterling). The Brit Government may supplement salary range of £4,278 to 4,818 pa (sterling) f married appointee or £2,730 to 3,156 (sterling) for single appointee (review annually and normally free from tax) a provide children's education allowan and holiday visit passages. Fam passages; non-contributory medi scheme; FSSU or SSSF; subsidiz housing/housing allowance.

Detailed applications (2 copies) w curriculum vitae and naming 3 referees be sent direct to Registrar (Recruitm and Training) University of Nairobi, P Box 30197, Nairobi, Kenya by 8 Aug 1979. Applicants resident in the UK sho also send one copy to Inter Univers Council, 90/91 Tottenham Court Ro London, W1P 0DT. Further details may obtained from either address. 2481(A

UNIVERSITY OF SYDNEY

LECTURESHIP IN GEOGRAPHY

Specialist in Coastal Zone Resources and Management required for Coastal Studies Unit, which is incorporated in the inter-departmental Marine Studies Centre. Appointee will be required to participate in undergraduate and postgraduate teaching and research. He/she should have a substantial background in economic, political and social aspects of land use management and have demonstrated ability to work with decision making bodies. He/she must also have sufficient expertise in coastal morphology and dynamics to work with the Coastal Studies Unit on comprehensive projects concerned with aspects such as beach erosion and longshore and onshore sediment transport. Preference may be given to applicants with experience of the Australian coast although applications from outside Australia welcome. A PhD or equivalent in postgraduate experience required.

The position is expected to be filled by a probationary appointment of three years, capable of leading to tenure, but if all the University's requirements for tenure are deemed to be satisfactorily met tenure may be granted at the time of appointment.

Salary range: $A15,786 — $20,737 p.a.

Applications including curriculum vitae, list of publications and names of three referees by *31 August 1979* to the Registrar, University of Sydney, NSW 2006, Australia, from whom further information available. Information also available from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF. 2509(A)

THE MEDICAL COLLEGE OF ST. BARTHOLOMEW'S HOSPITAL
(University of London)
West Smithfield,
London, EC1A 7BE

DEPARTMENT OF HAEMATOLOGY

GRADUATE RESEARCH ASSISTANT

Applications are invited from graduates in Biological Sciences to work on a project investigating the control of erythropoiesis. An interest in immunology and/or differentation would be an advantage. The project is funded by a Wellcome Trust for three years under the supervision of Dr. T.A. Horton.

The post is tenable from October 1st 1979, and the salary will be in the Research Assistant Range 1B.

Applications, with the names of two referees and curriculum vitae should be sent not later than 27th July 1979 to the Secretary of the Medical College at the above address, quoting reference 843. 2549(A)

TWO MEDICAL LABORATORY SCIENTIFIC OFFICERS

required in the Jerry Lewis Muscle Research Centre, Hammersmith Hospital to participate in research projects on neuromuscular disorders.

a) To work on electron microscopy of muscle. Previous EM experience preferable.

b) To carry out tissue culture of human tissues. Previous experience of tissue culture preferable.

Both posts on MLSO scale of £3,621 to £4,680 per annum plus £354 London Allowance. Initially according to age and experience.

Applications with names and addresses of two referees to Professor V Dubowitz, Department of Paediatrics, Hammersmith Hospital, Du Cane Road, London W12 0HS. 2524(A)

ICI Pharmaceuticals Division Alderley Park

have vacancies for:

Graduate Pharmacologist

To work in Central Nervous System Research. The successful applicant will join a research team engaged in the search for novel psychotropic drugs, in particular drugs which may be of use in the treatment of schizophrenia.

Post graduate experience in neuropharmacology and a capacity for independent work will be an advantage. Ref: DB 4.

Experimental Toxicologist

To work in our Safety of Medicines Department. The successful applicant will supervise a small team of laboratory staff and will be responsible for the running of short term and long term toxicity studies in rodents, lagomorphs and marmosets from the receipt of protocol to the writing of the final report.

Applicants must be Home Office licensees with a wide range of experience of toxicology on rodents or lagomorphs. Experience with marmosets and other primates would also be desirable. Applicants must also have had experience in managing work groups, with a minimum amount of supervision and be used to working to the highest standards of G.L.P. Education to H.N.D. level is also desirable. Ref: DB 5.

The Division's research laboratories are situated in pleasant surroundings but within easy reach of main road and rail routes. Conditions of service, salary and assistance given to married staff in moving house are designed to attract and retain staff of high calibre.

Applicants, male or female, should write quoting the appropriate reference number to:

Mrs D Bass, Assistant Personnel Officer, ICI Pharmaceuticals Division, Mereside Research Laboratories, Alderley Park, Macclesfield, Cheshire. 2499(A)

UNIVERSITY OF REGINA
(Canada)

ASSISTANT PROFESSOR DEVELOPMENTAL BIOLOGY

Applications are invited from candidates who have training and experience in the biology of development at the molecular, cellular and organismic level. Expertise in plant development and regeneration from somatic cells is desirable, but not essential. Applicants must have at least two years of postdoctoral experience.

The appointee will share in the teaching of undergraduate and graduate students and is expected to develop an active research programme. Minimum salary: $18,829. Starting date: as soon as possible.

Applications including a curriculum vitae, list of publications, a statement of research interests, and the names of at least three referees should be sent to Dr. H. Bertrand, Department of Biology, University of Regina, Regina, Saskatchewan S4S 0A2, Canada before August 20, 1979. W207A

THE UNIVERSITY OF MANCHESTER

DEPARTMENT OF PHYSICS

Applications are invited for the one-year post of Postdoctoral

RESEARCH ASSISTANT

in a team studying molecular collision process by high speed pulsed microwave spectroscopy. Initial salary range pa: £4,232 to £5,850 (£4,333 to £6,066 from October 1st) according to age, experience and qualifications. Superannuation.

Applications should be sent as soon as possible to Dr. M. A. H. McCausland, Department of Physics, Schuster Laboratory, The University, Manchester M13 9PL, from whom further details are available. 2479(A)

The New South Wales Institute of Technology
Sydney, Australia
SCHOOL OF LIFE SCIENCES

Principal Lecturer and Head
Department of Biochemistry

The New South Wales Institute of Technology is a corporate tertiary institution, established to provide a wide range of professional courses for those entering or already employed in industry, Government, and technological fields.

The School of Life Sciences occupies a six-storey building at Gore Hill adjacent to the Royal North Shore Hospital. There are well equipped laboratories for teaching and research in biochemistry, pathology, microbiology, environmental biology, physical biology and general biology. The School also jointly operates the Gore Hill Research Laboratories and an Electron Microscopy Unit with the Royal North Shore Hospital.

The appointee will be responsible to the Head of School for the teaching and development of undergraduate and postgraduate programs in biochemistry. This will include the continuing development of a postgraduate course in clinical biochemistry.

It is anticipated that the appointee will possess substantial post-graduate qualifications and have relevant teaching and administrative experience. A professional interest in clinical biochemistry or biochemistry related to industrial applications would be an advantage.

The President of the Institute, Dr. R.L. Werner, will be available in London for preliminary interviews from 10-20 September, inclusive.

Further information on the Institute and conditions of employment may also be obtained from Miss Challoner, at the address below.

Conditions and method of application.

The position carries a salary of $A 26,644 per annum.

Fares and a contribution toward removal and initial accommodation expenses will be provided for an overseas appointee. A housing loan scheme is also available. With consent of Council, academic staff are permitted to undertake limited consulting work.

Applications close on 24th August, 1979. Applicants should arrange for three confidential referees' reports to arrive by the same date. Applications should include: address, telephone number, personal details, experience, qualifications, affiliations, publications, research work conducted, and the names and addresses of those referees contacted. Applications and referees' reports are to be sent to:-

The Official Secretary, N.S.W. Government Offices, 66 Strand, London, WC2N 5LZ. Telephone: 01-839 6651. 2547(A)

ASSOCIATE DIRECTOR

EISCAT Scientific Association

The position of Associate Director for Scientific Programmes with the European Incoherent Scatter (EISCAT) Scientific Association will become vacant on 1 January, 1980, and must be filled as soon as possible after that date.

EISCAT is an organisation set up by scientific research bodies in six European countries. The UK partner is the Science Research Council. The purpose of the organization is to construct and operate a research facility in Northern Scandinavia for the purpose of conducting research related to the auroral ionosphere by means of incoherent scatter radar technique.

The person appointed will be required to work at the headquarters in Kiruna, Sweden. He/she must direct the scientific activity which will include reviewing proposals for experiments from scientists within the member countries and aid in the implementation of these experiments. He/she must also help organize cooperative experiments with groups conducting related research with other types of instruments, and be responsible for the operation of the observing stations in Kiruna and in Sodankyla, Finland, in close cooperation with the Associate Director responsible for the station in Tromso, Norway.

Applications are invited from persons with several years of experience in ionospheric and magnetospheric research. In addition to research experience administrative ability is desirable. Good knowledge of or experience in several of the following fields is required:

The physics of the troposphere, mesosphere, ionosphere and the magnetosphere;

Plasma physcis;

Measurement techniques, particularly involving the application of radio waves for diagnostic purposes;

Radar techniques and technology;

Digital computers, real time and off-line processing.

The appointment in Sweden will be for a two-year period. Remuneration and benefits will be in accordance with qualifications and experience; the terms and conditions of appointment will be negotiable.

Applications by letter, including a full c.v., should be sent by no later than 17 August to:

Mr. A. CHALLIS, SCIENCE RESEARCH COUNCIL, P.O. Box 18, SWINDON, WILTS., SN2 1ET.

2542(A)

BIOLOGICAL CHEMISTRY POLYSACCHARIDES

Applications are invited for posts of Scientist and Technician in a section of the Research and Development unit of Weddel Pharmaceuticals Ltd, a subsidiary of the Union International Company Ltd. The section is part of the Chemical Services Department at the Union International Research Centre, St. Albans and is engaged in investigation of complex carbohydrates for medicinal uses.

THE SCIENTIST will require a good honours degree in Biochemistry or Chemistry and an interest in the isolation and characterisation of natural polymers. Some industrial experience would be advantageous but not essential.

THE TECHNICIAN should be qualified to about HNC standard in science and have an aptitude for careful experimental work. Training in specific techniques will be given.

The laboratories are pleasantly situated and within easy reach of St. Albans city centre.

Applications please in writing, giving full career details to:

J. E. Dawes (AD 6093),
Weddel Pharmaceuticals Ltd.,
14 West Smithfield,
London,
EC1A 9JN

2480(A)

SCOTTISH UNIVERSITIES RESEARCH AND REACTOR CENTRF

1. REACTOR HEALTH PHYSICIST

2. RESEARCH FELLOW IN NUCLEAR MEDICINE

Applications are invited for the above posts which are at present temporary although there is a possibility of the first becoming permanent in the future. In both cases candidates should possess an honours degree in a physical science and have a knowledge of radioactivity and nuclear techniques. For the first post experience in health physics is necessary and for both some expertise in nuclear medicine, radiochemistry or a related topic is desirable. Successful candidates will be expected to pursue research in collaboration with Scottish University Departments. Applications including curriculum vitae and the names of two referees should be sent to the Director, Professor H. W. Wilson, Scottish Universities Research and Reactor Centre, East Kilbride, Glasgow, G75 0QU, not later than 3rd August 1979.

Salaries: Post 1: £4,232 to £8,452 pa (Lecturer Scale)
Post 2: £3,689 to £7,145 pa

University Superannuation Scheme. 2507(A)

UNIVERSITY OF NAIROBI — KENYA

Applications are invited for the post of LECTURER IN CLIMATOLOGY — DEPARTMENT OF GEOGRAPHY. Applicants should possess Ph.D. qualifications in Geography (with climatology as their principal areas of specialization). Interest in the studies of arid zones may be an advantage but not a condition. Applicants should also demonstrate interest in high level research evidenced by publications.

Besides the teaching of climatology at both undergraduate and postgraduate levels, the appointee should be able to participate in the teaching of at least two other areas of Geography. Salary scale: Lecturer K£2016 - 3312 pa (K£1 = £1.24 sterling). Family passages; FSSU or SSSF; non-contributory medical scheme; subsidized housing/housing allowance. Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Registrar (Recruitment and Training), University of Nairobi, P O Box 30197, Nairobi, Kenya by 8 August 1979. Applicants resident in the UK should also send one copy to Inter University Council, 90/91 Tottenham Court Road, London, W1P 0DT. Further details may be obtained from either address. 2484(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL

(UNIVERSITY OF LONDON)
DENMARK HILL, LONDON, SE5 8RX.

LIVER UNIT

Applications are invited for the post of POSTDOCTORAL SCIENTIFIC RESEARCH FELLOW

to join a multidisciplinary team investigating factors governing susceptibility to alcoholic liver disease. The successful applicant would be responsible for setting up tissue typing in the Liver Unit in collaboration with Professor R. Batchelor at the Hammersmith Hospital, and some previous experience in immunological techniques is essential.

Salary on the scale for Research and Analogous Staff Grade 1A (up to £7145 + London Weighting).

For further details, contact Dr. Roger Williams, Director of the Liver Unit at the above address.

Applications to the Secretary of the Medical School by 3rd August 1979.

2459(A)

INSTITUTE OF CANCER RESEARCH: Technician required at the Chester Beatty Research Institute, Fulham Road, London, SW3, to join a research team to work on the development of new chemotherapeutic agents for use in cancer. Cytotoxic drugs will be covalently linked to antibodies raised against tumour cells in order to target the drug specifically to the malignant tissues in the mammalian body. Candidates should possess an HNC, university degree or equivalent qualification in pharmacology, microbiology, biochemistry or other relevant subject. Experience in tissue culture methods would be an advantage. The post is available immediately and is tenable until July, 1983. Starting salary will be in accordance with MRC scales in range £3,261 to £4,293 p.a. (under review) plus London Allowance of £354 p.a. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref. 301/B/78. 2478(A)

THE LONDON HOSPITAL MEDICAL COLLEGE (University of London)

LABORATORY SCIENTIFIC OFFICER

Required in a research laboratory a technical assistant in a team with Professo Holborow investigating immunologic aspects of rheumatic and other disease The vacancy is suitable for someon interested in training in immunoenzym methods. Salary range (currently und review) £3,615/£4,284 including Londo Weighting, according to qualification The post is available from 1st September

Applications as soon as possible t Professor E. J. Holborow, Bone and Joi Research Unit, The London Hospit Medical College, Turner Street, Londo E1 2AD, quoting reference n BJRU/7/79. 2476(A)

ANGLO-AUSTRALIAN OBSERVATORY

Research Astronomers

The Anglo-Australian Observatory comprises the 3.9 metre telescope near Coonabarabran, NSW, and a laboratory at Epping near Sydney, Australia. There is a scientific staff of six astronomers nominated by the United Kingdom and Australia. Three of these positions become vacant prior to October 1980.

Candidates should have a strong background in physics or astrophysics. Appointments will be fixed terms of 1 to 5 years, with salaries in the range A15,422 to A30,530 per year, plus other allowances, fares, etc in addition.

The successful applicants will have the choice of being based at the laboratory or the telescope. They will provide assistance to visiting astronomers, and also contribute to the development, maintenance and general running of the AAT and its instrumentation. In addition they will have opportunities for personal research and collaboration with Australian and UK astronomers.

Applications should include:

1. Age, citizenship, country of residence and marital status.
2. Qualifications and experience, including date of PhD.
3. Astronomical research projects for which telescope time will be sought. Sufficient information is required to show the proposals would be competitive in applications to a time assignment committee.
4. Any instrument development project in which the applicant is particularly interested.
5. When applicant would be available.
6. Length of appointment envisaged.
7. Names and addresses of three referees.

Enquiries and applications should be addressed to:
Mr A Challis, Manpower Section, Science Research Council, PO Box 18, North Star Avenue, Swindon, Wiltshire SN2 1ET by 31 July 1979.

The three references should also be addressed to Mr Challis.

2510(A)

UNIVERSITY OF SUSSEX

SCHOOL OF MOLECULAR SCIENCES

POSTDOCTORAL RESEARCH ASSISTANT

pplications are invited for a position as a ostdoctoral research assistant to work ith Professor C. Eaborn on synthetic and echanistic aspects of the chemistry of erically-hindered organosilicon mpounds. The appointment will be for 1 ar starting from October 1979, and the arting salary will be in the range 232-£5591 p.a. Applications, including rriculum vitae and the names and dresses of two referees, should be sent as on as possible to Professor C. Eaborn, hool of Molecular Sciences, University Sussex, Brighton BN1 9QJ. 2501(A)

UNIVERSITY OF NOTTINGHAM QUEEN'S MEDICAL CENTRE

TECHNICIAN

DEPARTMENT OF BIOCHEMISTRY

pplications, male or female, are invited r the position of Technician in the epartment of Biochemistry. The ccessful candidate will be required to rk on the study of cell free protein nthesis in bacteria. Applicants should be aduates or hold HNC/HND or uivalent qualifications. Salary range 261 to £4,680 per annum depending on age and qualifications.

Application forms from: Mr F. C. eeby, Chief Technical Adviser, Medical hool, Queen's Medical Centre, ttingham. Closing date 27th July, 1979. 2512(A)

AGRICULTURAL RESEARCH COUNCIL

INSTITUTE OF ANIMAL PHYSIOLOGY
Babraham, Cambridge, CB2 4AT

Two vacancies exist at the Institute of Animal Physiology, Animal Research Station, Cambridge, at Senior Scientific Officer level.

CELL BIOLOGIST/ DEVELOPMENTAL BIOLOGIST

interested in working on basic problems of the development and differentiation of the early mammalian embryo. The person appointed will be expected to pursue independent studies on some aspect of the egg during preimplantation development. Experience in cell fusion, membrane biology or nuclear-cytoplasmic interactions would be an advantage. Ref. TR7.

BIOCHEMIST/ MOLECULAR BIOLOGIST

with an interest in applying modern biochemical techniques to the study of the mammalian egg during early development. Preference will be given to applicants who will be willing to pursue independent studies on some aspect of oocyte maturation, fertilization or preimplantation differentiation. Experience in protein synthesis or gene expression would be an advantage. (Ref MFH6).

Candidates should possess a first or upper second class honours degree together with at least four years relevant post-graduate experience, with preferably a PhD degree and a good record of published work. Salary in scale, which is under review, £5,154 to £6,898 pa. Non-contributory pension scheme. Application forms and further details may be obtained from the Secretary of the Institute. Please quote reference number. Closing date for applications 6th August 1979. 2515(A)

EUROPEAN TRAINING PROGRAMME in BRAIN AND BEHAVIOUR RESEARCH

TRAINEESHIPS

Traineeships of three, six or rarely nine months are offered to promising young scientists working in the area of brain and behaviour research in order to broaden their skills and knowledge in a field other than but related to their own. The grants are given to allow them to learn a particular technique abroad for which training is not offered in their own country. The trainee is expected to return to her/his original post upon termination of her/his training so that her/his institute will in turn be able to benefit from newly acquired skills.

All applications have to be accompanied by a letter of recommendation by the sending institute and by a letter of acceptance from the receiving institute. Preference is given to post-docs under the age of 35.

TRAVEL GRANTS

Travel grants are awarded to allow young research workers to visit another laboratory in connection with an on-going research project or to participate in international meetings and symposia. In addition special grants are to be awarded to students to enable them to take part in the yearly meetings of the European Neuroscience Association (ENA) which this year will take place in Rome on September 10-14.

Applications should be accompanied by letters of recommendation from the sending institute and of invitation from the institute to be visited. Applicants should be postgraduate research workers with the exception of applications to attend the yearly ENA meeting. In this particular case preference is given to postgraduate students.

The deadline for completed applications is *March 15 and September 15*. Further information and application forms may be obtained from:

Dr Stephanie Zobrist
European Science Foundation
European Training Programme in
Brain and Behaviour Research
1, quai Lezay Marnésia
F-67000 STRASBOURG
FRANCE
Tel: (88) 35 30 63
Telex: 890440

EUROPEAN SCIENCE FOUNDATION

W218(A)

INTERNATIONAL LABORATORY FOR RESEARCH ON ANIMAL DISEASES (ILRAD)
Nairobi, Kenya

IMMUNOLOGIST TO WORK ON PARASITIC DISEASES

There are in the laboratory of the Director of ILRAD two vacancies for immunologists to work on theileriosis. The research will include the infection and transformation of bovine lymphocytes and other cell types by theileria parasites and analysis of cell-mediated immune responses to the parasites and parasitized cells.

Applicants should have experience of cellular immunology and of parasitic or other infectious diseases.

Salary will depend on age and experience. Perquisites include moving allowance, housing and commutation allowances, medical and retirement benefits.

Applications, with *curriculum vitae*, list of publications and the names of three referees, should be submitted to the Director, ILRAD, PO Box 30709, Nairobi, Kenya by 12th August 1979. W203(A)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

DEPARTMENT OF ONCOLOGY

POSTDOCTORAL RESEARCH ASSISTANT

A postdoctoral research assistant is required, for a period of 3 years, to work on an MRC funded project. This will study the late effects of X-irradiation on the response of normal tissues to chemotherapeutic drugs. Applicants should have experience either in mammalian radiobiology, or in the study of cell kinetics in vivo.

Starting salary, according to age and experience, up to £5,034, inclusive of London Allowance. Applications, together with a curriculum vitae and the names of 2 referees should be addressed to Dr. E. Hamilton, Department of Oncology, The Middlesex Hospital Medical School, London W1P 7PN. 2517(A)

UNIVERSITY OF BRISTOL

DEPARTMENT OF PHYSICS

COSMIC RAY RESEARCH

Applications are invited for three posts of RESEARCH ASSISTANT, each of 24 months duration, for work within the cosmic ray studies group under Professor P.H. Fowler, FRS. These posts will be concerned with experiments to measure the high energy component of the ultra-heavy cosmic rays and the "anomalous" component of the low energy cosmic rays.

Experience with nuclear or spacecraft instrumentation, especially the use of drift chambers and microprocessor based data acquisition systems, would be appropriate.

Salaries will be on the scales for research staff grades 1A (2 posts) £4232 x £273 and 1B (1 post) £3689 x £272.

Applications should be made by 3rd August to Dr. R.M. Redfern, H.H. Wills Physics Laboratory, Tyndall Avenue, Bristol BS8 1TL. Telephone: Bristol 24161 ext. 110. 2506(A)

UNIVERSITY OF WARWICK

RESEARCH TECHNICIANS

The Department of Biological Sciences has vacancies in its four Research Groups, namely Chloroplast development, Developmental Biology (Molecular Genetics), Microbiology and Virology. The posts involve working under the direction of a senior scientist on a specific research project using the modern experimental techniques and instrumentation of molecular biology. The posts are for contract periods of one to three years and are available from October, 1979. Applications are invited from graduates and others with appropriate qualifications in biochemistry and/or microbiology. Salary either on Technician Grade 2B scale £2,759 to £3,138 p.a., or Grade 3 scale £2,931 to £3,336 p.a. Applications by letter giving full details of qualifications, experience etc to the Personnel Office, University of Warwick, Coventry CV4 7AL, quoting Ref. No: 47/D/79 by 20th July, 1979. 2396(A)

UNIVERSITY OF THE WEST INDIES
Trinidad

Applications are invited for the post of:

TEMPORARY LECTURER

AGRICULTURAL ENGINEERING
DEPARTMENT OF CROP SCIENCE

for two years. Specialization in soil and water management or mechanization of crop production is desirable. Salary scale 1977/78 (under review) TT$19,071 to 29,799 pa (£1 sterling = TT$5.18). Family passages; FSSU; unfurnished accommodation if available at 10% or furnished at 12½% or housing allowance of 20% of pensionable salary; study and travel grant. Detailed applications (2 copies) with curriculum vitae and naming three referees to be sent direct to Secretary, UWI, St Augustine, Trinidad as soon as possible. Applicants resident in the UK should also send one copy to Inter University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 2538(A)

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF BIOCHEMISTRY
SCHOOL OF BIOCHEMICAL AND PHYSIOLOGICAL SCIENCES

GRADUATE RESEARCH ASSISTANT

Applications are invited for a MRC funded graduate research assistant to work with Dr C R Lowe on the development of specific electrode systems for proteins, carbohydrates and steroids of clinical importance. Applicants should have a good Honours Degree in Biochemistry or Chemistry.

The appointment is for one year in the first instance, with the possibility of renewal for a further two years. The salary will be £3,775 pa on scale 1B for Research and Analogous Staff; USS benefits.

Applications giving date of birth, curriculum vitae and the names and addresses of two referees, should be sent to Mrs P. Vaughan-Smith, The University, Southampton, SO9 5NH by 30 July 1979, quoting reference 1085/R/N. 2495(A)

UNIVERSITY OF EAST ANGLIA

Norwich

RESEARCH IN ENVIRONMENTAL CHEMISTRY

Applications are invited for the position of Senior Research Associate on a project to investigate the chemistry of the air-sea interface, more especially the measurement of natural volatile organo-metallic compounds in surface waters with a view to evaluating their role in the global cycling of trace metals. The post is suitable for someone with a first degree training in chemistry and relevant postgraduate experience in chemistry or oceanographic/atmospheric services. The appointment is for two years on the salary scale £4232 — £7145 per annum plus USS.

Further information is available from Dr. P.S. Liss, School of Environmental Sciences, University of East Anglia, Norwich NR4 7TJ, to whom applications, including the names of two referees, should be sent as soon as possible. 2502(A)

UNIVERSITY OF LIVERPOOL

TWO TECHNICIANS (Grade 2B)

DEPARTMENT OF BIOCHEMISTRY

To assist groups studying (a) The biosynthesis and metabolism of sterols in higher plants and algae. (b) Hormonal control of insect development. Minimum qualification ONC or equivalent relevant qualifications. Minimum experience of 3 years.

The posts are tenable for 2½ years with possibility of renewal.

Salary within a range of £2757 — £3138 p.a., according to qualifications and experience.

Application forms may be obtained from The Registrar, The University, P.O Box 147, Liverpool, L69 3BX. Quote ref RV/686. 2468(A)

THE FACULTY OF MEDICINE Memorial University of Newfoundland requires a RESEARCH SCIENTIST who will be a staff member. The successful candidate must have considerable research experience involving messenger ribonucleoprotein complexes and translational control of protein synthesis in euckaryotic cells. In addition, the successful candidate must have graduate with a Ph.D. Degree from an approved college or university with major course work in Biochemistry or Molecular Biology. The incumbent will be primarily involved in conducting a research program in the characterization of the messenger ribonucleoprotein complex of euckaryotic cells. Current emphasis is on the role of these complexes in translational control of protein synthesis during myogenesis. Duties will also include responsibility for supervising research projects on the regulation of protein synthesizing machinery in both euckaryotic and prokaryotic cells. The salary for the position will be at the rate of $16,254.00 per annum. Applications or enquiries should be forwarded, not later than July 31, 1979 to Dr. Bruce Sells, Professor, Laboratories of Molecular Biology, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada. W221(A)

SUB-EDITOR

required to work on POLYMER, the international journal for the science and technology of polymers, within an expanding group of scientific research journals based in Guildford. Minimum qualification: first degree or equivalent, preferably in chemistry or the physical sciences; preference will be given to candidates with experience in sub-editing, proof-reading and handling scientific texts from edited version to publication. Good salary and career opportunities. Salary £3,880 + L.V.'s and reading allowance.

For further details apply to The Secretary to Publisher, POLYMER, IPC Science and Technology Press Ltd. P O Box 63, Westbury House, Bury Street, Guildford GU2 5BH, Tel: Guildford 31261 2543(A)

IPC BUSINESS PRESS

MEDICAL RESEARCH COUNCIL

MRC NEUROCHEMICAL PHARMACOLOGY UNIT

MRC Non-clinical Scientific Staff

A vacancy exists on the Scientific staff of the Unit for a NEUROPHYSIOLOGIST to undertake research on the actions of drugs and neurotransmitters at the single cell level in mammalian CNS. The Unit's research programme focuses particularly on the mode of action of anti-psychotic and other psychoactive drugs, and studies of neuropeptides.

A well equipped neurophysiology laboratory with computer facilities is available, and candidates should have several years postdoctoral experience in this or a related field of research.

Salary depending on age and experience, is expected to be within the range £5048 (-£6627) or £6886 (-£8452) but an appointment at a more senior level is not excluded. MRC Conditions of service, including superannuation.

Contact: Dr L Iversen, Director, MRC Neurochemical Pharmacology Unit, Department of Pharmacology, Medical School, Hills Road, Cambridge, CB2 2QD. 2486(A)

UNIVERSITY OF WARWICK

SENIOR RESEARCH TECHNICIAN

Required in the Department of Biological Sciences to organise the daily running of the technical services of a Virology Research Group and to assist with research under the personal direction of Professor D.C. Burke. The research is aimed at cloning of the interferon gene in bacteria.

Applications are invited from suitably qualified persons with several years experience of biochemistry and preferably tissue culture. Salary on the Technician Grade 5 scale: £3474 to £4056 p.a. The post is available initially for 12 months with the probability of extension for a further 5 years.

Applications in writing giving full details of age, experience etc., to the Personnel Office, University of Warwick, Coventry CV4 7AL quoting Ref. No: 48/3D/79, as soon as possible. 2487(A)

INSTITUTE OF CHILD HEALTH

SENIOR MEDICAL LABORATORY SCIENTIFIC OFFICER OR MEDICAL LABORATORY

SCIENTIFIC OFFICER

Applications are invited for the single post of SMLSO or MLSO to take part in a 4 year, Leukaemia Research Fund supported research project examining the molecular basis for the synthesis of onco-fetal proteins (particularly-feto protein) in human tumours. Experience with DNA and/or RNA extraction and assay a major advantage. Tissue culture experience also helpful.

The grading of the post (SMLSO or MLSO) would depend on the experience and qualifications of the successful applicant. SMLSO salary scale is £4347 to £4769; MLSO scale is £3366 to £4680 plus London Weighting.

Applications, accompanied by full curriculum vitae and addresses of two referees, to: Dr. J. Pritchard, Department of Haematology and Oncology, The Hospital for Sick Children, Great Ormond Street, London, WC1N 3JH. Enquiries about the post to Dr. Pritchard on 01-405 6200 Ext. 248. Closing date for applications 25 July 1979. 2548(A)

UNIVERSITY OF MANCHESTER

DEPARTMENT OF PHYSICS

Applications are invited for the post of

LECTURER

in the Department of Physics. The successful candidate will be expected to carry out experimental work within the Atomic, Molecular and Polymer Physics Group of the Department. Some preference will be given to candidates intending to pursue studies in the fields of coherent interactions or laser spectroscopy, but candidates having other interests which are related to those of the Group will also be considered. The appointment is from October 1st, 1979 or as soon as possible thereafter at an initial salary normally within the range £4,333 to £5,199 p.a. Further particulars and application forms are obtainable from the Registrar, the University, Manchester M13 9PL, to whom completed forms should be returned by August 3rd. Quote ref: 147/79/N. 2526(A)

UNIVERSITY OF BRISTOL

SCHOOL OF CHEMISTRY, ORGANIC GEOCHEMISTRY UNIT

RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant (Grade 1B) to assist in the application and development of computerised gas chromatography-mass spectrometry and related techniques to the analysis of organic compounds in sediments and petroleums. Instrumentation includes Finnigan 4000, MS 30 and CH-7 spectrometers interfaced to an INCOS 2300 data system. Applicants should have a qualification in chemistry or physics and an interest in instrumentation. Previous experience in mass spectrometry and/or gas chromatography would be an advantage.

This post is funded by the Natural Environment Research Council (starting salary £3689 - £4232 per annum).

Please send applications with details and the names of two referees to Professor G. Eglinton, University of Bristol, School of Chemistry, Cantock's Close, Bristol BS8 1TS. 2490(A)

MEDICAL RESEARCH COUNCIL

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

Applications are invited for a three-year POSTDOCTORAL appointment to work with Dr D.G. Smyth in the Laboratory of Peptide Chemistry, available from October 1st 1979. The position will be filled by a candidate with interest and preferably experience in the field of neuroactive peptides, with particular emphasis on the study of processing enzymes and control mechanisms. Some training in the techniques of protein chemistry or membrane biochemistry would be an asset.

The appointment will be in the salary range £4940 - £6627 per annum according to age and qualifications plus £502 p.a. London Allowance; superannuation provision.

Applications, giving details of qualifications, experience and the names of two referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA before 31st August 1979, quoting reference PC/1. 2485(A)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF THERAPEUTICS AND CLINICAL PHARMACOLOGY

Applications are invited for the post of

TEMPORARY LECTURER

(non-medical) in Therapeutics and Clinical Pharmacology. The appointment will be for a period of three years. The duties will be to undertake pharmacokinetic studies in collaboration with the medical and scientific staff of the Department. An interest and experience in measuring the drug content of biological fluids by chromatographic or enzymatic methods will be an advantage.

Salary in the range £4,333 to £8,992 (from 1 October) plus superannuation. Further details may be obtained from Professor O. L. Wade, The Department of Therapeutics and Clinical Pharmacology, The Medical School, Birmingham B15 2TJ (Tel: 021 472 1301 Ext 3201).

Applications, naming three referees, should be sent by 10 August 1979 to the Assistant Registrar, The Medical School, Birmingham B15 2TJ. 2521(A)

UNIVERSITY OF GLASGOW

POSTDOCTORAL RESEARCH ASSISTANT

PROTEIN CHEMISTRY/ VISION RESEARCH

Applications are invited for a Postdoctoral Research Assistant for an M.R.C. funded project on the isolation and characterization of specific visual pigment proteins. The post is available from 1st September, 1979 for a period of 14 months. Salary will be on Range 1A (Research and Analogous Staff) up to a maximum of £6627 (interim), depending on age and qualifications.

Experience in membrane proteins and/or protein chemistry essential.

Please submit applications, including resume, publications and names and addresses of at least two academic referees to: Dr. Alan Cooper, Department of Chemistry, University of Glasgow, Glasgow, G12 8QQ (Tel. 041-339-8855, ext. 7278).

In reply please quote Ref. No. 4483M. 2466(A)

STUDENTSHIPS

Athrofa Gogledd-dd Cymru
The North E Wales Institute
of higher education

KELSTERTON COLLEGE, CONNAH'S QUAY, DEESIDE, CLWYD

SRC CASE STUDENTSHIPS

Applications are invited for two SRC CASE Studentships, tenable from 1st October, 1979 for work on the following projects:

1. "Mechanism of lipid oxidation in aqueous emulsions", in collaboration with Unigate Ltd. Supervisor: Dr. J. C. Allen.
2. "Radioimmunoassay of polyamines", in collaboration with Ortho Diagnostics, Ltd. Supervisors: Drs. J. C. Allen and C. J. Smith.

The co-operating bodies will supplement the SRC Studentships by £250 p.a. Applicants should possess or expect to obtain 1st or upper 2nd class Honours, and should write as soon as possible, with curriculum vitae, to the Institute Registrar, North E Wales Institute, Kelsterton College, Connah's Quay, Deeside, Clwyd. CH5 4BR. 2426(F)

AGRICULTURAL RESEARCH COUNCIL

POSTGRADUATE STUDENSHIPS

(Standard Telecommunication Laboratories Limited)

Applications are invited from students interested in research designed to develop new methods for preventing trace element deficiency diseases in cattle and sheep.

Applicants should have a physiological background and experience of radioisotopic and stable balance methods and will be required to register for a higher degree, usually at the University of Reading.

Candidates should have a 1st or Upper Second class honours degree in a relevant subject.

The Institute is situated in a pleasant rural area with a staff restaurant and good recreational facilities. If required, accommodation for a single person could be provided in the Institute's hostel.

Non-contributory pension scheme.

Application forms from: Secretary, Institute for Research on Animal Diseases, Compton, Nr. Newbury, Berks. RG16 0NN. Quoting ref. 400. 2460(F)

UNIVERSITY OF GLASGOW

DEPARTMENT OF FORENSIC MEDICINE AND SCIENCE

N.E.R.C. RESEARCH STUDENTSHIP

Applications are invited from persons who hold or expect to hold a first or upper second class honours degree in chemistry, environmental sciences or a related discipline for a research studentship, commencing 1/10/79, funded by the Natural Environment Research Council.

The research, leading to the degree of Ph.D., will be on the topic "Environmental Geochemistry of Trace Metal Pollutants in the Urban Area of Glasgow". Analytical techniques available include AAS, FAAS, NAA, EDXRF and GLC.

Applications, including a curriculum vitae and the names of two referees, should be sent immediately to Dr. J.G. Farmer, Department of Forensic Medicine and Science, University of Glasgow, Glasgow G12 8QQ, Scotland.

In reply please quote Ref.No.4479M. 2467(F)

FELLOWSHIPS and STUDENTSHIPS continued

Commonwealth of Australia

QUEEN ELIZABETH II FELLOWSHIPS

Physical & Biological Sciences

To commemorate the Royal Visit to Australia in 1963 the Australian Government established the Queen Elizabeth II Fellowships scheme. Under this scheme up to ten fellowships may be awarded each year for full-time research by young scientists of exceptional promise and proven capacity for original work. These are post-doctorate awards tenable in an Australian university or approved research institution normally for two years. Tenure of a Fellowship is expected to commence within nine months of the announcement of the award.

QUALIFICATIONS: Queen Elizabeth II Fellows must be either Australian or United Kingdom citizens. They should have a Ph.D., or equivalent qualifications, in one of the physical or biological sciences (which are deemed to include mathematics and the scientific aspects of statistics, engineering, metallurgy, agriculture and medicine). Awards will, in general be restricted to applicants who are not more than 30 years of age on the date when applications close. Applications will also be considered from candidates for the Ph.D. degree if their thesis has been submitted for examination.

STIPEND: $17 131 (Australian) per annum – increased to $18 403 per annum at age of 28 years.

ALLOWANCES: Are payable in respect of a Fellow's dependent spouse ($500 p.a.) each dependent child ($200), superannuation payments (up to 10 per cent of stipend), appropriate insurance coverage and necessary travel expenses.

APPLICATIONS: *Persons interested in applying for the above fellowships should obtain application forms and a statement of the conditions of award from the Secretary, Queen Elizabeth Fellowships Committee, Department of Science and the Environment, P.O. Box 449, Woden A.C.T. 2606, Australia; the Minister (Scientific), Canberra House, 10-16 Maltravers Street, London WC2R 3EH, U.K.; or the Australian Embassy, 1601 Massachusetts Avenue, Washington D.C. 20036, U.S.A. Applications for the next round of awards, which will be announced in December 1979 close at the Canberra address on 24 August 1979.*

2469(E)

UNIVERSITY OF DUBLIN
Trinity College

UNIVERSITY OF DUBLIN
TRINITY COLLEGE
and
ST. PATRICK'S COLLEGE, MAYNOOTH
POST — DOCTORAL FELLOWSHIPS IN CHEMISTRY

Applications are invited for two positions supported by the National Board for Science and Technology.

One of these, with Professor D.R. Lloyd (Trinity College) and Professor C.M. Quinn (St. Patrick's College, Maynooth), is for work on photoemission from adsorbates. Experimental work will be carried out at Trinity College, using an angle-dispersive spectrometer. Previous experience with ultra-high vacuum work is highly desirable, and familiarity with some of the experimental and theoretical techniques of surface science would be advantageous. This fellowship is available from 1st October 1979 for one year initially, with extensions possible up to a total of three years, at a salary of £3837-3994-4153 P.A. (presently under review).

The other fellowship is for work with Dr. J.M. Kelly and Dr. D.H. Grayson, both at Trinity College, on the synthesis and properties of polymer-bound coordination compounds, and porphyrins. In order to assess their potential as solar energy converters. Previous experience with polymers, coordination compounds, heterocyclic compounds or in photo-chemistry would be helpful.

Intending applicants should contact the Establishment Officer, Trinity College, telephone Dublin 772941, extension 1775, in the first instance. Enquiries may also be made by telex 5442 TCD E1. The closing date for receipt of completed applications will be Friday, 17th August 1979.

2470(E)

UNIVERSITY OF KENT AT CANTERBURY

POST-DOCTORAL FELLOWSHIP IN MICROPROCESSOR CONTROL OF FERMENTATION

Applications are invited for a post-doctoral research fellowship in connection with a project involving the application of microprocessors to the continuous culture of micro-organisms. The successful candidate would be expected to study culture optimisation and strain selection using an apparatus consisting of a microprocessor interfaced with various monitoring and control devices attached to a fermenter. The appointment is for one year and is available immediately. Candidates should have a good honours degree in microbiology, biochemistry, bio-engineering or a related subject and post-graduate experience in an allied area. Previous experience with computer applications and programming would be an advantage.

Application forms and particulars may be obtained from the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH to whom completed applications should be submitted by 10 August, 1979. Please quote Ref: A40/79. 2518(E)

THE UNIVERSITY OF LEEDS
DEPARTMENT OF PLANT SCIENCES

RESEARCH FELLOW
RESEARCH ASSISTANT

Applications are invited for the above posts, which are funded by the SRC, in an established research group concerned with gamete recognition systems in algae. Both appointments are available for a fixed period of up to three years.

The Research Fellowship is suitable for candidates who already hold, or who expect to be awarded a PhD degree, preferably in the area of cell surface biochemistry (plants or animals) although candidates with experience in other areas will be considered. The successful applicant will be involved in the isolation and characterisation of fertilization receptors from the surfaces of algal gametes.

The Research Assistantship is suitable for a recent graduate in a biological subject. The successful applicant will be expected to work closely with other members of the group and will be particularly responsible for the development of cytochemical aspects of the project.

Starting salary at an appropriate point on the IB scale (£3,775 to £5,488) (Research Assistant) or the IA scale (£4,333 to £7,521 (Research Fellow) for Research and Analogous Staff according to age qualifications and experience. Both scales are under review with effect from 1 October 1979.

Informal enquiries about the posts will be welcomed and may be made through Drs J A Callow or L V Evans (Tel: 0532 31751 ext. 6580 or 6576).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 53/11. Closing date for applications 25 August 1979. 2522(E)

Southampton THE UNIVERSITY

APPLIED ELECTROCHEMISTRY and ELECTROCHEMICAL ENGINEERING

A number of Studentships are available within the Chemistry Department for postgraduate research on topics in applied electrochemistry and electrochemical engineering in the fields of organosynthesis, metal recovery and pollution control. Successful candidates will be expected to register for a higher degree.

Suitably qualified scientists and engineers should apply to Dr R E W Jansson, Director, Wolfson Centre for Electrochemical Science, Chemistry Department, The University, Southampton SO9 5NH, sending a curriculum vitae and the names of two referees. Please quote Ref N.

2463(F)

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF BIOCHEMISTRY

POSTGRADUATE STUDENTSHIP IN BIOCHEMISTRY

Applications are invited for a three-year studentship available from October 1979. Candidates should have a good degree in biochemistry or related subject and the successful candidate will be expected to register for a PhD in the above Department. The project, supported by Action Research for the Crippled Child, involves the use of cell culture studies in the study of homocystinuria, a genetic defect of methionine metabolism in man. The research methodology involved in the project will be multidisciplinary.

Applications, together with the names and addresses of two referees, should be sent to Dr R. Griffiths, Department of Biochemistry, University of St Andrews, North Street, St Andrews, Fife, Scotland, as soon as possible. 2529(F)

CHEMISTRY DEPARTMENT UNIVERSITY OF OTTAWA

Post-Doctoral Research Fellowship in Electrochemistry

Applications are invited internationally for an industrially sponsored Post-Doctoral Fellowship in the electrochemistry of specialized battery and energy storage systems. Candidates should have a recent PhD in electrochemical surface studies, electrode process work or materials science. The stipend will be $16,000 pa plus some assistance with moving expenses where appropriate.

Applications, with two letters of recommendation, are to be sent to Professor B E Conway, Chemistry Department, University of Ottawa, Ottawa, Canada. W217(E)

UNIVERSITY OF ALBERTA

BIOCHEMISTRY DEPARTMENT

POST-DOCTORAL FELLOWSHIP

Apply to Dr A. R. Morgan, Biochemistry Department, University of Alberta, Edmonton, Alberta, T6G 2H7 Canada. Candidates must be knowledgeable in physico-chemical and metabolic aspects of DNA. The two main areas in which the successful candidate would have a choice of doing research would be multi-stranded nucleic acids and their possible biological roles (nucleoids, chromosome condensation, tertiary structures) and DNA replication. The position is available immediately.

W213(E)

Redland Technology Limited

(in association with Kings College London and Surrey University)

NERC and SRC CASE Studentships

Redland Technology is a member of Redland Limited, the multi-national group of Companies which is principally concerned with the manufacture of building and construction materials.

Applications are invited for two CASE Studentships for research into the mechanism of colour development on firing clay building products. One Studentship is at Kings College London University and will study the mineralogy of the clay material. The other Studentship is at the University of Surrey and will study the properties and structure of the fired materials.

Both the successful applicants will spend two months each year at the Redland Technology Laboratories at Horsham in West Sussex and additional payment will be made for this activity.

Applicants should have a good Honours Degree in Ceramics/Mineralogy/Geology/Chemistry/Physics or other appropriate discipline.

Further information can be obtained from:

Dr. G. W. Aston, Redland Technology Limited, Graylands, Horsham, Sussex. Tel: 0403 2351

2461(F)

Redland Technology

THE HARTLEY FELLOWSHIPS of the UNIVERSITY OF SOUTHAMPTON

Applications are invited for three Fellowships normally tenable from 1 October 1980, for up to two years research, in any field for which facilities are available within the Faculties of Arts, Science, Engineering & Applied Science, Social Sciences, Law, Education, Medicine and Mathematical Studies.

Fellows will normally be appointed from persons of high promise whose records show evidence of capacity for original research, and who have up to five years post-doctoral experience or equivalent research record.

Salary will be related to age and experience and be within nationally agreed ranges for research staff in universities. (No provision for travel or removal expenses).

The closing date for applications for the 1980 competition is 1 November 1979.

Details and application forms from Mr E.D. Gordon, The University, Southampton SO9 5NH.

Please quote reference N. 2462(E)

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF PHYSICS

— RESEARCH STUDENTSHIP

LUMINESCENCE IN AMORPHOUS SEMICONDUCTORS

Applications are invited from men and women for an SRC CASE Research Studentship in collaboration with Dr. P.J. Dean, Royal Signals and Radar Establishment, Malvern, leading to the degree of Ph.D. The investigation will be concerned with electroluminescence and deep traps in amorphous semiconductors, within our established group studying laser excited luminescence in these materials.

Current work includes picosecond pulse exitation for time resolved studies. The programme will give experience in fundamental solid state physics and in electro-optical techniques, and presents an opportunity to study novel materials of current technological interest. The award will be subject to the usual SRC conditions with about £500 p.a. supplement by the RSRE. Applications (with the names of two referees) or enquiries to Dr. I.G. Austin/Dr. T.M. Searle, Department of Physics, The University, Sheffield S3 7RH. Tel. 78555 ext. 4288 or 4289. Quote ref: R 338(G. 2465(F)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL RESEARCH FELLOWSHIP IN BIOENERGETICS

Applications are invited for a Research Fellowship to investigate the factors controlling the synthesis of ATP after short flash excitation of membranes isolated from the photosynthetic bacteria. The principle aim of this SRC-supported project is to determine the relationship between electron transport reactions through defined segments of the electron transport chain, the development of the electrochemical proton gradient and the extent and rate of ATP synthesis after single and multiple flashes.

The appointment is for three years commencing October 1979 (or as soon as possible thereafter) in the range (under review) £4,333 to £5,488 (from 1 October). Starting salary not expected to exceed £4,910 pa. Candidates should preferably have some previous experience in the field of mitochondrial, chloroplast or bacterial bioenergetics.

Applications, including a short curriculum vitae and the names of three academic referees should be sent by 10 August to Miss L. R. Heyes, Senate Registry, University of Birmingham, PO Box 363, Birmingham B15 2TT. Further details may be obtained from Dr. J. B. Jackson, Department of Biochemistry. Tel: 021-472 1301 Ext. 3113.

2520(E)

Southampton THE UNIVERSITY

POST GRADUATE STUDENTSHIPS IN CRYOGENICS

Applications are invited from science and engineering graduates for one year. MSc and 2/3 year MPhil/PhD studentships in Cryogenics. Telephone Dr R G Scurlock, Southampton 559122 Ext. 2112/2068. 2464(F)

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF CHEMISTRY

Applications are invited from men and women for an SRC CASE Studentship: academic investigator: Dr. A.J. Smith; co-investigator: Dr. D. Brown, Chemistry Division, Harwell. Subject: "Very High Co-ordination Numbers in Actinide Compounds". The work will involve preparative chemistry and crystal structure analysis (with the appropriate computing) of compounds of (i) uranium and thorium and (ii) the α-active elements protactinium, neptunium, and/or plutonium. Training will be given in radiochemical and crystallographic techniques; previous experience in these fields is not essential. First or Upper Second degree required.

Details from, and applications to, Dr. A.J. Smith, Department of Chemistry, The University, Sheffield S3 7HF as soon as possible. Quote ref: R 339/G 2508(F)

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STUDENTSHIP IN BIOCHEMICAL PHARMACOLOGY

Applications are invited from graduates for a research studentship on the usual MRC/Research Council terms, to study the mechanisms by which environmental chemicals may cause cancer of the gastrointestinal tract.

Candidates should have a first or upper second class honours degree in Pharmacology, Biochemistry or a related subject and will be expected to register for an MPhil with possibility of transfer to PhD. The studentship will be tenable from October 1979.

Applications with curriculum vitae and names of two referees to Dr A. G. Renwick, Pharmacology, Medical & Biological Sciences Building, Bassett Crescent East, Southampton, SO9 3TU. 2496(F)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL (University of London)

MEDICAL RESEARCH COUNCIL STUDENTSHIP

Applications are invited from a candidate with a good honours degree to work on biophysical aspects of cell-substratum interactions or membrane fusion.

Applications, including curriculum vitae, and the names of two referees, to Dr. David Gingell, Department of Biology as Applied to Medicine, The Middlesex Hospital Medical School, London W1P 6DB. 2483(F)

THE CITY UNIVERSITY

DEPARTMENT OF CHEMISTRY

Applications are invited for Research Studentships tenable from 1st October 1979 and leading to a higher degree in physical chemistry.

Awards include (A) SRC CASE Studentship for work on the influence of organic sulphur compounds on hydrocarbon combustion (Professor C.F. Cullis, in collaboration with Esso Petroleum Company) (b) SRC CTA Studentship for work on organosilicon compounds as fuel additives (Professor C.F. Cullis, in collaboration with Dow-Corning) (c) SRC CASE Studentship for work on ion-exchange and detergency using zeolites (Dr. R. P. Townsend, in collaboration with Unilever Research) (d) SRC CASE Studentship for work on novel oxidation reactions of hydrocarbons in zeolites (Drs. R. P. Townsend and S. A. Matlin in collaboration with Johnson-Matthey) (e) Studentship for work on the emission of particulates during combustion processes and their interaction with other combustion products. Applications should be sent to Professor C. F. Cullis, Department of Chemistry, The City University, Northampton Square, London EC1V 0HB (Tel: 01-253 4399 Ext. 367) as soon as possible. 2532(F)

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Applications are invited for a three-year MRC-financed research fellowship. Salary will be on the RF IA scale £4,333 to £7,521 (to operate from 1st October 1979) plus superannuation. Starting salary will be up to £5,488 according to age and previous (not necessarily related) postdoctoral experience.

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Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham, P O Box 363, Birmingham B15 2TT, to whom applications (three copies) including full curriculum vitae and naming three referees, should be sent by Friday 27 July, 1979. Please quote ref NM6. 2397(E)

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FACULTY OF MEDICINE

RESEARCH STUDENTSHIP

Applications are invited from graduates with a good honours degree for a Southampton and Wessex Medical School Trust Research Studentship tenable for three years from 1 October 1979. Successful applicants will be offered the opportunity to study for the degree of PhD, on an ongoing project investigating biochemical and immunological properties of hepatitis B virus.

Further details may be obtained from Professor Ralph Wright, Department of Medicine, Level F, Centre Block, Southampton General Hospital, Tremona Road, Southampton, SO9 4XY, telephone Southampton 777222, ext. 4299. Applications required by 23 July 1979. 2497(F)

ASSISTANTSHIPS

Centre for Applied Microbiology & Research, Porton Down
Diagnostic Reagents Laboratory

Post-Graduate Research Assistantship

A graduate research assistant with at least a 2nd Class Hons. Degree is required to investigate the use of triazine chromophores in protein and enzyme purification, immobilisation, assay and structural analysis. The successful candidate will be expected to register for a PhD. The post is funded by a research contract and is tenable for up to 3 years.

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This post may be discussed informally by contacting Dr. A. Atkinson, Director, Diagnostic Reagents Laboratory. Tel. 0980-610391, Ext. 406/365.

Applications, including curriculum vitae and two academic referees should be sent to Mrs M. Bushby, Personnel Officer, CAMR, Porton Down, Salisbury, Wilts. Closing date 27 July 1979.

PHLS Public Health Laboratory Service Board. 2489(P)

UNIVERSITY OF EXETER

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Applications are invited for the post of Research Assistant in the Department of Biological Sciences. The post is tenable for two years, commencing as soon as possible. The successful applicant will work under the supervision of Mr I J Linn on a project which will involve the use of miniature radio transmitters and a semi-automated data capture system to study the range, movements and social systems of short-tailed voles *Microtus agrestis* in the field.

The minimum qualification is a good Honours degree in Zoology, Biology or Ecology; an interest in or qualification in computing and/or electronics will be an advantage.

Salary will be on the Research Assistant scale: £3689 rising to £3961 p.a. (under review). The post is superannuable.

Further particulars may be obtained from Miss Doreen Watson, Northcote House, The Queen's Drive, Exeter, to whom applications (3 copies) should be sent by 3 August 1979. Please quote reference No.7163. 2504(P)

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September 5th to 7th, 1979

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Further information from Professor F. Deinhardt, Pettenkofer-Institut Pettenkoferstr. 9a, D8000 München 2 Western Germany, Tel: 089/53 93 21. W223(K)

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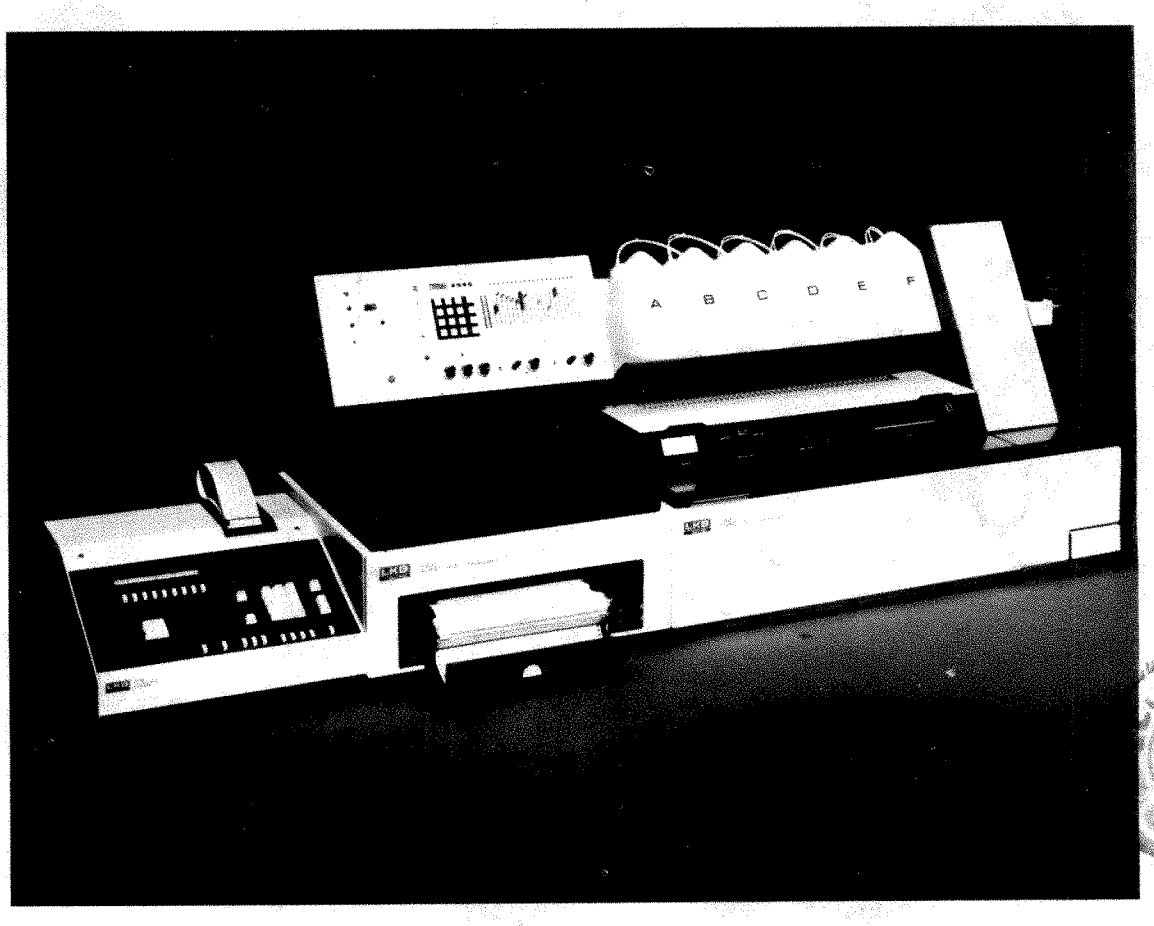
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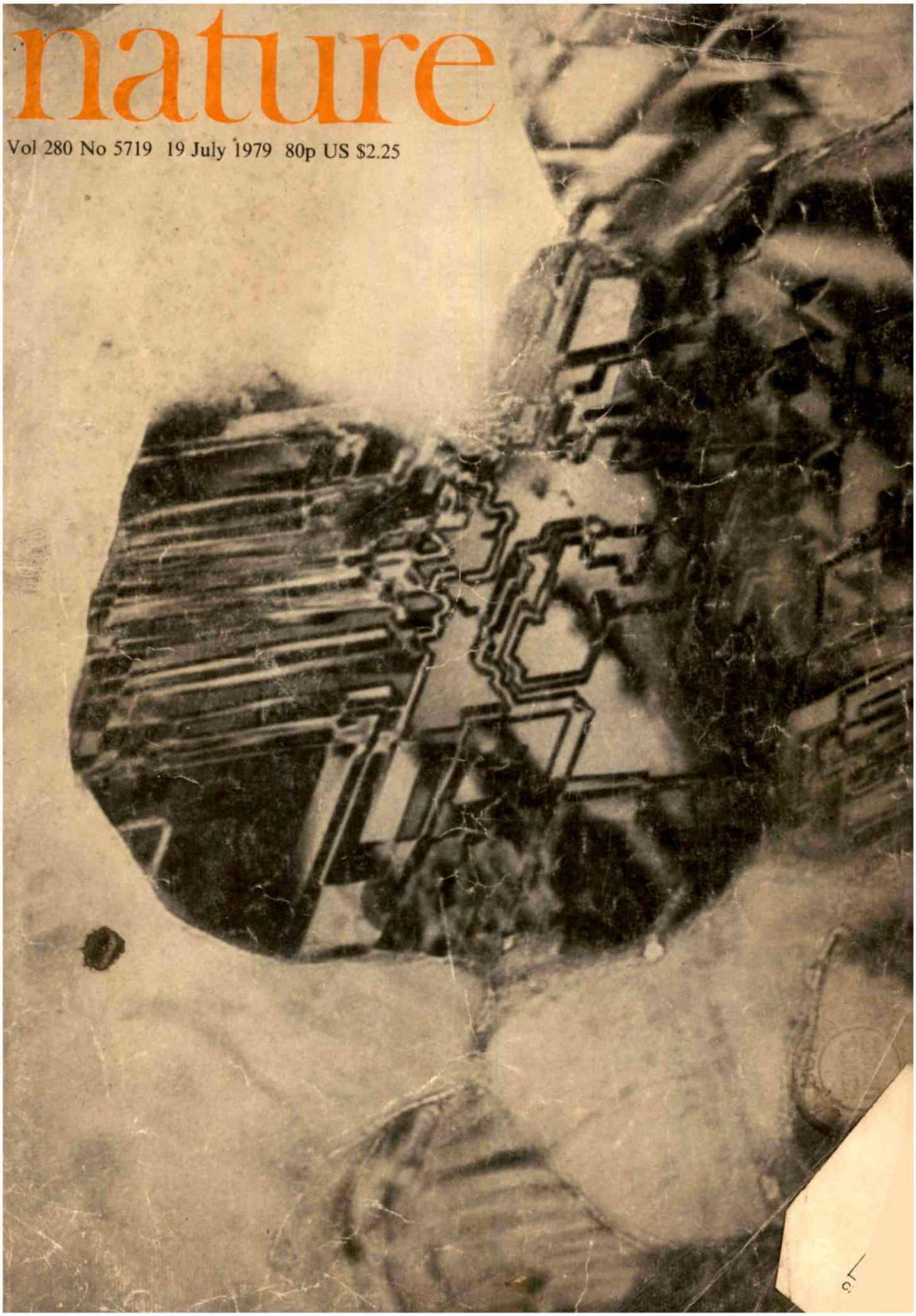
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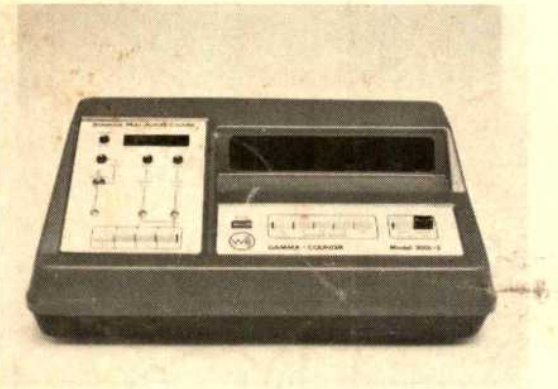
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Vol.280 No.5719 19 July 1979

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Volume 280 19 July 1979

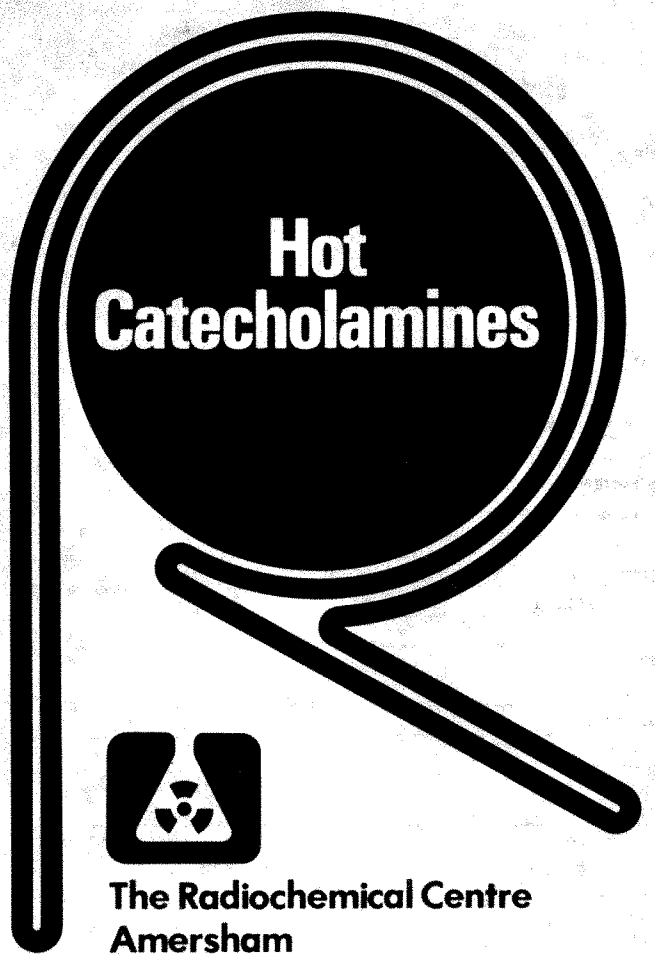
Carter's energy message 179
Aldermaston goes a little public 179
Sparks continue to fly in low-level radiation row 180
US scientists warn of environmental dangers from synthetic fuels 181
Congress slows growth of biomedical research 182
German research minister calls for 'critical science' 182
UK deports Japanese scientist 183
Controls urged on man-made mineral fibres 183
Asbestos killed woman, not glass fibre 183
In brief 184
UKAEA — boom or bust? 186
How the authority might survive 186
'Saving it' is easier said than done 188
A political view of CO_2 189

NEWS AND VIEWS
Longer perspectives in neutrino physics/Recognition, restriction and immunity/Ecology of photosynthesis/Viral pathogenesis and virulence/Seeing is believing in lineages/Mutants of the *lac* repressor/DNA gyrase and DNA unwinding/Oceanic heat flow, lithosphere age and thickness 191

REVIEW ARTICLE
The Loch Lomond Stadial in the British Isles — *J.B. Sissons* 199

ARTICLES
General relativity, thermodynamics, and the Poincaré cycle — *F.J. Tipler* 203
Geochemical problems of the Antarctic dry areas — *A.T. Wilson* 205
Embryonic development of identified neurones: differentiation from neuroblast to neurone — *C.S. Goodman and N.C. Spitzer* 208

LETTERS
Accretion on massive black holes in galactic nuclei — *V.G. Gurzadyan and L.M. Ozernoy* 214
Observation of large 2.2μm polarisation in 3C345 — *R.F. Knacke, R.W. Capps and M. Johns* 215
High-pressure $(Mg, Fe)_2SiO_4$ phases in the Tenham chondritic meteorite — *A. Putnis and G.D. Price* 217
New phyllosilicate types in a carbonaceous chondrite matrix — *I.D.R. Mackinnon and P.R. Buseck* 219
An experimental measurement of cation diffusion in almandine garnet — *R. Freer* 220
Palaeo-stress estimates in the Moine Thrust Zone, Eriboll, Scotland — *S. White* 222
Recent thrusting in the Appalachians — *K. Schäfer* 223
Direct measurements of secondary currents at a river inflexion point — *C.R. Thorne and R.D. Hey* 226
Aneuploidy in mouse fetuses after paternal exposure to X rays — *I. Hansmann, R. Zmarsly, H.D. Probeck, J. Schäfer and J. Jenderny* 228
Vitamin D and its analogues as a new class of plant growth substances affecting rhizogenesis — *A.J. Buchala and A. Schmid* 230
Effects of visual experience on the maturation of the efferent system to the corpus callosum — *G.M. Innocenti and D.O. Frost* 231
Electroconvulsive shock treatment decreases β-adrenergic receptor sensitivity in rat brain — *G.N. Pandey, W.J. Heinze, B.D. Brown and J.M. Davis* 234



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How does adrenaline accelerate the heart?	*H.F. Brown, D. DiFrancesco and J.S. Noble*	235
Role of the dendritic release of dopamine in the reciprocal control of the two nigro-striatal dopaminergic pathways	*V. Leviel, A. Chéramy and J. Glowinski*	236
The role of mitogenic lectins in T-cell triggering	*E. -L. Larsson and A. Coutinho*	239
Expression of V-region-like determinants on Ig-negative precursors in murine fetal liver and bone marrow	*L. Forni, P. -A. Cazenave H. Cosenza, K. Forsbeck and A. Coutinho*	241
Tumour promotor TPA enhances transformation of human leukocytes by Epstein–Barr virus	*N. Yamamoto and H. zur Hausen*	244
Oxidant damage mediates variant red cell resistance to malaria	*M.J. Friedman*	245
Nucleotide sequences of the attachment sites of bacteriophage Mu DNA	*R. Kahmann and D. Kamp*	247
Degradation of DL-leucine with longituinally polarised electrons	*L.A. Hodge, F.B. Dunning, G.K. Walters, R.H. White and G.J. Schroepfer*	250
Reply	*W.A. Bonner, M.R. Yearian M.A. Van Dort*	252

MATTERS ARISING

Mechanisms for radiation-induced shrinkage of voids	*K. Krishan and R.V. Nandedkar*	253
Reply	*J.H. Evans*	253
Possible Sun–weather correlation	*A.B. Pittock*	254
Reply	*J. Xanthakis*	254
Use of the solar limb effect to test photon decay and cosmological redshift theories	*J.M. Beckers and L.E. Cram*	255
The problem of thrown string	*A.P. Roberts and D. Sprevak*	256

BOOK REVIEWS

What Little I Remember (Otto R. Frisch)	*Rudolph Peierls*	257
Ecological and Sociological Studies of Gelada Baboons (M. Kawai, editor)	*T.H. Clutton-Brock*	259
Trace Elements and Iron in Human Metabolism (A.S. Prasad)	*N. Aldridge M. Webb*	260
Methods in Electromagnetic Wave Propagation (D.S. Jones)	*B.S. Westcott*	260

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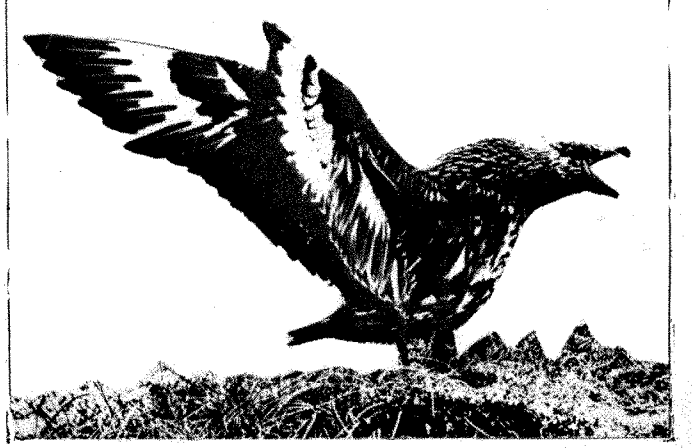
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19 July 1979

Carter's energy message

President Carter has emerged from his self-imposed period of isolation to announce a six-point plan for cutting dependence on foreign oil imports. Those who expected his long silence to be followed by some dramatic gesture were to be disappointed; the content of his speech to the nation was confined to proposals which had received wide currency in recent months — holding oil imports to 1977 levels (themselves the highest ever) and paring down imports by a half over the next decade, developing synthetic fuels, making a more positive start on conservation and so on. Nothing was said about nuclear power. No imaginative political moves were made — but this is not President Carter's style.

At least Carter's aims are relatively modest. At the time of the 1973 energy crisis President Nixon wheeled out a plan, 'Project Independence', of which was intended to make the United States independent of foreign energy suppliers by the year 1980.

Perhaps the most eyecatching proposal is that alternative fuels, notably synthetic fuels, should account for 2.5 million barrels per day by the year 1990. This would be rather more than a tenth of present-day oil consumption. Even this (as David Dickson points out on page 181) is little more than catching on to a recent optimistic public mood that expensive synthetic fuels will eventually be acceptable in the market place. And environmental objections, including those concerning carbon dioxide (see also page 189), will have to take a back seat.

President Carter has chosen to couple his energy message with a deeper warning to the American people that there is a crisis of confidence, moral and spiritual, in the nation. There may be a crisis of confidence in the presidency, and certainly Americans are worried about their future industrial competitiveness, but to bring in moral and spiritual values is surely to confuse the real message: that a whole raft of institutional and social changes are going to be necessary to meet a new era of energy austerity. □

Aldermaston goes a little public

The invitation for a number of members of the press to visit Aldermaston, the centre of Britain's atomic weapons' research, was an unexpected and pleasant surprise. Defence research establishments rarely open their doors, and as far as anyone could remember this was the first time a contingent from the press had been inside the security fence. The purpose of the visit was, from Aldermaston's point of view, to demonstrate their new laser compression facility recently opened by the Queen — a facility which seemed strangely similar to another laser compression facility just 30 kilometres away at the Rutherford Laboratory. Why can't the weapons people and the academics pool their resources (the price tag is, after all, several million pounds) and share a bigger and better facility? The Americans seem to have far less inhibitions about doing defence and academic work in the same building.

But the purpose of the visit, from many of the journalists' point of view, was not so much to look at a laser, nor even at the splendid display of non-weapons-related research gathered in a giant marquee and discretely draped with reminders that a pay dispute was still on. Rather, it was a unique opportunity to fire at the Director of the establishment a whole range of questions about broader issues concerned with nuclear weapons.

The most persistent questioning concerned the return to action of the plutonium facility which had to be closed last August when some workers in it were found to have been exposed to higher than permitted doses of radiation. It seems that as yet a substantial fraction of the facility is still not fully in operation. One of the big problems is manpower — not just skilled craftsmen but also health physicists prescribed by the Pochin report.

Even on this matter the Director and his colleagues were the very soul of discretion. When it came to questions such as 'What is happening under the Polaris improvement programme?' 'What is going to happen to the US/UK agreement on nuclear exchanges, due for renewal this year' and 'How would a comprehensive test ban pose problems for nuclear weapons stockpiles', the discretion was absolute — each was answered with a 'no comment'

It would have been a somewhat disgruntled team of journalists which would have left Aldermston under these circumstances were it not that the message did seem to have registered that the lack of public information on the British nuclear deterrent, particularly at a time at which major decisions are being made, is a matter of some concern. Promises were made that the Ministry of Defence would look into giving the press a fuller briefing in the near future on nuclear policy. The sooner the better. □

Sparks continue to fly in low-level radiation row

David Dickson describes a conflict in paradigms behind differing interpretations of the health hazards of nuclear power

Two and a half months after the National Academy of Sciences submitted to the Environmental Protection Agency the "final draft" of a long-awaited report on the biological effects of ionising radiation, an *ad hoc* committee within the academy is still meeting to re-write the most controversial section of the report, that discussing the appropriate model for interpreting the dose-response relationship for low levels of human exposure.

With public concern at the possible health hazards of nuclear power fuelled by the recent accident at Three Mile Island — and the nuclear industry no less concerned about meeting the costs of a possible tightening of occupational exposure levels — the issue is inevitably a hotly-debated one. Hopes that the temperature might be reduced by referring the issue to the academy, however, have been frustrated by a dispute over the interpretation of scientific evidence as heated as the public controversy outside.

The scientific dispute emerged briefly into public view at a press conference in May, when presentation of the draft conclusions of the academy study was accompanied by a strongly-worded minority statement claiming that the report's conclusions could lead to "detrimental apprehension" over radiation hazards; when additional members of the drafting committee indicated their own reservations, academy president Dr Philip Handler decided that the disputed section should be re-written.

Controversy already surrounds two other academy reports on politically sensitive issues, one on methods of nuclear waste management which was withheld from publication partly at the request of the Department of Energy, the other a massive study on energy policy which is already two years behind schedule. And Dr Handler has now announced that top NAS officials will be taking a close look at procedures for handling such issues over the summer "to see that these type of problems don't happen again."

Concern over the health effects of radiation is as old as knowledge of radiation itself. Until the mid-1960s, the main issue of controversy was whether a threshold exists below which radiation can be assumed to have negligible effect; since then, most scientists have accepted that there is probably no such threshold, and the debate has been over the shape of the dose-response relationship at low levels of exposure.

In a report in 1972, the academy's Committee on the Biological Effects of Radiation (BEIR) suggested that a linear hypothesis, extrapolating backwards from the known relationship at high levels, was the most appropriate to adopt for regulatory purposes — given all the uncertainties involved — and this has been accepted by various standard setting bodies.

However, this hypothesis has been attacked from both sides. Suporters of the nuclear industry claim that it is too restrictive, considerably overstating the risks; on the other hand studies of nuclear workers and others have been reported which, industry critics claim, show even existing exposure levels to be too high.

The BEIR committee, in its new report, sticks to the linear hypothesis as the most "prudent" assumption. Within the committee, however, a fierce battle has raged over whether, and if so to what extent, this assumption overstates the "true" risk. Both sides in the dispute agree that there is insufficient human data to provide statistically valid risk estimates for exposures of less than about 10 rads; where they disagree, with almost religious fervour, is what type of models can be used to fill the gap.

Supporters of the linear hypothesis tend to rely predominantly on epidemiological and statistical arguments. They claim, firstly that there is sufficient evidence from other studies, such as the effects of the medical uses of radiation, to make the linear hypothesis plausible; and secondly that the lack of human data makes it statistically impossible to reach any more precise assessment.

Their opponents, in contrast, tend to rely more heavily on radiobiological data obtained from studies of the effects of radiation on lower organisms. In the minority report attached to the draft of the committee's report, it is stated that "even if the linear model *were* in fact to afford a substantially better fit to epidemiological data, this would be insufficient justification for rejection of models that are consistent with a vast body of radiobiological evidence."

According to the latter arguments, a more appropriate dose-response model (at least for "low energy transfer" radiation, such as gamma rays) would be a quadratic one, possibly with a linear component. The linear hypothesis, they suggest, should only be accepted as an upper limit, with suitable recognition of a low limit of equal validity. "It should then be stated that the true risk is between these limits, and very likely near the lower limit".

Radiobiological data is also given greater prominence in deliberations within the National Council for Radiobiological Protection, a body which, although private, has been attacked by critics for its links to the nuclear establishment. Dr Victor Bond, for example, chairman of an NCRP committee which is soon to produce a report on low level radiation expected to reach different conclusions to the BEIR committee, told a Congressional committee last month that there were "good biological reasons" to support the lower estimates.

Dr Bond referred in particular to studies such as those carried by Dr Harald Rossi, one of the most outspoken critics of the linear hypothesis on the BEIR committee, and colleagues at the Columbia University College of Physicians and Surgeons in New York, on genetic changes in cells of the plant Tradescantia. "Radiobiology in simple systems is important to the establishment of the most-probable dose-effect relationships for the human being" he said, adding that the linear hypothesis for low level radiation was "biologically unsubstantiated".

Nowhere is the difference between the supporters and the critics of the linear hypothesis more marked, however, than in their different interpretations of studies on survivors of the atomic bombs dropped on Hiroshima and Nagasaki.

The critics point for support of their curvilinear hypothesis to studies of the mortality rates from all forms of cancer carried out between 1950 and 1974 on 80,000 survivors in the two cities. Concentrating in particular on the Nagasaki mortality data, this reveals an effect "about ten times lower than that based on proportional projections from persons receiving high X-ray doses" according to Dr Edward Webster of Harvard Medical School, co-author with Dr Rossi of the original minority report.

Supporters of the linear hypothesis, however, look at the Japanese data differently. They criticise the mortality data for its lack of statistical precision (in particular in the light of uncertainties over the relative effects of gamma and neutron components), arguing that "it is difficult to define a functional form in the dose reponse relationship" and that a linear-quadratic curve "is not demonstrably superior to a simple linear fit."

As an alternative, the original draft of the report suggests that it is more useful to look at data on cancer incidence rather than on mortality; and that a registry of solid tumours among Nagasaki survivors — evidence whose validity is questioned by the critics — supports their general hypothesis.

A further dimension to the dispute — and, one that some argue, is largely fuelling it — has been a sharp difference in attitude towards the nuclear power industry. Dr Edward Radford of Pittsburgh University, chairman of the BEIR committee, has made little secret of his personal views that current permitted exposure levels for nuclear workers are unnecessarily and perhaps dangerously high, suggesting that exposure levels be

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reduced by a factor of up to ten.

"The purpose of requiring more restrictive occupational exposure limits is to provide clear design criteria to allow engineers to design facilities to meet these kinds of standards, rather than rely on the somewhat nebulous and legally dangerous to the industry 'as low as reasonably achievable' concept," he says.

The dissenting group on the BEIR committee has made it equally clear that it feels too restrictive exposure levels could place an unnecessarily heavy burden on the nuclear industry. The original minority report warns that overestimates of radiation hazard could result in "serious detriment" to national energy policy. "If the guide line levels were reduced in the way [Radford] wants them, there would not be any nuclear industry at all" one committee member has been quoted as saying.

The BEIR committee dispute has been characterised as a conventional scientific disagreement. "Such controversies have existed within the scientific community since time immemorial. We will truly be in trouble when scientists passively accept each other's work without challenge," says Dr Sidney Marks of the Battelle Memorial Institute's Pacific North-West Laboratory.

In this case, however, the incentive has been primarily provided not by a desire for knowledge, but by a specific regulatory need; and the resulting impasse therefore carries both scientific and political dimensions. Initially the academy had hoped that this could be accommodated in the minority report; however when it emerged that over half of the BEIR committee's somatic effects subcommittee shared the reservations about the degree of support given to the linear hypothesis, it was decided that a redrafting of the whole section to produce "a more balanced expression of differing interpretations" would be more appropriate.

The new version will, it is hoped, be ready for re-submission to the full committee within a few weeks. The academy still says that "dissenting views will be published in the final report"; so whatever is agreed is unlikely to be taken as the last word. □

US scientists warn of environmental dangers from synthetic fuels

As President Carter was preparing the details of a major national programme to stimulate the production of synthetic fuels, announced in his energy speech last Sunday, the Council of Environmental Quality released a report warning that a rapid increase in the use of synthetic fuels could have serious environmental consequences through speeding up the accumulation of CO_2 in the atmosphere (see also page 189).

Synthetic fuels, previously a relatively unfashionable area of research, have suddenly been receiving the enthusiastic attention both of the President and of the US Congress.

Despite wide uncertainties over both economic and technical viability, the House of Representatives has already passed a bill authorising a crash development programme aimed at a target of producing 2 million barrels of synthetic fuel a day by the year 1990. And in his energy speech, delivered after almost two weeks of closeted discussion with his top advisers, President Carter proposed a programme that would develop America's alternative fuel sources from coal, oil-shale, plant products and the Sun to replace 2.5 million barrels a day of imported oil by 1990.

However both environmentalists and scientists have expressed concern at the environmental implications of such programmes, urging the President to concentrate on means for achieving energy conservation rather than the search for substitute fuels.

In a report to the CEQ, the four scientists say that the production of CO_2 resulting from the use of synthetic fuels (such as gas or oil) made from coal is almost 50% higher than the CO_2 emissions resulting from the production of energy directly from coal, and twice as high as the result of obtaining an equivalent amount of energy from natural gas.

"It is our conviction that an appropriate reaction to the mounting worldwide squeeze on supplies of energy requires consideration of the CO_2 problem as an intrinsic part of any proposed policy on energy," the four scientists say.

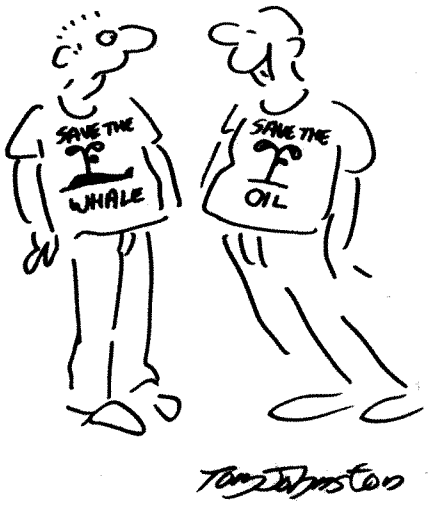
Environmental groups have warned the President about other potentially damaging aspects of a massive synthetics fuels programmes, ranging from the disruption caused by the construction of large production plants to the creation of large quantities of carcinogenic substances.

In a letter sent to the President last week, five such groups urged that he not allow himself to be caught up in what they described as a "synfuel panic", and consider lower-cost conservation programmes as an alternative to a massive commitment to synthetic fuels.

Many of the environmental risks associated with such a commitment were "unacceptable", the groups said in their letter; emphasis should rather be placed on more solar development programmes, less costly programmes for synfuel development, and more oil and gas exploration.

Support for such a policy also came from a different direction, namely a study prepared by the Harvard Business School, soon to be published by Random House under the title "Energy Future". According to this study, the need to constrain oil imports is much greater than most economists and energy analysts have realised, since the political and other costs of dependence on a few producing countries has to be included on top of the market price for oil.

The Harvard study suggests a diverse national energy policy encouraging modest growth in the use of coal, some reliance on nuclear power, and continued efforts to develop synthetic fuels; however it argues that in the current political climate, a stepped-up programme to accelerate conservation and solar energy would prove far more rewarding than any other energy path.



Tom Johnston

The environmental hazards associated with a major synthetic fuels programme were also covered by a report published last week by the Department of Energy's Office of Technology Impacts. Although the report says that it should be possible to find acceptable sites for large synthetic fuels production plants, it warns that successful commercialisation does have environmental risks, and that stringent environmental controls, management practices and "permitting conditions" should be maintained.

The Department report says that deployment of synthetic liquid facilities on an accelerated schedule to 1990 appears feasible in terms of current environmental constraints, although a target of 2 million barrels a day, rather than 500,000, would bring "rapid siting difficulties".

In reaching its conclusion, the report says there is some risk that environmental research and development programmes cannot fully satisfy all existing and expected regulatory demands, but that these risks should be known by 1985, and it is expected that appropriate control adjustments can be made.

"Reduction of these uncertainties requires refocusing of environmental research and assessment programmes to aggressively address these areas", the report says. **David Dickson**

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Congress slows growth of biomedical research

Appropriations committees in both the US House of Representatives and the Senate have recommended increases in the amount of money requested by President Carter for biomedical research in his budget proposals for the fiscal year 1980, submitted to Congress at the beginning of the year. However the increases, which average about 6% over the amount spent by the US on such research in the current financial year, is less than the expected rate of inflation — and considerably lower than the 24% increase over the President's proposal voted by Congress last year.

In his budget message, the President proposed that the research budget for the National Institutes of Health should remain virtually static, at about $3.2 billion, between 1979 and 1980. Of particular concern to many research workers had been the proposal to reduce from 5,673 to 3,062 the number of new grants and competing renewals for investigator-initiated research (implying a drop from 42 to 20% of those approved for funding actually receiving support).

On the advice of its appropriations committee, the House has recommended that the NIH budget be increased next year by $202 million — or 6.4% over the current year; the major proportion of this increase will go to support investigator-initiated research in the various institutes, with the aim of increasing the number of new grants to 5,000.

"Thus the bill continues the real growth in federal support for basic biomedical research which has prevailed in recent years", the committee says in its report, adding that its primary objective "is to allocate funds to the most promising research endeavours as determined by the peer review system".

The committee also says that the present balance between non-competing continuations, competing renewals, and new grants "warrants a careful review to determine whether current procedures and mechanisms leave enough room for the support of unconventional ideas and new investigators without proven 'track-records' ". It has requested the director of NIH, Dr Donald Fredrickson, to undertake such as review and to make a report before 1 October.

It also suggests that the NIH should explore the possibility of starting a programme to awarding stipends to medical or dental students to spend brief periods engaged in health research projects, with the hope of attracting them to research careers and thus increase the number of MDs and DMDs going into basic and clinical research.

The Senate is yet to vote on appropriations for biomedical research. However the Senate's appropriations committee last week accepted the proposal of its subcommittee that would cut $13 million of the increase proposed by the House, still leaving intact an increase of 6% over the current year's funding. A single figure has to be agreed by both the House and the Senate before the appropriation can become law. □

Germany's research minister calls for 'critical science'

WEST GERMANY wants its scientists to be more critical in their research and to give due consideration to the disadvantages of technological advances as well as the advantages. There should be more discussion with pressure groups and the public before fundamental decisions on, say, nuclear energy are made.

This change of emphasis in Bonn's research and technology policy was outlined by Research Minister Volker Hauff when he released a major directory of research in West Germany recently. The 650-page report — *Bundesbericht Forschung VI* — is the Bonn government's sixth assessment of research policy, institutions and financing.

Hauff said the risks of research and technical progress should enter into decisions more than before. The government wanted to be more critical, to analyse and take account of the advantages and the disadvantages of new developments and to hold a dialogue with trade unions, individual population groups and pressure groups *before* taking decisions, not afterwards.

There is now a growing protest against almost every new large-scale technical construction in West Germany, even down to pressure groups against new roads and railway lines. Among the fields whose disadvantages need more critical examination, Hauff mentioned genetic engineering, the investigation of new sources of energy, and the widespread introduction of microelectronics. On the other hand, the research report, despite all the criticisms and objections surrounding Gorleben, still stands by the government's controversial "integrated disposal" policy for getting rid of the waste from nuclear power stations.

The report records a total expenditure of DM30,000 million for research and development last year, of which half came from the state and half came from industry. It amounted to a 2.3% share of the gross national product. The main focus of R&D expenditure by the federal government (totalling DM8,500 million) was on the Federal Ministry of Research and Technology's promotion programmes (DM4,700 million) and on the Defence Ministry's research expenditure (DM1,700 million). The Länder on the other hand finance research mainly in the universities and other colleges.

The growth rate of the total R&D expenditure in the Federal Republic has declined from 20% per year in 1969 to 8% in 1978.

Within the R&D expenditure, the proportion for defence research and other government departmental research is declining, while that of the Ministry of Research and Technology's promotion programmes is rising slightly. The proportion of basic research fell from 19.5% in 1975 to 16.5% in 1979, but should rise again slightly to 17.1% by 1981.

The report also explicity catalogues the aims of research and technology policy. In addition to the earlier three aims — expansion of scientific knowledge, increase in economic efficiency and improvement in citizens' living and working conditions — there are now a further two: the conservation of resources, and the "improvement of knowledge about technological risk", to provide a better basis for decision making. As far as possible, all five aims are to be pursued with equal vigour. The inference is that the previously dominating emphasis on economic interests is to yield gradually in favour of a broader outlook. To what extent the non-economic aims stand a chance of realisation is, however, questionable.

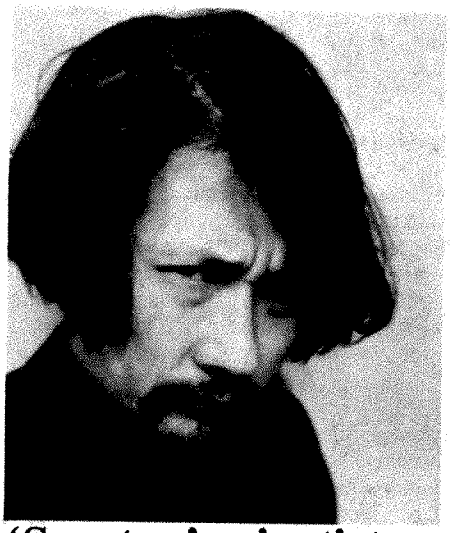
This is evident in the Federal government's stress on how little latitude there is for 'going it alone' in the face of international competition, and in the increasing recognition that national decision-making mechanisms set narrow limits on its research and technology policy. While Research Report V was still saying briskly in 1975: "Research policy must investigate needs, evaluate various possible solutions . . . and set priorities"; this time the language is more reserved: "In an organisation of research which has, for good reasons, been designed pluralistically, government research promotion can and may lay emphasis on matters of content within a limited compass only."

Klaus Höpfner

●According to their annual report for 1978, just published, the Deutsche Forschungsgemeinschaft (which funds much of Germany's research in universities) spent over 700 million DM last year for more than 10,000 projects in all branches of science and research. The biosciences shared the largest fraction of the money, although their share sank from 37.1% (1977) to 36.2% (1978). The other natural sciences augmented their share slightly to 24.7%, while engineering sciences fall 0.5% to 20.9%. The most rapid increase came in the social sciences, which grew 17% from 1977 to 1978. □

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'Spartan' scientist must go, says UK

Dr Tetz Yoshimura (above), the Japanese mathematical physicist, who seeks to stay in Britain and pursue his research undisturbed by the 'rat race' of modern Japanese life, is to be deported from Britain. His appeal against deportation was rejected last week by the Home Office on the grounds that his position "presents insufficient unusual features to justify giving him favourable treatment".

This represents a shift in standpoint from previous Home Office notifications, which rejected his application to stay on the grounds that his income from freelance coaching and translation work was insufficient to maintain him. Although Dr Yoshimura's spartan life-style allows him to maintain himself on £12 per week (half his average income), further guarantees were required. When the author Russell Hoban came forward as financial guarantor, the Home Office dropped the financial aspect, and turned to the fact that Dr Yoshimura's various academic committments had terminated — not surprisingly, since coaching and the marking of examination papers is not normally required during the long vacation.

It seems strange that the Home Office claims the case exhibits no unusual features. Dr Yoshimura is an example of a now almost extinct race of scientists who hope to work without the support of major public funding bodies. When he came to Britain in 1971, he hoped to find a haven of academic detachment. Even the bulky file of correspondence on his case contains the occasional off-print or letter from an academic publisher about the proper placing of a bracket, that have somehow crept into the official hassling.

Faced with the final deportation order, Lord Avebury, chairman of the inter-party parliamentary Human Rights Group told *Nature* that he hoped that Dr Yoshimura's scientific colleagues would bring all possible pressure on the Home Secretary to reverse his decision.

Controls urged on mineral fibre

A unique government-industry-trade union working party organised by the UK Health and Safety Executive has proposed controls on mineral insulation wools used as substitutes for asbestos, and called for a programme of research to define further the hazards of fibres.

The report estimates that 15,000 workers are involved in the production and steady use of man-made mineral fibres (MMMFs) with up to 250,000 workers coming into "occasional" contact with MMMFs. In addition, the Chairman of the Party, D J Evans estimates that perhaps 20 times this number handle the materials privately.

The fibres are produced from glass, rock, slag and metallic oxides and include fibreglass and rock and slag wools which are widely used for home insulation as a substitute for asbestos. The proposed limits are a fibre limit of five fibres/ml and a mass limit of 5 mg/m^3. (The present limits for asbestos exposure are 2 fibres/ml which is expected to be lowered to 1 fibre/ml shortly. The National Institute for Occupational Safety and Health in the US has proposed a new limit of 0.1 fibre/ml.) The time for introduction of the MMMF standard is "not to exceed three years".

In separate appendices, the trade union and industry representatives express the fundamental conflict in hazards legislation over who is to pay — industry in the form of safer equipment, or the work force in terms of poor health and possibly early death. Industry contends that no hazard has been shown to exist and as such controls must be based strictly on cost. "In the absence of a scientific basis upon which any changes in the (general dust standard) can be made, any change must be based upon what is considered to be the best practicable engineering controls."

Industry is willing however to support the introduction of a standard. "While emphasising the absence of a scientific basis for the figure we accept the proposed standard of 5 fibres/ml." This standard is not expected to affect industry operations significantly.

In their statement the TUC states that it would prefer a standard of 1 fibre/ml and feels it could live with a standard of 3 fibres/ml as "an acceptable and attainable standard under present engineering control knowledge." The TUC disagrees with the 5 fibre/ml standard and it also disagrees with the three year lead-in period, calling instead for adoption of the standards within 12 months.

In the middle of the political fight between those who assign the limits, and those who bear the risks, stand the scientific specialists. In the UK, research into the hazards of fibreglass is being conducted at three places. In Southampton, Professor Donald Acheson will conduct human epidemiological studies. In Edinburgh, the Institute of Occupational Medicine is working on standardisation techniques, and at Penarth a Medical Research Council unit will conduct animal studies as well as designing measurement devices. Additional research is being conducted by the industry funded. International Agency for Research into Cancer with support of the World Health Organisation.

Experienced health and safety observers, however, point out that the situation bears an uncomfortable resemblance to the history of asbestos. In the UK, the first cases of asbestosis were reported by lay people in 1898, and the government noted cases of death in 1900. But the first legislation was not introduced until 1931, and widespread public awareness of the extent of the hazard did not occur until the 1970s. Fibreglass and its cousins are relatively recent materials and the historical data may not even exist.

Dr Morris Greenberg, medical adviser to the Health and Safety Executive thinks that the situation is difficult. "We think that MMMFs are not in the same league as asbestos but we don't know how far down the leage table they are." **Joe Schwartz**

Man-made Mineral Fibres. Report of a Working Party to the Advisory Committee on Toxic Substances. HMSO. 50p.

Asbestos, not glass fibre, killed woman

Scientists at the US National Institutes of Health announced last week that a case of pneumoconiosis reported by Japanese research workers last autumn as possibly being related to exposure to glass fibre had turned out to be the result of previous exposure to asbestos. The link between glass fibre and pneumoconiosis had been suggested by a Japanese research worker at a meeting last year held under the auspices of the National Cancer Institute, following his discovery of physiological damage in the lungs of a woman who had worked with glass wool similar to the damage caused by asbestos fibres.

The report received considerable publicity, and generated wide concern in the glass fibre industry. However the NIH reported last week that microscopical analysis of biopsy material carried out by Dr I J Sellikoff of the Mount Sinai School of Medicine showed the presence in the lung of asbestos fibres, but not glass fibres.

The NIH report says that during discussion of the original report, it was understood that six other cases of pulmonary disease had been found. "The existence of these cases cannot be confirmed and is attributed to a linguistic error", the NIH says.

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news in brief

IWC offers some hope for sperm whales: Conservationists won a half-victory at last week's International Whaling Commission meeting after years of unsuccessful campaigning. There will now be an indefinite ban on factory-ship whaling for sperm whales, and a whale sanctuary will be set up in the Indian Ocean for ten years. About 7,000 sperm whales will be saved by the factory-ship whaling ban, which will probably put an end to the Russian whaling fleet. The small minke whale, however, has not received the same protection. Commercial minke whaling has been excluded from the IWC moratorium, and the Antarctic end of the Indian Ocean where the minke is found in great numbers will not form part of the sanctuary.

The IWC also set a new quota of 16,000, some 4,000 less than last season's kill. Recommendations were made by IWC's technical committee on humane methods for slaughtering whales but no nation offered to allow independent observers on to their ships, so research on death times and killing methods will be difficult. The IWC also agreed that scientific permits and quotas on the number of whales needed for research would now have to be approved by a science committee. Previously each nation set their own quotas.

Conservationists were disappointed that a proposal to ban all sperm whaling was rejected by the IWC and that shore-based sperm whaling will continue. The commission also rejected a proposal to prohibit trade between IWC and non-IWC nations, and a proposal to add the narwhal and white whales to the IWC's list of protected species. The commission also failed to come to a decision on pirate whaling.

Malformations at birth increased in Séveso last year: Figures issued by the special regional commissioner for Séveso, Mr Antonio Spallino, suggest that there has been an increase in the past year in the number of local children born malformed. According to Spallino, of 2,777 births recorded in the Séveso region in 1978, 90 infants were born malformed and 24 were classed as 'doubtful cases'. The comparable figures for 1977 were 2,756 births, 41 malformations and 25 doubtful cases. In July 1976, a trichlorophenol reactor belonging to the Givaudan company at Séveso, overheated, discharging its contents — including a teratogen 2,3,7,8 — tetrachloro-dibenzo-p-dioxin (dioxin) — over a populated area near Séveso in Italy. Concern was expressed at the time that the presence of dioxin, a well-known teratogen, could lead to an increase in birth malformations. The current figures do suggest such an increase, although the rate of malformations reported for 1978 is still comparable with the norm for Western Europe and bears little relation to the concentration of dioxin.

MRC staff committee criticises MRC management for budget cut inaction: Officers of the National Staff Side of the UK Medical Research Council have mounted a campaign to oppose cuts in the medical research budget, that includes strong criticism of MRC officials. The committee cites the closure of two MRC units and the future loss of 380 jobs or 10% of the workforce as evidence of the damage being done to the nation's research effort. "We do not agree that the Council should implement or even accept the imposed cuts. The MRC seem to have done nothing to offer resistance on their own account and have refused to be associated with our protests." Officials in the MRC claim that further assessment is needed and that management has its own separate channels of communication with government. "No figures yet exist for losses due to budget cuts and the two unit closures were for scientific not economic reasons" an MRC official told *Nature*. "The official side has made its views known to the government through the Advisory Board for the Research Councils." The Council's views have not been disclosed pending a Council meeting called for 19 July.

Aldermaston unions to file contamination lawsuits: Four trade unions at Aldermaston, the UK's nuclear weapon laboratory, are considering more than 70 claims for damages against the Ministry of Defence for plutonium contamination. The action follows the unions' refusal last February to work in contaminated areas identified by the Pochin report. The report was produced by Sir Edward Pochin, a radiologist, after plutonium had been found in the lungs of three workers in the laboratory's laundry room. Nine workers in the industry-chemistry section were also contaminated.

Sussex students now expelled: Sussex University students Richard Flint and Shaun Fensom have been expelled from the university for their part in preliminary science examination disruptions. The expulsion was the result of a second disciplinary committee action announced on 6 July. A faculty committee consisting of pro-vice Chancellor Professor T Nuttal as chairman and four faculty members, M Hawkins, W Lamont, L Cook, and J Maynard-Smith, appointed by the university Senate had reinstated the students on 25 June — provided they were "not found guilty of a further breach of discipline as defined by the disciplinary code after this judgement". One week later the committee reconvened to consider the student disruptions of the week of 18 June as a separate series of events from the first disruptions in May. The June demonstrations saw the breaking down of doors and the first arrests and injuries, when faculty members confronted a picket line of 150 students in front of the Building of Engineering and Applied Science. A faculty petition signed by over one hundred faculty members — few of them from the science faculty — called for the reinstatement of the two students (as well as a withdrawal of threatening letters to university student rent strikers (21 June)) to no avail.

In addition criminal charges of malicious intent to cause damage have been filed against five students and the university has taken out a £20,000 order of compensation against five students and a £10,000 order of compensation against the student union.

UK government scientists win claim; strike goes on. Scientists of the striking Institution of Professional Civil Servants have won their pay claim for this year. The Civil Service Department has granted pay rises in five scientific grades ranging down from 34.1% for the top grade of Principal Scientific Officer to 22% for Assistant Scientific Officer. The rises are linked to those for administrative grades — a hotly contested point. In what has become an increasingly bitter series of negotiations the IPCS has also won its demand that next year's salaries are to be negotiable. The CSD had demanded the union accept without negotiation the results of a pay research exercise to determine their pay.

In the meantime the IPCS in still on strike in support of claims for the governments 40,000 technologists — an action which is supported strongly by the scientists. Government scientists at Royal Ordnance factories at Glascoed, South Wales and Bradeway Green and Chorley both near Manchester have brought shell manufacture to a halt. In addition scientists at the government's Forensic Laboratory withdrew their labour in a two week strike but, because of the Official Secrets Act, could not disclose the effect of their action even to the union leadership. The IPCS has called for arbitration and is continuing its tactics of selective strikes, half day strike-demonstrations and withdrawal of good will.

NASA orders a Spacelab from ESA: On 5 July, NASA signed a contract with the European Space Agency for the supply of components for a second Spacelab flight unit identical to those of the first Spacelab unit which ESA is currently building. The cost to NASA of the second Spacelab will be announced by ERNO, the

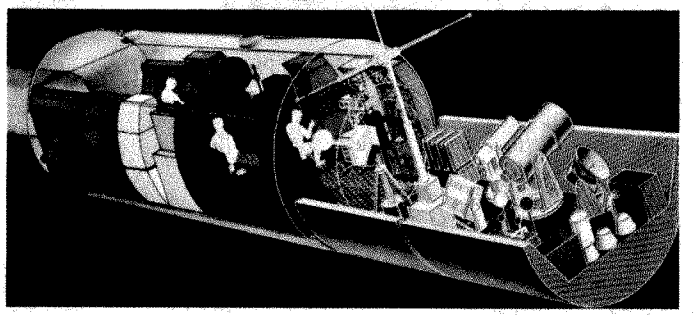
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German head of the consortium which will be building it, at the end of this month. NASA hopes however, that it will not exceed 110 million accounting units (1 a.u. is $1.25).

A memorandum of understanding that NASA should buy a second Spacelab from ESA was signed when the two agencies first embarked on the Spacelab programme. During much of last year, ESA and NASA spent time trying to think of ways in which NASA could procure the second Spacelab without any exchange of funds. A system of bartering, however, was unattractive to ESA which was reluctant to provide a Spacelab in exchange for free shuttle flights for some of the first Spacelab's demonstrations missions. Difficulty in filling some of the demonstration missions with sufficient experiments, mainly because of costs, meant that ESA could not guarantee in advance that it would use the shuttle flights it had bartered for. The two agencies therefore finally agreed to revert to the normal way of selling and buying by exchange of funds.

Funding of the first Spacelab is currently undergoing some difficulties, however. Present estimates of the total cost are now 680 million accounting units, about 140% of the original estimated cost. Under the Spacelab agreement, any participating state is entitled to leave the programme when costs rise to 120% of the original estimate. In principle, however all states want to continue despite the 40% overrun — but some, notably Italy, are finding difficulty in raising the funds.



Another factor which could add further to costs is a possible further delay to the Spacelab launch caused by difficulties with the space shuttle. Despite the recent statement by Dr Robert Frosch, NASA's administrator, that the space shuttle's first operational flight will now be delayed until September 1981, however, ESA has received no formal notification of any further delay to the first Spacelab launch most recently scheduled for August 1981.

Religious leaders criticise links between knowledge and power: Scientists and technologists have weakened their claims to intellectual freedom by showing bias on the side of those who wield economic power, according to Dr Philip Potter, general secretary of the World Council of Churches. Addressing the opening session of a two-week conference on science and religion in Boston last Thursday, Dr Potter said that there was little sign that scientists were on the side of the oppressed, the deprived and the "marginalised".

"Science and technology might claim to be objective and universal, but this claim is not born out in reality," said Dr Potter, who is a methodist minister in Jamaica. "Science and technology are not neutral or value-free but are instruments of power, and that means political power"; a leading issue of the conference was therefore how ways could be found of allowing the public a greater share of decisions on how science should be employed.

In contrast to Dr Potter's comments, Dr Robert H Brown, an astronomer from the University of Sydney, Australia, defended science against claims that it had been corrupted through colluding with the powerful against the interests of the poor. "Some tell us that the ideals of science have been so eroded by its alliance with power that it has little to offer us," he said. "I suggest that it is no more a valid reason for turning away from the values of science than it was for scientists to turn away from the Christian faith."

Laetrile claimed to increase size of tumours: Doses of the controversial drug Laetrile, which some claim to have anti-cancer properties, has been shown to increase the size of tumours in rats, and eventually lead to cyanide poisoning, according to the report of two US medical research workers in the *Journal of the American Medical Association*. The researchers, from the Northwestern University medical school, say that doses described as "realistic in terms of human ingestion" led to a progressive increase in the size of tumours when administered to rats, and as the dosage was increased, so to did the incidence of death from cyanide poisoning. "The findings seriously question the use of amygdalin [Laetrile] in clinical medicine under any circumstances" said Dr Janardan D Khandekar, one of the scientists involved.

Meanwhile the Food and Drug Administration has rejected the charge from one of its staff scientists that it is unethical for the agency to study the effects of Laetrile, as has been requested by the National Cancer Institute, even though there is no scientific evidence of its efficacy.

In reply to a citizen's petition filed by Dr Robert Young, the agency has said that it does not consider such studies to be unethical since the tests might provide some useful information. "The ethical aspects of failing to discover a useful cancer drug are complex and not well explored, but the prospect of such an outcome occurring because of intellectual rigidity or over adherence to an ethical dogma is unsettling," replied Dr Richard Crout, director of the Bureau of Drugs, although admitting to "a great deal of ambivalence" on the matter.

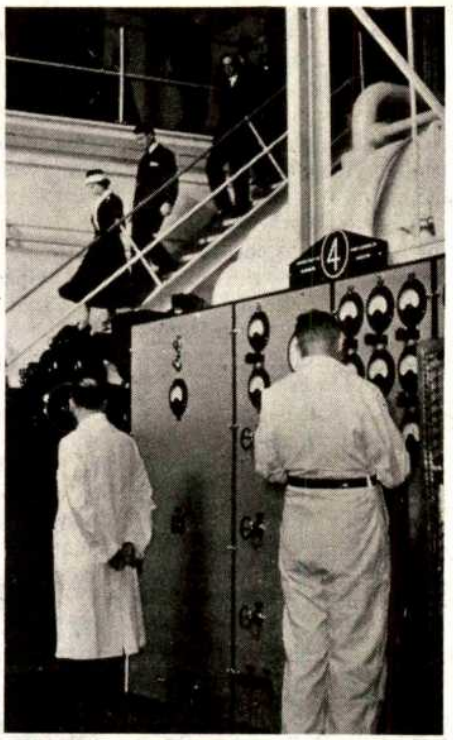
Germany's science council recommends sites for new polar research institute: The federal government of West Germany, which aims to join the consultative round of the Antarctic Agreement, wants to strengthen polar research activities in the FRG and therefore decided some time ago to establish a central polar research institute "as soon as possible". Last month, the Science Council (Wissenschaftsrat), which was asked to advise, welcomed the initiative and made some detailed suggestions for the new 30-scientist establishment.

The Science Council recommends that the institute should set up as a foundation under public law and financed equally by the federal government (the *Bund*) and the *Land* where it is situated. It should be responsible for the planning, construction and operation of the German research station in the Antarctic and direct the operation of the ice-breaking research and supply ship in the polar regions. Moreoever, it should have an important function in co-ordinating national and international polar research activities, thereby co-operating with other institutions acting in this field and building on a long-standing tradition of German polar research. Concerning its location, the Science Council recommends that it should be established in the neighbourhood of a large university with internationally proven research in the relevant fields. The Science Council therefore put Kiel at the top of the list, Hamburg next, and Bremen (including Bremerhafen) third. Among the three towns, Hamburg has rejected the institute, as it is fully occupied with establishing a new technical university. Kiel, on the other hand, is showing a strong interest, but faces strong political objections in Bonn.

India to launch oceanographic research vessel: An oceanographic research vessel will be commissioned by 1981 for research and development of marine resources in India. The Indian government's decision to acquire the vessel is in line with the government's recognition of the importance of ocean waters and the sea bed as store of living and nonliving resources. Earlier, the Government of India had enacted the Maritime Zone 1976 which reserved the right of survey and exploration of the marine resources of a 320 km 'Exclusive Economic Zone' and the continental shelf exclusively for the Indian people. To ensure optimum utilisation of research facilities and proper co-ordination the Ocean Science and Technology Agency (OSTA) was set up in 1978. The research vessel is to be manufactured in the Federal Republic of Germany.

UKAEA: boom or bust?

Two years after the establishment of the United Kingdom Atomic Energy Authority 25 years ago, the Queen was opening the world's first full-scale nuclear power station at Calder Hall (see right). But these early boom years of British nuclear power are over. Energy forecasts are drastically lower, and uncertainty about the future of the industry has led to the loss of key staff. The fast breeder design team, for example, has been virtually disbanded. So even if the new government wished to re-invest in the nuclear industry — as the Prime Minister indicated at the recent summit meeting in Japan — it would first have to re-establish confidence in the industry itself. So while Britain's new energy ministers, headed by Secretary of State David Howell, consider the formation of a new energy policy for Britain, we asked **John Surrey** of the Science Policy Research Unit at the University of Sussex to give us his view of the future of nuclear power in this country. On a following page, **Paul McDonald** considers the difficulties facing another major energy option — conservation.



How the UKAEA might survive

LONG-term forecasts given at the UK National Energy Conference in June 1976 pointed to a future 'energy gap', due to rapid growth in demand and rapid depletion of British North Sea oil and gas reserves. They indicated that without major investment in coal and especially nuclear power, the UK would have to rely on increasingly large and expensive oil imports after about 1990. Only the gas industry challenged the forecasts, arguing that North Sea supplies would last until at least 2000. For the other fuel industries, high demand forecasts promised security and expansion.

Coal's target capacity for 2000 was set at 170 million tonnes — the maximum attainable given the need to replace a large proportion of current capacity and the long lead times for major projects such as the development of the newly discovered coal-fields at Selby and Belvoir. The same climate encouraged the UKAEA (Atomic Energy Authority) to present a 'reference' programme which envisaged 104,000 MW of nuclear power in operation by 2000, including 33,000 MW of fast reactors. These were to be preceded by a 1,300 MW demonstration plant, originally code-named CFR-1 and later CDFR. The 'reference' programme would require about 6,000 MW of nuclear plant to be built *each year* — somewhat more than the total nuclear generating capacity in service today.

A recent consultative document, the 1978 Green Paper on energy policy, contained much lower demand estimates for 2000 — 450-560 mtce (million tonnes of coal equivalent). For comparison, the mid-point of the 1976 forecasts was 558 mtce and the top of the range was 760 mtce. The 1978 official forecasts were very close in several respects to those published by Chesshire and me shortly before the Green Paper appeared. The two sets of estimates were very close for final energy consumption and, in the 'high' case, for primary energy. But in the 'low' case the Green Paper estimate of primary energy demand was 115 mtce higher than ours (see Table 1). Given the other similarities, this big difference can only stem from an assumption behind the official forecasts that electricity consumption, and therefore conversion losses, will be much greater than we estimated under the 'low' case. The discrepancy simply illustrates that the prospects for electricity are one of the biggest uncertainties in energy planning — which is of key importance for coal and nuclear power, given their dependence upon electricity generation.

Until nuclear power stations can work satisfactorily and economically under highly variable load conditions, the scope for nuclear power will be limited to base load generation. In the UK, it is further restricted by a large plant surplus, including a large amount of plant still under construction. Furthermore, electricity is expensive relative to other fuels: in the industrial market the price ratio of electricity is 5.4 relative to gas, 6.1 relative to coal and 3.8 relative to oil.

As long as the relative price is so high, electricity will not substitute for oil and coal in competitive crude heat uses, or for gas in many premium uses. Even assuming that nuclear-generated electricity will henceforth be cheap relative to oil and coal, the fact that nuclear power currently

Table 1 1978 Forecasts of UK Energy Consumption in 2000

	1977 Actual	Forecasts for 2000 SPRU[1]	Green Paper[2]
		billion therms	
Domestic	15.0	14.2—16.2	(13.6)—15.8
Transport	13.1	17.2—19.9	(15.8)—17.0
Other final consumers	7.6	6.0— 8.7	(7.0)— 9.0
Iron and steel	4.9	3.7— 8.4	(7.0)— 9.9
Manufacturing industry	17.9	19.6—28.2	(20.0)—25.4
FINAL ENERGY	58.5	60.8—81.4	(63.4)—76.8
		million tonnes of coal equivalent	
Coal	122.7	83—162	170[3]
Oil	136.6	124—224	150
Natural gas	62.8	88	50—90
Nuclear and hydro	14.3	40—102	95
PRIMARY ENERGY	338.4	335—577	(450)—560

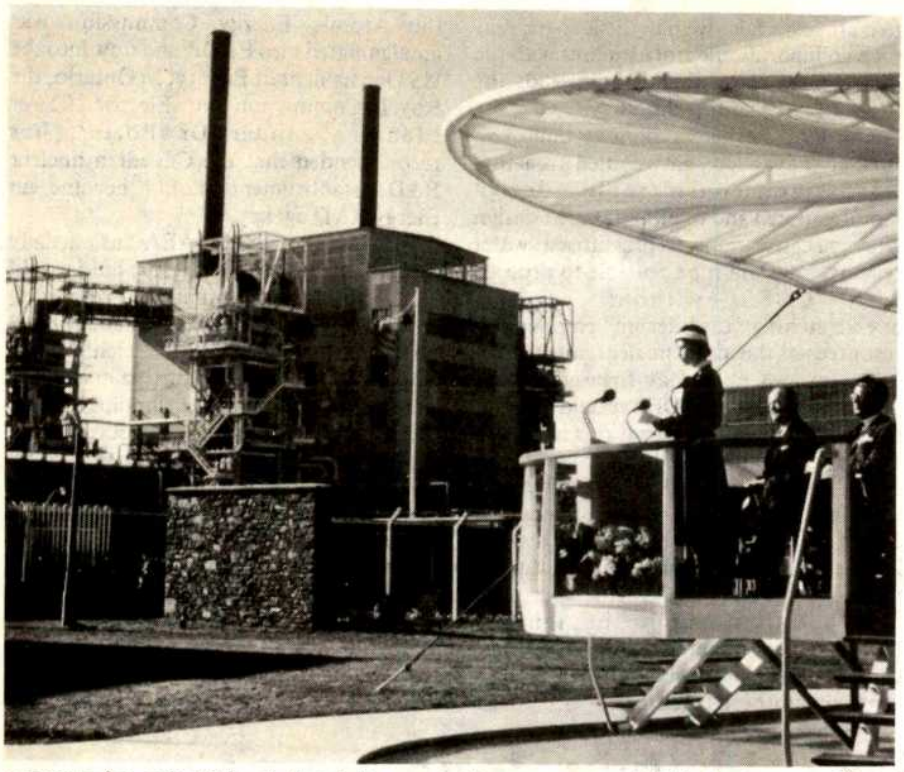
[1] J. H. Chesshire and A. J. Surrey, *Estimating UK Energy Demand for the Year 2000: A Sectoral Approach*, SPRU Occasional Paper No. 5, February 1978.

[2] *Energy Policy: A Consultative Document*, Cmnd 7101, HMSO, February 1978. The figures in brackets apply to a 'low' case which is only partly specified.

[3] These are estimated availabilities of indigenous fuels only. They sum to 465 mtce, in the 'high' case, leaving net imports of 45 mtce and a 'policy gap' of 50 mtce.

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accounts for only 13% of electricity supplied to the British grid means that the price of electricity will not fall appreciably relative to the price of oil and coal until base load is supplied predominantly by nuclear power stations. That, of course, would involve a large nuclear programme to replace the fossil fuel power stations built in the 1950s and 1960s and — since two-thirds of British coal output goes to power stations — a big fall in coal demand.

One is inescapably drawn to the conclusion that electricity demand growth over the next two decades will continue to be low, especially if the supply of North Sea gas reaches 6,000 million cubic feet a day in the early 1980s and remains at that level until 2000. In these circumstances, electricity demand growth will be chiefly in certain premium uses where electricity offers advantages which outweigh its high price. Information available on domestic appliance ownership levels and industrial process applications indicates that the growth potential from these electricity-specific uses is quite small.

Compared with the earlier 104,000 MW 'reference' programme the 1978 Green Paper sees a nuclear component of 25-40,000 MW in 2000, implying the construction of only 1-2,000 MW annually from 1985. The central question now facing the nuclear industry is no longer whether it can muster the skills and resources to build a large programme, but whether it can survive a long period of low home ordering and continued difficulty in exporting.

Following Ince B, Drax B and two new advanced gas cooled reactor orders, further advance orders for power stations should be out of the question on cost grounds. It is therefore urgent to identify the resources and skills that are specific to nuclear design and engineering and to examine how they can best be organised and retained against the time that series ordering can take place. This problem must have precedence over the fast reactor question, for it would be sheer folly to develop fast reactor technology without ensuring that the design and manufacturing base is there so that nuclear power stations can be built efficiently when they are needed. If there is a solution, it is likely to require the rationalisation of the whole power plant manufacturing industry, including nuclear design and construction and large steam turbine generators.

The recognition that long lead times make it impossible to install a significant fast reactor programme by 2000 now seems to have brought a corresponding change in the reason adduced for proceeding with the demonstration plant. When CFR-1 was regarded as the first of a large fast reactor programme, it could be held that costs of the demonstration plant over and above the costs of the cheapest alternative power source (a light water reactor, for example) would be recouped from the benefits accruing from the commercial fast reactors that were soon to follow.

Since the justification was primarily economic, it was possible to argue that CFR-1 would carry no opportunity cost in terms of other R&D projects. Questions about resource allocation were answered by the claim that the programme as a whole would show a large and positive net present value after applying the test rate of discount that is applied to public sector investments. As long as it appeared tenable to argue that the cost of CFR-1 would be recouped from the subsequent commercial programme, the relevant economic questions concerned the estimated capital and operating costs of fast reactors compared with thermal reactors and the estimated long-run supply and price of uranium. Both questions involve major uncertainties and evoke widely differing views.

If, however, approval for CDFR is seen as carrying no commitment to build a programme of fast reactors before the turn of the century, the choice must be seen primarily in the context of R&D strategy. If the commitment which is sought is for CDFR alone, not for a subsequent programme, the question of R&D opportunity costs looms large — the resources must come from somewhere and something will have to be foregone. Unless it is reasonably certain that a highly economic programme will follow in the not-too-distant future, the question of economic risk becomes very important with an R&D project costing £1,500 million or more. Such a commitment to any single R&D project can scarcely fail to affect the climate of opinion and the funds available for alternative R&D options. We therefore need to be quite sure that an equivalent funding of alternative technologies (not only in the energy field, of course) will be less rewarding than building CDFR.

The argument that Britain should acquire the capability to build commercial fast reactors as an insurance against long-term uranium scarcity involves a political judgement. It requires a decision on the acceptability of the whole range of risks and the appropriate rate of social time preference, how much the present generation is prepared to forego for the well-being of future generations.

Another argument in favour of CDFR, the 'export' justification, is wholly spurious for no one can say whether there will be opportunities 20 years hence to export 1,300 MW fast reactors. So far, domestic markets in the USA, Japan and most of Western Europe have been closed to imports of nuclear reactors. Whether the developing countries can accommodate 1,300 MW generating units on their power systems is a moot point. And on foreign policy grounds it is surely unwise for the UK to appear willing to promote exports of fast reactors (and the plutonium to fuel them) before rigorous international safeguards for reprocessing and the commercial use of plutonium are agreed and implemented.

If the political judgement is that Britain should acquire the capability to build fast reactors in the future, the relevant question then is how to proceed. Several further questions must then be answered, because building CDFR is only one of the options for acquiring this capability.

Firstly, why is it necessary to scale up to 1,300 MW? Doesn't the ability to replicate and perhaps stretch the 250 MW Dounreay prototype fast reactor give sufficient insurance against the risk of long-term

uranium scarcity? If there are compelling technical reasons for scaling up, why not concentrate R&D effort upon the specific, critical metallurgical and engineering problems, as opposed to building a 1,300 MW power station costing at least £1,500 million? In any case, the performance of very large conventional and nuclear generating units hardly inspires confidence that the proposed scaling-up is economically justified.

Second, since France and Germany are intent upon building demonstration plants which are broadly similar to CDFR, why not wait and license from them when their designs are proven? After all, other countries have acquired the ability to build thermal nuclear reactors on the basis of foreign licences, whilst Britain has spent untold sums in developing indigenous reactor designs. Alternatively, why not collaborate with France and Germany, learning through participation, so as to be able to incorporate any necessary modifications in an eventual British version? The argument that Britain must go it alone because we have little to offer in such collaboration hardly squares with the oft-repeated claim that Britain leads the field in fast reactor technology.

Third, given the decisions to build two further advanced gas cooled reactors (involving considerable design modifications) and to undertake the design work necessary for a pressurised water reactor, how will it be possible to proceed with CDFR without severely overstretching the design engineering resources of the ailing nuclear industry?

So far, attention has focused on the question of whether CDFR should be built, rather than what happens if it is not built. Fast reactor work accounts for two-thirds of the scientists and engineers employed by the UKAEA on reactor development. A decision not to build CDFR will immediately call into question the future of the UKAEA. Even if CDFR is approved, this question cannot be ignored indefinitely. Sooner or later, it must confront any single-mission R&D agency. It has already been faced in the USA, where the Atomic Energy Commission was amalgamated into ERDA and now into the US Department of Energy. In Ontario, the Royal Commission on Electric Power Planning, under Dr Porter, has recommended that the Canadian nuclear R&D establishment should become an energy R&D agency.

It is true that the UKAEA has partially diversified into ancillary work; but I would like to think that its skills and resources, which are unique in Britain, can be applied on a much larger scale to other technologies with the same brilliance and dynamism that were applied to nuclear technology in the 1950s. This is not to suggest that the UKAEA must become an energy R&D agency. Its new role must be defined in the context of overall industrial and R&D strategy and the need to make the best use of all our public laboratories.

The assurance of a future rôle which is nationally and personally worthwhile will remove much of the uncertainty for the scientists and will make it less likely that a breeder is built for the wrong reason. □

'Saving it' is easier said than done

With the oil crisis in full cry and nuclear energy still an uncertain option, the UK government this week called on industrialists to make energy saving a priority. **Paul McDonald** assesses the potential of this new policy.

Energy conservation, according to Mr David Howell, the Secretary of State for Energy, is "now at the centre of energy policy". Faced with the task of trying to cut oil consumption by five per cent, the Government is relying heavily on industry to make significant improvements in the efficiency with which it uses its energy. However, apart from the widely publicised efforts of certain individual companies, there has been little progress in this sphere.

There are no major technical reasons why much of industry could not improve the efficiency of its use of energy substantially. Such is the inefficiency of the average industry, that its energy can usually be reduced by 10% with little or even no capital investment, simply by goodhousekeeping measures, such as insulation, or turning off machinery not in use. Savings of a further 10% are usually possible from improvements in processes; and a further 10% on top of that from major reorganizations of processing and wholesale re-equipping. The actual proportion that can be saved varies from industry to industry. The Department of Energy's estimates of potential savings vary from a total of 10% in the pottery industry to 61% in aluminium smelting.

The Department of Energy has, unfortunately, no way of assessing accurately how industry is progressing in this. The most optimistic estimates put the average level of improvement in the efficiency of industrial energy consumption since 1974 at 10%; but this is no more than could be achieved by general goodhousekeeping measures in most industries — and certainly below the level of our overseas competitors such as West Germany and Japan. In spite of an upsurge of interest in energy conservation amongst industrialists since January, there are still many factors inhibiting any major improvement in the use of energy, which are going to make the Department's target of 5% very difficult to achieve.

On the surface, there appears to be a good deal of activity: over 4,000 energy managers have been appointed; but their functions and responsibilities vary enormously, and, even in some large companies, a number of them are little more than token appointments.

A major constraint on improving energy efficiency is the lack of capital for measures designed to improve energy use, such as waste heat recovery and improving processes. This is particularly the case for the large number of industries where energy accounts for less than about 2% of manufacturing costs. There is, in any case, a preference in most industries to invest in production rather than conservation. Whilst the latter brings savings, these do not necessarily show up in reduced expenditure on energy in subsequent years, since they may easily be cancelled out by the general rise in energy prices. Given the preference for increasing output, conservation measures are often penalised by the imposition of shorter payback times than those for other items of capital expenditure.

Continuing economic recession and low levels of productivity greatly inhibit the efficient use of energy by forcing industries to work well below their full capacity. Furthermore, in a number of industries, such as food, drink and tobacco, there is a trend towards increased processing, which will increase the consumption of energy for the same level of output.

Improvements in energy use are further hindered by the slow rate of re-equipping in British industry. Many companies wait until equipment is due for rebuilding or replacement before trying to improve its energy consumption. In the case of a furnace, this is only once every 5-10 years.

Proper records of fuel consumption are vital to the sucess of any serious attempt to improve energy efficiency. In a great many companies these simply do not exist, making it impossible to set targets or monitor progress. Futhermore, the high costs of installing and maintaining adequate metering for this task will make the accurate control of energy use a continuing problem for a large number of firms.

The aim of company energy policies is not always to save energy. For certain firms operating continuous processes, security of supply may be the paramount consideration. For others, energy policy may be part of a general cost cutting exercise, and can just as easily involve renegotiating tariffs or switching to other fuels, as actually trying to reduce consumption by requiring the installation of energy using anti-pollution equipment as part of its health and safety policies. Energy conservation is clearly not going to be the easy part of the Government's energy policy.

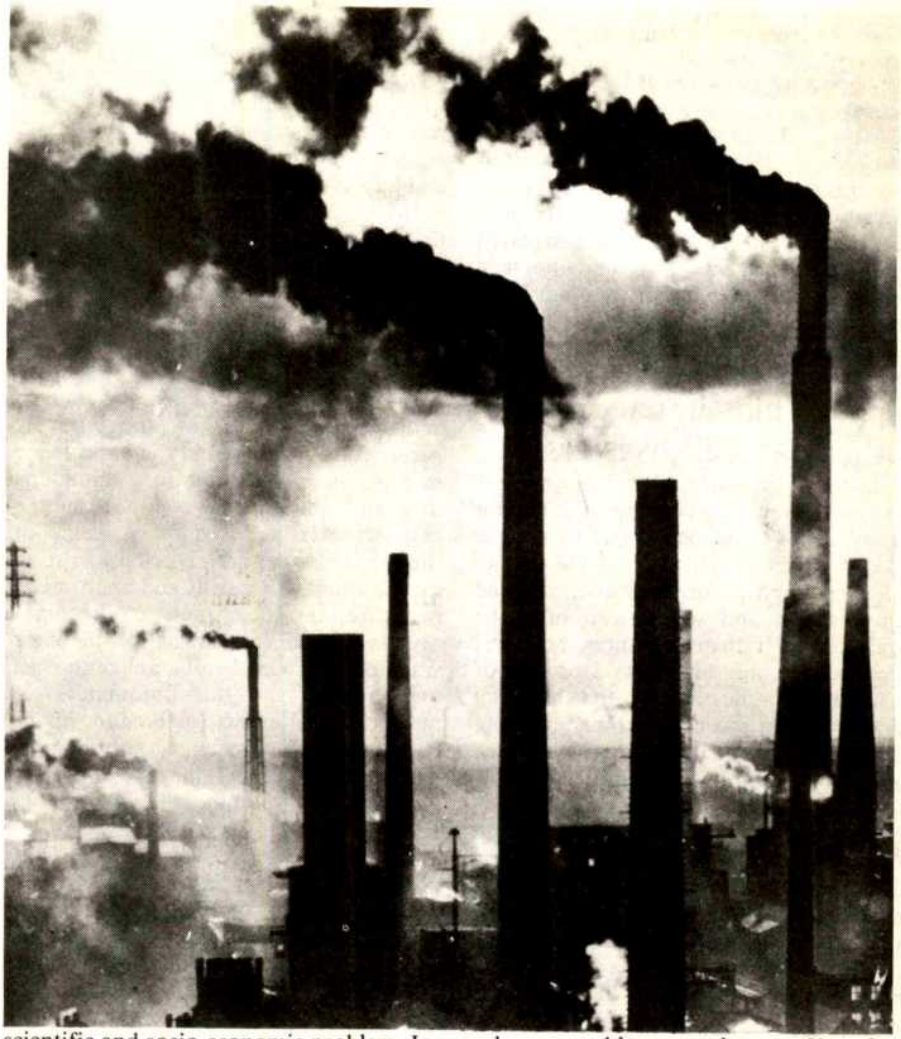
Dr McDonald is a writer on energy matters

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A political view of CO_2

The increase in atmospheric carbon dioxide may be accelerated by President Carter's new-found enthusiasm for synthetic fuel. But the atmospheric 'crisis' may come too slowly to bother the politicians, argues **Michael Glantz.**



FOR those interested in the study of climate — including its impact on society and society's impact on it — 1972 was an extremely important year. In that year a collection of weather anomalies occurred adversely affecting global food production and therefore availability. At that time some blamed the food shortages on the weather. More recently, however, those claims have been reevaluated and the blame is being apportioned more correctly between weather and society. The anomalies of 1972 included the fourth consecutive year of drought in the Sahelian zone of West Africa, the failure of the Peruvian coastal fisheries, droughts in Central America, the Soviet Union, India and China, along with excessive rains in parts of the Philippines, Australia and Kenya.

At that time the sharp increase in grain prices on the international market, the largest grain purchase by the USSR to that date, and the general scarcity of foodstuffs in the international marketplace, were all blamed on fluctuations in the weather and led to the concern that the weather had gone haywire. Concern about what was happening to the weather can be evidenced by citing such publications as "Ominous changes in the weather," "What's gone wrong with the weather?", "When the Sahel freezes over," The Cooling, Blizzard, Ice, The Genesis Strategy, Hot House Earth, Climatic Change, The Weather Conspiracy and so on.

Was the climate regime getting cooler? Was it getting warmer? Was it staying the same? Was climate variability increasing? Climate (and weather) have become recognised as important variables in the activities of societies, regardless of the level of industrial development, geographic location, or ideological bias of the respective society. This new-found importance is due partly to the media's acceptance that such factors are newsworthy, and partly as a result of the raised consciousness of some political and economic leaders that climate factors must be considered in the food-population equation.

A projected CO_2-induced global warming became part of the debate and is increasingly being viewed as an important scientific and socio-economic problem. In addition to the natural processes, CO_2 enters the atmosphere as a result of human activities, primarily the burning of fossil fuels. The current rate of increase of CO_2 content in the atmosphere has been measured at about several tenths of one per cent per year. With projected energy use increases it has been suggested that a doubling of the CO_2 content in the atmosphere can be expected by the middle of the 21st century.

A doubling is expected to warm the lower atmosphere (as a result of various physical processes) with estimates ranging from 1 to 4 °C.* This warming is expected to affect weather patterns and climate regimes around the globe, changing precipitation and temperature patterns resulting in the shifting of agricultural zones. Such shifts would adversely affect some regions while favourably affecting others, with no assurances as to who the winners and losers will be. Thus, much attention has been focused on the ultimate effects of the increased CO_2 loading of the atmosphere.

In fact, the increase in the CO_2 content of the atmosphere takes place each day and, measurably so, each year. Yet, the annual increments are perceived by most observers to present little immediate danger to society and are considered non-threatening. No immediate attention, let alone rectification, of the CO_2 problem is perceived to be necessary.

The CO_2 impacts are especially an important area of study for the social sciences because they represent, as well as highlight, a relatively new (some would say neglected) research area of interactions between climate and society. Many consider the study of the CO_2 content of the atmosphere timely even though it is still only a potential problem with what appears to be a relatively long lead time, that is, the major impacts are projected in terms of decades. The "luxury" of a long lead time is not accepted as such by everyone, since society will require a long time to make adjustments that would be in line with attempts to develop alternative sources of energy to reduce the use of fossil fuels.

It may be useful to separate the CO_2 problem in to the two predominant perspectives: as an event — the doubling, and as a process — the annual 1 p.p.m. increase. In reality both views are correct but each by itself presents an incomplete picture of the problem. To better understand and, therefore, deal with the potential societal and environmental

*It is important to keep in mind that the locations, types, magnitudes and directions of change as well as the importance of certain variables in CO_2-related processes are in most instances educated speculation at this time.

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problems associated with an increased CO_2 content of the atmosphere, the problem must be viewed as both event and process.

Most of the attention of the media, the public and the policy-makers is directed toward the CO_2 problem as an event because of the spectacular aspects of a CO_2 doubling. For example, an often referred to impact of a doubling is a surging into the ocean of the West Antarctic Ice Sheets resulting eventually in an increase in sea level on the order of 5-8 metres.

Spectacular impact of other natural disasters

The CO_2 problem viewed as an event has been likened to other specific natural disasters — earthquakes, droughts, floods, severe or dry winters. The impact of a drought, for example, on the environment and society can often be spectacular. In many instances, however, the drought only highlights a process of environmental degradation that has been under way for a period of time preceding the drought event itself; a process which at any one point in time represents only a low level abuse of the environment.

So, the dust storms in the American Great Plains during the droughts in the 1930s and briefly in the 1970s, while spectacular, were not unique (except for their magnitude). Each year during the period between such major drought events, the top soil was also blown away at a much less spectacular level. The cumulative effect of such storms in the interdrought periods may in fact be much greater than that of the specific drought event.

One might argue by analogy that the tendency to overfocus on the more spectacular event (the CO_2 doubling) takes place at the expense of attention to the slow but steady annual increases in CO_2 (the process).

The example of urban air pollution, as a low-level but continually increasing insult to the environment, in some ways represents a microcosm of the CO_2 problem as a process. It also suggests how society might react to such a problem. There are very gradual, but definite, changes that take place in the environment (especially weather and climate) as a result of urban air pollution, a large part of which results from the burning of fossil fuels.

Yet, in many instances the changes that have occurred are low level and have apparently taken place gradually over an extended period of time. One might ask, how well have societies and their political leaders dealt with those incremental changes in air pollution levels and with the social changes they may have brought about? How well have they dealt with drought episodes? How well can they be expected to deal with CO_2 problems?

Studies of societal responses to spectacular events such as major droughts and their impacts suggest that political interest in those kinds of problems varies with the presence of other problems that compete for time and scarce resources. In addition, interest in such events also rises and falls as a result of the different lengths in office of different decision-makers (2,4,6 year cycles for politicians). This fluctuation in interest in manifested in a relatively abrupt swing between two types of decision-making processes:

- "crisis management" and
- "muddling through."

In crisis decision-making, the time to act is perceived to be short, the stakes are perceived to be high, and the impacts are seen to be severe. As for "muddling through," the opposite is seen to be the case; there is perceived to be plenty of time to act, society is expected to rise to the occasion with a swift and appropriate response if and when it becomes necessary, and the impact of the next situation is expected to be unlike the last one because of the uniqueness of meteorological events (or because of the belief that society tends to learn from its past mistakes), or as is the case of a CO_2-induced global warming, the situation has never happened in the past.

The two perspectives of the CO_2 problem mentioned earlier — event and process — are useful for a better understanding of not only the problem and its impacts but of the political decision-making responses to it. For example, perceiving the increase in CO_2 levels as an event may be valuable for creating an awareness of the potential consequences. It may also be helpful in generating public and political concern about the risks associated with a continued dependence on fossil fuels. However, if the analogy of urban air pollution as a microcosm of the CO_2 problem is accepted, then it can be seen that spectacular arguments about the CO_2 doubling will not bring about the desired public policy decisions concerning alternative energy systems.

Muddling through on long-term problems

What is needed is a decision-making approach that matches the environmental situation. While the concern with a doubling might initially create a crisis decision-making atmosphere, it would be an ineffective approach to an environmental problem which is essentially low level but cumulative, a not so spectacular problem that requires sustained interest over a long period of time.

In the past the decision-making approach to low level environmental problems such as air pollution has generally been one of muddling through. That may have been adequate in dealing with some political and social problems, but it has not been an effective way to deal with low-level cumulative environmental problems. Eventually such problems increasingly worsen until a crisis situation is perceived to prevail. Then policy making grows out of crisis management.

Crisis decision making from the outset has also been ineffective as a way to deal with low-level cumulative insults to the environment. After a brief flurry of decision-making in a crisis atmosphere, the crisis (perceived high threat, short time to act, high cost) in this case does not immediately materialise. As a result, interest in the problem dissipates and those who again attempt to generate interest receive little response from the media, the public and the politicians. Environmental degradation, however, continues to worsen steadily.

Crisis thinking may be the only way

What is needed is a way to deal with the two aspects of the CO_2 problem simultaneously. The CO_2 problem, not unlike the more localised urban air pollution problem, might be seen as an "impending" crisis and, therefore, in need of a special type of decision-making process, one that combined the positive elements of a "crisis awareness" with cautious decision-making.

An interesting question that arises is whether a democratic society can effectively deal with a CO_2-like environmental problem. Of the competing interests in such a pluralistic society, some of those interests will be winners and some will be losers. The demand of society for continually increasing amounts of energy derived from the burning of fossil fuels may be more immediate and pressing than the environmental effects of such activities. In addition, in every society where resources are scarce, there is a great competition among interest groups for attention, time, and resources to deal with their particular problem. In light of the hydra-headed crises that confront most societies (water, food, population, energy, disease, etc.) interest in any one problem (especially the low level ones) lasts only for a short time before society shifts its attention to the other problems that confront it. So it is possible that a pluralistic society may best be equipped to deal with environmental problems on a crisis basis, that is, it is best (if not only) capable of fighting brush fires as they arise.

The question then becomes one of developing a policy-making approach for a pluralistic society (including the international community), one which can sustain the interest and concern of various segments of society in a low-level but cumulative environmental problem such as the effects of the continually increasing burning of fossil fuels. □

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news and views

Longer perspectives in neutrino physics

from D. J. Miller

THE honeymoon is drawing to an end. The charms of the 'new physics'—weak neutral currents, the J/ψ, the upsilon, parton-like scattering and jets — are now part of the everyday world. At the Bergen conference 'Neutrino '79'* this change of outlook showed in a number of ways.

J.D. Bjorken (Stanford Linear Accelerator Center) summarised the situation. A year ago individual experiments, such as the Gargamelle neutrino-electron scattering result (see *News and Views* **273**, 98; **274**, 531; 1978) looked as if they might change our whole understanding of the field. But as such fluctuations are superseded by accumulating statistics, the 'standard model' has shown great elasticity. The same picture always reasserts itself. The strongly interacting particles (hadrons) are explained very economically in terms of fractionally charged 'coloured' quarks, fundamental particles at the same level as the leptons (electron, muon, tau and the neutrinos), but confined in threes or in quark-antiquark pairs. The electromagnetic and weak couplings of quarks and leptons have settled into a simple pattern. Last year's questions were about the neutral weak currents. This year three theorists stood up one after another (J.J. Sakurai, University of California, Los Angeles, P. Langacker, University of Pennsylvania and M. Roos from Helsinki) and, with a variety of starting assumptions — discarding part of the data, varying the theoretical model, fitting 10 parameters, 7, 5, 2 or 1 parameter — they all came back to the same values for the couplings. There is still a lot of checking to be done, but many of the experimental reports on neutrino results from accelerators are beginning to sound routine.

All theoretical discussions of the immediate experimental picture are dominated by the two gauge theories which together now constitute the standard model; QCD (quantum chromodynamics, see Marciano and Pagels *Nature* **279**, 479; 1979) for the strong interaction with quark-binding, and $SU(2) \times U(1)$ (the 'Glashow-Weinberg-Salam model', see *News and Views* **279**, 675; 1979) for the weak and electromagnetic interactions. Neither of them is proved to be correct but each offers an economical and comprehensive explanation of a large group of phenomena. Neutrino results which test the predictions of QCD were reported by a number of groups and it is clear that the breakdown of the simplest quark-parton model of neutrino or muon scattering is consistent with QCD. A few results, notably some new muon scattering data from Fermilab experiments, did not quite fit in — but people were inclined to wait and see whether such disagreements would just go away, as so many others have done.

Two working gauge theories would seem to imply an underlying unified gauge theory. 'Grand unified models' are being put forward with increasing confidence — the simplest being the SU(5) theory due to Georgi and Glashow. The reaction to such models last year was lukewarm. How could we test a unified theory when the forces it describes (strong, weak and electromagnetic) only become equal at an energy of around 10^{15} GeV? Now, however, it has been realised that an important experimental test lies within easy reach. The credit for pointing this out belongs, among others, to Cecilia Jarlskog of Bergen — chief organiser of the conference and a uniquely good-humoured review-speaker. In her talk she reminded us that if the 'grand unification mass' is 5×10^{14} GeV then the proton lifetime should be between 10^{30} and 10^{33} years (see *News and Views* **278**, 687; 1979). Experimental limits on this lifetime are already more than 10^{29} years — a by-product of cosmic-ray neutrino experiments. The most interesting experimental session of the conference to me was the one which contained no new results at all but in which L.R. Sulak (Harvard University) and E.C. Fowler (Purdue University) talked about plans for experiments to push the limit on the proton lifetime to beyond 10^{33}, perhaps even to 10^{35} years. Sulak presented a scheme which is likely to be working within a year. It involved no new technology — just a tank of water 20 m × 20 m × 20 m, with a grid of photomultipliers over all of its faces to detect Cerenkov light from the electromagnetic showers which would be produced by such decays as proton → positron + pi-zero.

The implications of the new physics, in particular those of the neutral weak currents, are now being worked out in other fields than high energy particles. An important series of experiments is taking place on the quantum-optical effects of parity violating couplings. E.D. Commins (University of California, Berkeley) reported that the Novosibirsk result on parity-violating optical rotation in bismuth vapour is looking increasingly secure and is agreeing with the $SU(2) \times U(1)$ model predictions. He presented his own preliminary but positive results on dichroism in thallium and he suggested that the null results seen by some groups working with bismuth may have been due to such subtleties as variable side-bands in their laser beams. The present motivation for all such experiments is still to check the weak neutral current couplings, but I wonder if this is not likely to become a 'spin-off' field from particle physics, comparable to the 'spin-offs' of the Mossbauer effect and nuclear magnetic resonance from nuclear physics. Perhaps in a dozen years optical parity-violating effects will be another tool in atomic physics or in chemistry; the apparatus is not expensive and laser technology is advancing rapidly. Low energy nuclear physicists are also now joining in the search for neutral-current effects and some positive results were reported.

But astrophysicists provided the widest horizons. There is still a problem in the observation of neutrinos from the Sun. M.S. Davis's group from the Brookhaven Laboratory see about one-half or one-third of the predicted rate of transitions $^{37}Cl + \nu_e \rightarrow {}^{37}Ar + e^-$. This reaction is only sensitive to higher energy neutrinos which come mostly from a subsidiary branch of the solar nucleosynthesis cycle. For years there has been a debate as to whether the solar models, the nuclear transition rates or the properties of neutrinos were to blame for the anomaly. Now a new collaboration has been formed — reported by Kirsten from Munich and including Brookhaven,

* 'Neutrino '79' was held in Bergen on 18-22 June and organised by the University of Bergen and NORDITA.

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Pennsylvania and the Weizmann Institute, which will look for the more copious lower energy neutrinos that must be produced by the basic solar hydrogen-burning process $p + p \rightarrow d e^+ \nu_e$. They will collect 50 tons of gallium over the next few years and search for the expected production of one atom per day of radioactive germanium by way of the process $^{71}Ga + \nu_e \rightarrow {}^{71}Ge + e^-$.

Other astrophysicists reported on the apparent regress of the theory of stellar collapse. This has followed from a serious treatment of the equation of state of highly condensed matter. It had been thought that neutrinos could provide the mechanism for transporting energy from the core of the supernova to the exploding envelope, thus allowing the collapsed core to get rid of its binding energy and form a neutron star. But present values for neutral and charged current cross-sections require that during the crucial last second of collapse the neutrinos are trapped in the core and form yet another degenerate Fermi gas, in equilibrium with neutrons, protons, electrons and nuclei. As a consequence of this, a number of speakers reported, it is not now possible to show how a supernova envelope can be formed by the collapse of a massive star. New ideas and calculations are clearly needed, but the theorists are hampered by a poverty of experimental constraints. Supernovae are seen only about once per century in our Galaxy and no one has yet observed the expected 'neutrino flash' that should go with the visible flash. Such an observation would be extremely useful.

The biggest accelerator of them all, the primaeval big bang, provides the most speculative field of application for the new physics. Cosmologists, D. Schramm (University of Chicago) told us, have embraced the Grand Unified models as a potential answer to the question of why the universe seems asymmetric in baryon number. Why is all stable matter apparently not matched by equal amounts of antimatter? If the massive (10^{15} GeV/c^2) particles exist which would mediate proton decay they must have been produced copiously in the very earliest stages of big bang. Their decays could be affected by substantial CP symmetry violation, leading, as in K° processes, to a time asymmetry in the production of positive and negative particles. In thermodynamic equilibrium, such effects would be cancelled out, but in an expanding and cooling system an overall baryon excess over antibaryons might well be produced. Variations on this theory have been suggested, involving such contracting mechanisms as a 'foam' of mini black holes or supercluster-sized zones of baryon and anti-baryon matter caused by local differences in spontaneous symmetry-breaking.

In the final sessions Glashow (Harvard University) gave his customary shopping list of work still to be done. Despite the widening influence of recent results, there are many gaps in our understanding. In particular, why do we have the same sort of family of quarks and leptons three times over? Why is each generation so much heavier than the last? How many more generations are there? (If more generations mean more massless neutrinos then the cosmologists cannot account for the amount of helium in the universe). Accelerator and storage-ring physics may spend the next few years tidying up at the present level of understanding but we should be building new machines at the same time. The CERN $\bar{p}p$ collider, the Isabelle pp collider at Brookhaven, the suggested European LEP e^+e^- machine and an electron–proton collider are all needed if we are to penetrate into the world of gauge bosons which promises to exhibit a rich variety of phenomena, as well as leading us closer to a unified model. Though, as Glashow reminded us, gravity should also be included before we have the truly 'Grand' unified theory. □

Recognition, restriction and immunity

by Miranda Robertson

THE immune response of animals is controlled by two sets of genes: the immunoglobulin variable region genes (V genes) which determine the responses of lymphocytes to antigens; and the major histocompatibility complex genes (MHC genes), which determine their responses to one another. It is now clear that while B cells see antigen *per se*, by means of surface immunoglobulin coded by V genes, T cells see antigen only in association with the appropriate syngeneic MHC determinant, and it is this combination that induces the helper, suppressor or killer response. But whereas the molecular basis of antigen recognition by B cells is well understood, that of T cells has remained elusive. The attempt to define at the molecular level both what T cells see with, and exactly what they see, was therefore one of the main preoccupations of participants at a recent meeting on recognition and function in T and B lymphocytes.*

One crucial question is the contribution of the V genes to T cell recognition. Eichmann and Rajewski, and Binz and Wigzell have provided strong circumstantial evidence for the expression of V genes in T cell receptors with the demonstration that both helper and suppressor responses to specific antigens can be induced by antibodies against V-gene coded antigenic determinants (idiotypes)[1,2]. This is important not only for the light it sheds on T cell receptors, but also because it implies in principle that T cells as well as B cells may be subject to anti-idiotypic control as proposed in Jerne's network theory (reports of whose demise[3] are greatly exaggerated).

More recently, D. Givol (Weizmann Institute) has raised antisera in rabbits that react with framework determinants of V_H chains of mouse immunoglobulin[4]. These antisera will prevent the binding of antigen to some T cells and inhibit T cell help *in vitro*, and have recently been exploited by T. Tada (Tokyo University) in the characterisation of an antigen-specific suppressor factor isolated from a T cell hybridoma[5].

Soluble factors derived from T cells have been the focus of much recent investigation on T cell functions. It is not yet clear how they are related to the T cell receptor: they may, by analogy with surface and secreted immunoglobulin on B cells, be a secreted version of the membrane receptor; or they may be the receptors themselves, shed into the culture medium *in vitro* but with no normal function as a soluble molecule *in vivo*.

The data from Tada's laboratory strongly suggest that the T cell receptor and the soluble factor are very closely related, and that they are determined both by immunoglobulin V genes and by genes in the I region of the MHC. The basis for this conclusion is that antisera against 'I-region-associated' (Ia) antigens, as well as anti-V_H antiserum and specific antigen can all bind both to suppressor T cells and to the antigen-specific suppressor factor. A further extremely important point is that the Ia antibodies are specific for the mouse strain that donated the suppressor cell, and the factor acts only on cells from mice with the same I-J antigens. This so-called restriction by MHC antigens is characteristic of T cell interactions and it is therefore important to know whether it applies to soluble factors.

An important issue which hybridomas should help to resolve is that of the part played by antigen nonspecific factors in lymphocyte interactions. Although there have been many reports of such factors, some feel that such nonspecificity in a system whose principal characteristic is exquisite specificity must make their functional relevance suspect. Others, including Tada, have incorporated them into hypothetical nets in which both specific and nonspecific factors play a part, acting on different subsets of cells. Tada's laboratory has developed a number of hybridomas, some producing specific and some nonspecific suppressor factors. The fact that the nonspecific factors are

*The ICN-UCLA Symposium on T and B Lymphocytes — Recognition and Function, was held at Keystone, Colorado, on 26-30 March.

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monoclonal eliminates the possibility that their nonspecificity is apparent rather than real, and that the cells are really producing a very large number of different specific factors.

A similar collection of hybridomas producing helper factors would be a great asset. The biochemical elucidation of helper factors lags well behind that of suppressors. There is now evidence for idiotype-bearing, antigen-specific factors which replace T cell help in the production of antibodies against phosphorylcholine (M. Feldman, University College London) and (T,G)-A--L (ref.6) but the relationship of such specific factors to nonspecific helper factors remains unclear. J.D. Watson (University of California at Irvine), for example, reported that T cell growth factor(s) from supernatant of spleen cells treated with concanavalin A would act both as a nonspecific growth factor and as a nonspecific helper factor in antibody production or the generation of cytotoxic T cells; but if microcultures of ten or so cells are expanded with the use of the growth factor, their supernatants are active only on some cells. This experiment, which is similar to that of Waldmann and Lefkowitz[7] suggests that 'nonspecific' growth factors from concanavalin A supernatants may be mixtures of antigen or idiotype-specific factors; it remains to be seen whether such mixtures include a genuinely nonspecific T cell growth factor.

MHC restriction, which limits T cell recognition of viral antigens to infected cells bearing the same MHC antigens, can be seen as a special case of antigen specificity applied to T cells. It has now become clear that, like any other kind of antigen recognition, MHC restricted recognition is subject to cross-reaction. Cross-reactions have been demonstrated in experiments in which allogeneic virus-infected cells are killed by cytotoxic cells depleted of alloreactivity by passage through the appropriate mouse strain (J.R. Bennink, University of Philadelphia). The cross-reaction may not be reciprocal: for example, b haplotype cells may kill infected k haplotype targets while k cells will not kill infected b targets[8].

The converse can also occur: certain combinations of haplotype and virus can fail to elicit a cytotoxic response even when MHC restriction requirements are met. Much of the interest in such failures of recognition is of course due to their possible bearing on susceptibility to disease in man. A McMichael (Oxford University) reported that human lymphocytes of certain haplotypes will not show MHC-restricted killing in association with influenza virus antigens, whereas others do. Similarly, S. Shaw (US National Institute of Health) reported that some HLA haplotypes elicit much stronger anti-influenza cytotoxic responses than others.

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These data lend support to the proposal that the immense polymorphism of the MHC antigens is due to the massive selective advantage of heterozygosity: if the combination of one parent's MHC antigen and virus does not elicit a response, the other parent's may (indeed this has been shown[9]).

However, this begs the question of why antigens that fail to elicit an immune response should survive in the population. The answer may lie in the mechanism of selective recognition. It is now clear that some combinations of MHC antigen and virus cross-react with alloantigens[10]: it is thus highly likely that the combination of some viral antigens with self antigens will cross-react with other self antigens. Since any cell reacting strongly against self must be eliminated, it follows that cells reacting against certain combinations of self and virus will sometimes be eliminated too.

While it is clear that to avoid autoimmune reactions T cells with high-affinity receptors for self must be eliminated, it has been suggested that receptors with weak affinity for self antigens are the basis for MHC restricted recognition [11]. Tentative evidence in support of this theory was presented by H. Wigzell (Uppsala University), who has used an anti-idiotypic serum raised against immune T cells specific for an allogeneic antigen to extract the T cell receptor for that antigen. He then labelled internally the allogeneic antigen, the self antigen of the immune rat and a third-party antigen, and ran them on Sepharose columns to which the receptor was bound. Three labelled polypeptides from the third-party control passed straight through the column; two chains from the cells of the immunising strain were stopped; and one chain from the self strain was retarded.

Wigzell interprets this as evidence not only for weak binding of alloreactive receptors to self, but also as evidence that different polypeptides are involved in the recognition of self MHC and allo- or viral determinants. It is still a matter of disupte whether T cell receptors 'see' virus and MHC antigen as a complex, with only one receptor for both ('altered self'), or whether they see the two antigens with different receptors ('dual recognition')[12]. Attempts to resolve this question at a cellular level are all open to doubt: it is probable that only biochemistry can produce the answer, and Wigzell's experiment may be a first step. □

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Ecology of photosynthesis

from Peter D. Moore

Resource partitioning in plants is less well documented than among animals and this is not surprising, for the resources on which plants depend are fewer and adaptations which could be referred to as partitioning are generally more subtle than is the case with animals. This is probably why plant ecophysiologists have eagerly grasped the opportunities presented by the gradual exposition of the extent of the C_4 and crassulacean acid metabolism (CAM) photosynthetic adaptations among plants. It is well established that both of these modifications to the basic C_3 Calvin photosynthetic system are associated with certain ecologically selective advantages in conditions of high temperature, high light intensity and water stress. This has led to many investigations into the biogeographical significance of the adaptations, mainly by mapping their distributions on a continental scale (see *Nature* **272**, 400; 1978). Information is now accumulating concerning the ecological value of such adaptations in the local composition of plant communities.

To search for correlations between the success of one particular type of photosynthetic system and an environmental gradient, such as aridity or elevation, is not new. In 1974, Mooney, Troughton and Berry (*Carnegie Inst. Ybk.* **73**, 793; 1974) reported a survey they had conducted along the coastal strips of California and Chile, where the climate shifts from a Mediterranean to a desert type with decreasing latitude. They found in both locations that increasing aridity was associated with an increasing proportion of CAM plants in the flora. Among C_3 plants, there was a change from evergreen to drought-deciduous forms, but C_4 plants were virtually absent from all stations. They explain this as a consequence of the low temperatures prevailing at the times of water availability.

More recent work in the cold winter deserts of Utah by Caldwell, White, Moore and Camp (*Oecologia, Berl.* **29**, 275; 1977) has also shown that such C_4 species as *Atriplex confertifolia* fail to achieve many of the advantages normally associated with the C_4 system when they commence growth in the cool of spring. They may, however, still match the growth of their C_3 competitors and gain the advantage of a longer growing season as summer aridity sets in. It is difficult, therefore, to explain the lack of C_4 species in the Californian and Chilean coastal deserts.

Eickmeier has now published the results of a similar environmental gradient study from Texas (*Photosynthetica* **12**, 290;

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1978). Of 88 non-herbaceous species found along a 1,300-m elevation gradient, 22 were CAM, three were C_4 and the remaining 63 were C_3. Ten samples were taken at 150-m altitude intervals; density, frequency and area cover values were used to determine an importance value for each species at each elevation. The main peak in the importance of CAM species was found to occur at low altitude, that of C_3 species at higher altitudes and the C_4 peak was intermediate between the other two. Eickmeier considers this sequence to reflect both the adaptational significance of the physiological variations and also the respective responses to biotic, competitive factors. For example, the CAM plants are most suited to the low altitude, arid environment, where competitive interplay with other plant species may be regarded as minimal (but see *Nature op. cit*). C_3 plants, he claims are evolutionarily attuned to competitive situations with low abiotic stress; C_4 plants can cope with both, having potentially high productivity and the capacity to withstand water stress.

The greater competitive success of C_3 species in higher altitude, more mesic conditions in the tropics has now been demonstrated by Tieszen, Senyimba, Imbamba and Troughton (*Oecologia, Berl.* **37**, 337; 1979) along an altitudinal and moisture gradient in Kenya. They have found that nearly all the grass species of the low altitude grasslands in Kenya are of a C_3 photosynthetic type. Some grass tribes, such as Paniceae and Andropogoneae (both exclusively C_4) were most frequently encountered at intermediate altitudes. The exclusively C_3 tribes (such as Festuceae, Aveneae and Agrostideae) were found only at high altitude. No C_3 species were found below an altitude of 2,000 m and no C_4 species above 3,000 m.

Evidently the complex adaptational significance of C_3, C_4 and CAM photosynthetic systems is profoundly influential on the floristic composition of plant communities both on a biogeographic and an ecological scale.

Viral pathogenesis and virulence

from D.A.J. Tyrrell

IT is easy to say "The influenza virus causes influenza", while having really no scientific explanation of many of the signs and symptoms of the disease, for instance the headache or the depressed white blood cell count. This meeting* would have jolted anyone out of such a complacent view and also gave some good examples of how patient, thoughtful research is building a more complete picture of what happens in some virus infections – how the virus enters and spreads through the body, enters cells, disturbs their function, interacts with the host defence mechanisms (such as interferon or humoral or cellular immunity) and disturbs normal body functions to produce what we recognise as a clinical disease.

What seem in the laboratory to be very similar viruses may nevertheless produce either different diseases or similar diseases by different methods. There was for instance a session on coronaviruses, which all look the same and are similar in biochemical composition. Yet the different coronaviruses are specific to particular species — mouse coronaviruses never seem to affect man or *vice versa*, for example — and sometimes even to a very limited variety of cells in one species. P.A. Bachmann (World Health Organization, Munich) described how the coronavirus of transmissible gastroenteritis of pigs (TGE) affects only the differentiated enterocytes of the villi of the small intestine which may almost disappear. The crypt cells, however, survive and migrate up to cover the villi again, but while they are doing this and differentiating into mature enterocytes, there are profound physiological disturbances — lack of absorption, excess secretion, lack of digestive function (for instance the splitting of lactose by disaccharidase) — leading to marked diarrhoea, which can kill a piglet from dehydration unless it is treated. On the other hand, another pig coronavirus causes vomiting and wasting disease. M. Pensaert and K.L. Andries (State University of Ghent) described how this virus affects the upper respiratory tract and lung, the tonsil and the jejunum; it affects the cells of the plexus of Auerbach and Meissner in the gut and seems to spread from them. It spreads through the nervous system to the brain stem and later to the spinal cord and the rest of the brain. All this was established in great detail by painstaking serial virus assay and immunofluorescent microscopy of experimentally infected animals. This suggests how the disease develops — probably the virus spreads from the respiratory tract and gut along peripheral nerves to the brain stem where the lesions give rise to vomiting, while the damage to neurones in the plexuses prevents normal peristalsis, leading to wasting. Furthermore, knowing that the virus invades the nervous system widely, it is not surprising that a virtually identical virus has been recovered from pigs diagnosed as having encephalitis, and so was named haemagglutinating encephalitis virus.

Explanations of these important and mysterious tropisms of viruses must lie at the molecular level, in the functioning of the peptides and nucleic acids of which viruses are made. B. Fields (Harvard Medical School) gave an example of what can be done, when he described his study of two pairs of genes, represented by the RNAs S1 and M2 of reoviruses types 1 and 3. He studied parent viruses and a collection of recombinants (or reassortants) and showed that S1 nucleic acid specifies the haemagglutinin protein of the virus and that this is responsible for the ability to agglutinate red cells and for serological specifity. It is also responsible for cell tropism in the animal, so that the type 3 virus causes a necrotising encephalitis due to a specific attack on the neurones. It also determines the extent to which the virus inhibits DNA synthesis when absorbed to cells. M2 RNA on the other hand, which is abundant on the surface of the virus particle determines whether the virus resists inactivation by chymotrypsin. M2 RNA from type 3 makes the virus resistant to host enzymes and hence able to infect when given by mouth. Discussion of this work showed that the next step was to determine at which site on the surface of the neurone the virus attached. This is probably a part of the chemical matrix which has some other physiological function characteristic of neurones. Reassortants were also made which were infectious when given by mouth, like type 1, and caused central nervous system disease, like type 3, and so, by the oral route, were more pathogenic than either parent. This type of manipulation confirms earlier conclusions about the functions of the genes.

It is very difficult to evaluate the way in which the host defences interact with the virus, sometimes preventing its multiplication, sometimes causing immunological damage to infected tissue. For instance, P.A. Neighbour and B.R. Bloom (Albert Einstein College of Medicine, New York) described some of their work which started when they analysed why the lymphocytes of patients with multiple sclerosis show an unusual reactivity to measles virus; they tracked down the fault to a defect in the ability of their lymphocytes to produce type 1 interferon (Neighbour & Bloom *Proc. natn. Acad. Sci. U.S.A.* **76**, 476; 1979).

The success of the meeting was obvious, and there were many other interesting papers besides those mentioned here, including whole sessions on herpes viruses and rhabdoviruses. There were clear signs that pathogenesis not only holds scientific interest, but may be a source of practically useful knowledge: vaccines and antiviral treatments do not always work – sometimes because we guess too much and know too little about the process by which viruses produce disease. □

*A symposium on Mechanisms of Viral Pathogenesis and Virulence was held in Munich on 6 and 7 June, 1979 and organised by P.A. Bachmann.

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Seeing is believing in lineages

from a Correspondent

AT last a directly observed and accurate cell lineage is available for the development of one organism, the nematode *Caenorhabditis*. As reported at a recent meeting*, the lineage runs from the fertilised zygote to the completion of embryogenesis (550 cells), and then there is a gap of one or two cell divisions, which have not yet been studied, before the lineage runs directly through to adult structures. This description is now up for analysis and G.von Ehrenstein (Max-Planck Institute for Experimental Medicine, Göttingen) has isolated at least seven temperature-sensitive mutants whose maternal gene expression is necessary and sufficient for normal embryogenesis, and which eventually block development at the 'lima bean' stage. Three of these produce abnormal guts without obviously disturbing the rest of the lineage; in the gut lineage one changes the direction of a single division while the other two change the relative timing of division and migration of gut primordial cells to the interior. It is, however, unlikely that the regularity of the lineage merely depends on control of cell division rate and cell division direction for there are even more extensive cell migrations across four or five cell diameters in other parts of the embryonic lineage. Certainly there is regulation in the post-embryonic lineage for, following laser ablation of single cells, their lineage and adult structures may be replaced by neighbouring cells within an 'equivalence group' (J. Kimble, MRC Laboratory of Molecular Biology, Cambridge). It remained unclear whether the replacing cell had to repeat the lineage of the ablated cell in order to replace its adult structures because within an equivalence group it always did so. (This and related work with *Caenorhabditis* was mentioned in *News and Views*, **279**, 758; 1979.)

The masterplans in 'mosaic' eggs were certainly under attack even though there are nice sets of computer simulations which could manufacture such plans (E. Parisi, Laboratory of Molecular Embryology, Naples). P. Guerrier (Biological Station, Roscoff) reviewed his own and van den Biggelaar's beautiful manipulation experiments on the 'mosaic eggs' of annelids and molluscs and made it difficult to see how anybody could still believe in mosaicism in these embryos (for example, Bigelaar & Guerrier, *Devl Biol.* **68**, 462; 1978). The *Drosophila* egg must at least be allowed its polarities, and to go with 'bicaudal', there is now a maternal effect mutant, dorsal (*dl*), which leaves the anterior–posterior polarity intact but which lacks lateral and ventral structures (C. Nüsslein-Volhard, EMBL, Heidelberg). It may be 'all dorsal' for no dorsal midline can be detected, and in order to show formally that the laser ablation fate map was altered by this dorsal hypodermis embryo it was necessary to put the mutation on a background which reduced its penetrance so that some ventral structures were formed. The polar tendencies of the *Drosophila* egg must arise during oogenesis and E. Weischaus (EMBL, Heidelberg) has now made use of another maternal effect mutant, *fs(1)K10*, in a neat mosaic analysis of stem cell behaviour during oogenesis. This mutation is expressed as a chorion defect (allowing immediate recognition of affected eggs) but its expression is restricted to the germ line; consequently recombination induced by X-irradiation during the formation of eggs can be used to follow the behaviour of stem cells which are egg precursors. From blastoderm to pupa, the stem cells increase to about 100 and this level is maintained through egg production. This technique could be exceptionally useful for analysing the effect of characters expressed in the nurse-cell oocyte clusters on subsequent development.

Even those working on haemopoietic stem cells could now see lineage diversification with their eyes and could provide direct evidence for multipotential stem cells. G.R. Johnson (Walter and Eliza Hall Institute, Melbourne) has now picked single cells, waited for them to divide twice in isolated liquid culture, separated the four cells, found that each could form colonies which contained a variety of cell types, and thus showed that one cell could form erythrocytes, macrophages and granulocytes. The implication is that cell lineages can separate without the special cell contacts and microenvironments which are supposed to pick out and diversify stem cells; nobody could decide if the behaviour of these clones was natural or perverted. The environmental lobby made a come-back with a study of the progressive education of T lymphocytes to attack virus-infected cells. It has now been shown that the major histocompatibility complex (MHC) type of both the thymus and the 'post-thymic environment' educates cytotoxic T cells to kill virus-infected cells with similar MHC (R.M. Zinkernagel, Scripps Clinic, La Jolla).

If lymphocytes can be educated, then nerve states can be altered and stabilised. The evidence that individual post-mitotic neurones can functionally convert from adrenergic to cholinergic cells in the presence of non-neuronal cells is now convincing and P.H. Patterson (Harvard University) went on to show that this change could be prevented by depolarising the neurone. This change could be seen directly in cell culture; could it be as simple as that in the adrenergic–cholinergic switch shown by neural crest cells transplanted to different levels of the axis of developing chick–quail chimaeras? Certainly these transplants illustrate beautifully the importance of the migratory path and final location in picking out the phenotype of neural crest cells (N. Le Douarin, Embryology Institute, Nogent-sur-Marne). Depolarising a neurone in culture may mimic normal neurone activity, and chronic stimulation of the spinal cord during embryogenesis may do the same. If it does, then the stabilisation of nerve synapses on muscle during development may be shown to depend on nerve activity. The system is the posterior latissimus dorsi muscle of the chick which early in development has several synapses per fibre and of these only one remains in the adult; stimulation of the spinal cord during embryogenesis maintains multiple connections (P. Changeux, Pasteur Institute, Paris). Is this the selective stabilisation theory of nerve connections in action? □

*The meeting on Cell Lineage, Stem Cells and Cell Determination was held in Seillac on 20-24 May 1979. The proceedings will be published by Elsevier-North Holland.

Mutants of the *lac* repressor

from Alan D. B. Malcolm

As part of their study of the binding of the *lac* repressor protein of *Escherichia coli* to the *lac* operator DNA, Miller and colleagues set out several years ago to produce mutants of the repressor protein by an ingenious technique, and the culmination of their work appears in three recent papers (*J. molec. Biol.* **130**, 191, 223 & 249; 1979). Miller's approach has been to use chemical mutagens to produce nonsense mutations (where a coding triplet is replaced by one of the three 'stop' signals, UAG, UAA or UGA) in a suppressor strain which possesses a tRNA able to substitute an amino acid in place of the stop. So far it is possible to insert Ser, Gln, Tyr, Lys or Leu at UAG; Gln, Tyr or Lys at UAA and Trp at UGA. This method has the advantage that it is easy to screen for proteins with minimal functional defects (this is difficult to achieve by conventional mutagenic techniques) and also that some of the mutations introduced would be expected to require two base changes. It also has some limitations. Mutations can be introduced only at positions where the wild type can be mutated into one of the three 'stop' signals. It is known that not all sites are equally susceptible to mutagenesis and the distribution of these 'hot spots' has been the subject of a previous paper (Farabaugh *et al. J. molec. Biol.* **126**, 847; 1978). The amino acids that may be introduced are limited by the availability of suppressor strains, although there seems to be no reason why the number of these could not be increased in the future. Miller *et al.* have collected more than 300 mutants at 90 out of the 360 positions in the *lac* repressor and these can be altered in various ways: inducer binding, ability to form tetramers and binding to DNA (either operator or

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nonspecific) can all be altered. It has been known for some time that it is the amino end of the protein that is involved in DNA binding (for example, *Nature*, **268**, 196; 1977) and it is therefore not surprising that mutations in this region frequently abolish DNA binding (*J. molec. Biol.* **130**, 191; 1979), whereas mutations at the carboxyl end are often without effect. Indeed a chimaera between the *lac* repressor and β-galactosidase produced by a deletion mutant of *E.coli* (Brake *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 4824; 1978) which is without the last four residues at the C terminus but has instead nearly 10^5 daltons of β-galactosidase, retains all the various properties of the repressor. It is therefore interesting that when the Tyr 273 is replaced with Gln, the tight operator binding of the repressor disappears. Tyrosine residues have already been implicated in specific protein–nucleic acid recognition both as a result of model studies (Wehling *et al. Nucleic Acid. Res.* **4**, 513; 1977; Brun *et al. Biochemistry* **14**, 558; 1975) and also from work on gene 5 protein from bacteriophage fd (Coleman & Armitage *Biochemistry*, **17**, 5038; 1978) on gene 32 protein from bacteriophage T4 (Anderson & Coleman *Biochemistry*, **14**, 5485; 1976), as well as in the N-terminal region of the *lac* repressor (Alexander *et al. Biochim. biophys. Acta* **493**, 367; 1977).

Miller and Schmeissner have also analysed many missense mutations in which the use of chemical mutagens has resulted simply in the substitution of one amino acid for another (*J. molec. Biol.* **130**, 223, 1979). As expected, the concentration of i^-mutants (where there is no active repressor, so that the *lac* enzymes are constitutive) is particularly high among the first 60 amino acids. The i^s mutants are those in which the *lac* enzymes are repressed even in the presence of inducer and none of these occurs in the early part of the gene. This is exactly as expected because it is known that the binding site for IPTG (a synthetic inducer) depends on residues 60-360 and not on 1-59 (for example, *Nature* **268**, 196; 1977).

When the results of the suppressed nonsense mutations are taken together with those for the missense mutations (*J. molec. Biol.* **130**, 249; 1979) some striking regularities appear. For example, the positions where i_s mutants occur in clusters about 26 amino acids apart (that is, around 193, 219, 245, 271 and apart (that is, around 193, 219, 245, 271 and 297) both for missense mutations and for suppressed nonsense mutations. The exact significance of this is not yet clear, but Miller speculates that the inducer binding site may resemble the antigen-binding site of immunoglobulins. This is a cavity formed by residues in hairpin turns. The hypervariable regions occur at the turns themselves and mutations here affect the antigen-binding properties without causing alteration of the overall structure of the protein. If a similar regular structure were used to make the inducer binding site then mutations at the turns (assuming them to be about 26 amino acids apart) might alter inducer binding while leaving the overall protein structure unaltered.

All this must remain speculative until physical studies define the three-dimensional structures of the repressor and its complexes with inducer and operator. Nuclear magnetic resonance (NMR) is giving increasingly detailed information about such structures. Lu and his colleagues have grown *E.coli* on 3-fluorotyrosine in place of tyrosine and have isolated a fluorinated *lac* repressor (*Proc. natn. Acad. Sci. U.S.A.* **73**, 3471; 1976) and have now succeeded in assigning most of the ^{19}F peaks in an NMR spectrum to individual tyrosine residues (P. Lu, personal communication). Comparison of the proton NMR spectrum of the intact protein with the amino tails (residues 1–51) and the remaining core, shows that the tails have the same structure whether on their own or as part of the complete repressor (Buck *et al. FEBS Lett.* **96**, 335; 1978). Further, the narrowness of the linewidths suggests that the tails have a faster tumbling rate than would be expected if they were an integral part of a protein. It seems as if there must be a very flexible 'hinge' between positions 50 and 60 which allows the core and the tails almost independent motion.

The other half of the problem of course is to study the operator DNA. Caruthers and colleagues (Goeddel *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 3578; 1978) have synthesised *lac* operators substituted with nucleotide analogues such as 5-bromouracil, 5-bromocytidine, uracil and hypoxanthine. These and previous binding studies (Ogata & Gilbert *Proc. natn. Acad. Sci. U.S.A.* **74**, 4973; 1977) suggest that the repressor binds in the major groove on one side of the DNA only. Narang has used his phosphotriester method to synthesise various sections of the operator (Narang *et al. Can. J. Biochem.* **55**, 1125; 1977) and has now studied amino acid binding to the central nine base pair sequence. (Patel *et al. Biochem. biophys. Res. Commun.* **85**, 1560, 1978) The free energies of binding tyrosine and glutamine to this oligonucleotide are both about 17 kJ mol^{-1}. Because the free energy for binding intact repressor to intact operator is only about 73 kJ mol^{-1}, it seems possible that only four or five amino acid–base interactions may be required to produce this free energy; it is already known from the mutation studies that nine amino acids in the tail section are essential (including Tyr 17, Gln 18 and Gln 54).

It looks as though the lac repressor is beginning to yield to the combined skills of geneticists, chemists and biophysicists. □

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DNA gyrase and DNA unwinding

from David T. Denhardt

WHEN DNA is replicated, transcribed, recombined or otherwise manipulated within the cell the two strands of the double helix have to be at least partially unwound. The physical problems inherent in unwinding the DNA duplex have long been recognised and led John Cairns some years ago to postulate a 'swivel' in the circular chromosome of *Escherichia coli* to enable it to be unwound. The 'swivel' has now been recast as the bacterial topoisomerases — topoisomerase I, or ω protein, and topoisomerase II, or DNA gyrase — and the latter has been implicated as an essential enzyme in DNA replication in bacteria. DNA gyrase introduces negative superhelical twists into relaxed closed circular DNA molecules; how it does this, and how this property is related to its role in DNA replication are now under intensive study. The best guess at the moment is that it relieves the positive supertwisting that builds up in front of the replication fork as the two strands are unwound during replication. Negatively superhelical DNA may also be essential for both the initiation of replication and some recombinational events.

The relation between the helical turns in a DNA duplex and the superhelical twists in a superhelical molecule are illustrated diagramatically in Fig. 1. A closed circular duplex is equivalent topologically to a linear molecule whose ends are unable to rotate, and despite appearances all three structures in the figure have the same net number (5) of twists of one strand about the other. The two negative superhelical twists in the molecule on the right are removed by unwinding two turns in the DNA duplex; if the duplex is unwound further positive superhelical twists are introduced. To understand the action of gyrase imagine the molecule in the centre to be a DNA molecule with its usual one duplex turn per roughly 10 base pairs. Gyrase binds to the DNA molecule in such a way as to introduce compensating positive and negative superhelical twists and then it breaks and reseals the DNA duplex so as to selectively remove the positive superhelical twist. Dissociation of gyrase from the DNA leaves a negatively superhelical molecule.

Evidence for the existence of DNA gyrase was first obtained by Kiyoshi Mizuuchi and Howard Nash who found that closed circular phage lambda DNA was much more efficient at undergoing an intramolecular recombination event than was nicked circular DNA (*Proc. natn. Acad. Sci. U.S.A.* **73**, 3524; 1976); a nick (a

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Fig. 1

single-strand break) in a DNA molecule prevents it from being made superhelical. Martin Gellert, together with Mizuuchi, O'Dea and Nash at the US National Institutes of Health, subsequently succeeded in purifying an ATP-dependent activity that converted relaxed closed circular DNA into negatively superhelical DNA (*Proc. natn. Acad. Sci. U.S.A.* **73**, 3872; 1976). They showed that the antibiotics novobiocin and its more active analogue coumermycin A_1 inhibited DNA supertwisting and DNA replication in *E. coli* by their ability to inhibit DNA gyrase; mutations in the *cou* gene simultaneously conferred resistance to both these drugs and produced a gyrase that was drug-resistant *in vitro* (*Proc. natn. Acad. Sci. U.S.A.* **73**, 4474; 1976).

At the same time Nicholas Cozzarelli and colleagues at the University of Chicago were approaching the discovery of gyrase from a different direction. They had purified the protein product — Pnal — which confers sensitivity to the antibiotic nalidixic acid on *E. coli*. Because nalidixic acid inhibited DNA gyrase *in vitro* Gellert suggested to Cozzarelli that Pnal might be related to DNA gyrase, and this was soon proved to be so.

Functional gyrase is a 400,000 molecular weight tetramer composed of two pairs of identical subunits of molecular weights of about 105,000 and 95,000 (Higgins *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 5960; 1978). These polypeptides are coded for by the *nalA* and *cou* genes respectively; it has been proposed that the genes now be called *gyrA* and *gyrB*. Either subunit alone is inactive but they readily associate to form the native enzyme. Leroy Liu and James Wang, at Harvard University, purified the analogous enzyme from *Micrococcus luteus* and the two enzymes seem to have similar properties (*Proc. natn. Acad. Sci. U.S.A.* **75**, 2098; 1978).

What does gyrase do? Dissection of its activity has revealed several aspects of gyrase action. First, it can bind to DNA. Second, it can hydrolyse ATP in a DNA duplex-dependent reaction; this is the activity inhibited by coumermycin (Sugino & Cozzarelli, *J. biol. Chem.* in the press). Third, in the absence of ATP it removes superhelical turns from DNA by the breakage and reunion of the two DNA strands. This is inhibited by nalidixic acid. It is not yet clear whether *in vivo*, gyrase would induce a single-stranded break only, allowing rotation of one strand around the other, or whether double-stranded breaks are transiently induced. Double-strand breaks are observed when dodecyl sulphate is added to a nalidixic acid-inhibited reaction. This has allowed preferred sites for gyrase action to be identified. The break in one of the DNA strands is separated by four base pairs from the break in the other producing a linear molecule with protruding 5′ ends to which the protein seems to be covalently bound. The 3′ ends in contrast are free and able to prime a limited amount of synthesis by DNA polymerase. This provides the handle needed to determine the sequence around the cleavage site. As shown in a paper in the May issue of *Cell* there seems to be little similarity in the nucleotide sequences around the sites of cleavage in the different molecules studied so far except that on at least one strand, cleavage usually occurs between a T and G (Morrison & Cozzarelli *Cell* **17**, 175; 1979).

The binding of gyrase to DNA has been studied in detail and strong evidence suggests that the DNA is wrapped around the outside of the enzyme. Liu and Wang bound nicked circular duplex DNA to gyrase in the absence of ATP and then closed the nick with polynucleotide ligase. When gyrase was removed they found that the DNA had acquired positive superhelical twists. This implied that either the DNA wraps around the protein or that when gyrase binds to DNA it induces a winding of the helix. As there is no precedent for the latter but the precedent of DNA wrapping around histones in eukaryotic DNA for the former this explanation seemed more likely. They have since shown that when DNA is in a complex with gyrase a 143 base-pair segment is protected from digestion by *Staphylococcus* nuclease. When the gyrase–DNA complex was digested instead with DNase I, electrophoresis of the protected DNA after denaturation revealed a set of six single-stranded molecules of lengths, 47,57,67,77,86 and 96 nucleotides. It seems likely that the DNA is lying largely on the surface of the enzyme in a form inaccessible to staphylococcal nuclease but partially accessible to DNase I such that the latter enzyme can nick the duplex over a limited region at one of several sites separated by 10-base pair intervals (Liu & Wang, *Cell* **15**, 979, 1978).

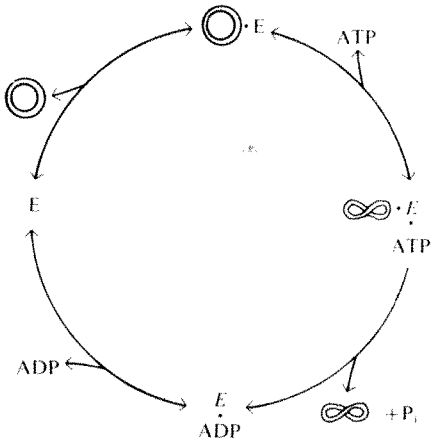


Fig. 2

Using a similar approach Peebles *et al.* (*Cold Spring Harbor Symp. quant. Biol*, **43**, in the press) also confirmed that gyrase introduced positive superhelical twists into relaxed closed circular DNA in the absence of ATP. They then went one step further. To the gyrase–DNA complex they added an ATP analogue which although it induces a conformational change in the gyrase molecule cannot support the cleavage reaction. Subsequent treatment with nalidixic acid and dodecyl sulphate to induce double-strand breaks showed that reaction with the ATP analogue had shifted the cleavage site some distance along the DNA molecule. Also, about half the positive superhelical twists introduced into the DNA on gyrase binding had disappeared.

There is now sufficient information to construct a model for gyrase action. All its elements are common to one or more of the models suggested by the several groups studying the enzyme. As proposed by Wang, on binding the DNA wraps around the enzyme. As proposed by Cozzarelli (Sugino *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 4838; 1978) ATP then interacts with the enzyme to induce a conformational change that results in the translocation of the DNA relative to the enzyme and formation of a positive superhelical loop of DNA. As proposed by Gellert *et al.*, (*Cold Spring Harbor Symp. quant. Biol.* **43**, in the press) a nicking–closing cycle (or breakage and reunion if a double-strand break is transiently introduced) removes the positive superhelical turn in the isolated section of DNA. ATP hydrolysis returns the enzyme to its initial conformation and leaves the DNA with a net negative superhelical twist. The end result is that the DNA has been partly unwound. The similarity of this mechanism to other ATP-coupled energy-transducing processes has been discussed by Sugino *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75**, 4838; 1978) and Peebles *et al.* (*Cold Spring Harbor Symp. quant. Biol.* **43**, in the press) and their model is illustrated in Fig. 2. E and *E* are two conformations of the enzyme.

In vivo evidence of the role of gyrase in the bacterial cell is provided by the inhibition of DNA replication by the antibiotics nalidixic acid and coumermycin and their analogues. Conditional lethal mutations have also been mapped in the *nalA* gene and possibly the *cou* gene (Kreuzer *et al. Molec. gen. genet.* **167**, 129; 1978; Projan & Wechsler in *DNA Synthesis: Present and Future*, 387, (ed Molineux & Kohiyama, Plenum, 1978).

The requirement for gyrase may simply be to remove positive superhelical twists

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induced in the DNA by the unwinding of the DNA during replication. Alternatively, or additionally, the DNA may need to be made negatively superhelical for DNA replication to be initiated. Negatively superhelical DNA is required for the initiation of replication of the bacteriophage ϕX174 replicative form DNA *in vitro* because the ϕX174 gene A protein, which must nick the DNA to intiate replication, does not act on relaxed DNA (Marians *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 1965; 1977).

Surprisingly gyrase seems to be required for the replication of the linear DNA of the phage T7 *in vivo* but not *in vitro* (DeWyngaert & Hinkle *J. Virol.* **29**, 529; 1979). The most likely explanation is that in the cell the DNA is collapsed into a compact structure as a result of its interaction with cellular molecules, whereas in its isolated form *in vitro* one end of the duplex is free to rotate relative to the other end and so superhelical twists cannot build up in the molecule.

McCarthy (*J. molec. biol.* **127**, 265; 1979) has discovered that the T4 DNA-delay mutants (*39,52,60*) require DNA gyrase for replication as they are unable to replicate in the presence of novobiocin or coumermycin in sensitive, but not drug-resistant, cells. Wild type T4 was not dependent on gyrase function, presumably because the products of one or more of the above genes form an enzyme that provides an equivalent function. From the kinetics of DNA synthesis under conditions of gyrase inhibition McCarthy deduced that the activity was required for the initiation of 'growing points' but not for the actual DNA elongation process.

On the assumption that transcription and recombination require local unwinding of the DNA it is hardly surprising that in certain cases gyrase seems to be required for both. However, the weight of the evidence suggests that gyrase is only required for transcription from some promoters (Smith *et al. Nature* **275**, 420; 1978). It is not obvious why. Harking back to the *in vitro* studies on integrative recombination in phage lambda from which the existence of gyrase was inferred it seems likely that the requirement for gyrase in recombination may be a consequence of the need for negatively superhelical DNA for this reaction, as well as for its role in DNA unwinding (Mizuuchi *et al. J. molec. Biol.* **121**, 375; 1978). One explanation for the requirement for negatively superhelical DNA is that the negative superhelical turns facilitate the uptake of pieces of single-stranded DNA to form partially triple-stranded structures. (Beattie *et al. J. molec Biol.* **116**, 783; 1977).

It is relevant to note that those DNA metabolic processes that are coupled to gyrase action become directly dependent on the ATP content of the cell, and a high ATP content at that, since the K_m of gyrase for ATP is of the order of 300 μm. If the cell cannot generate adequate supplies of energy the negative supertwisting of the DNA will rapidly disappear. With its disappearance those reactions which depend on having a negatively superhelical DNA substrate will not take place. The advantage of this as a control point in the prokaryotic cell is self-evident. Negative supertwisting, or its absence, is a signal transmitted throughout the entire DNA molecule, or at least that section of it that behaves as a topological unit.

So far gyrase has not been found in eukaryotic cells and there is no reason to expect it. The eukaryotic nicking–closing enzyme is sufficient to remove the positive superhelical twists induced in the DNA during replication. the superhelical twists present, for example, in purified SV40 DNA are accounted for by the number of nucleosomes in the DNA. (Each turn of the DNA around a nucleosome is approximately equivalent to one superhelical twist). Consequently eukaryotic DNA is not under an unwinding stress resulting from the presence of negative superhelical twists and this could be a partial explanation for its being replicated more slowly than prokaryotic DNA. □

Oceanic heat flow, lithosphere age and thickness

from Geoff Brown

THE systematic decrease of measured heat flow with increasing age of oceanic lithosphere was well documented by the classic work of Sclater and Francheteau (*Geophys. Jl. R. astr. Soc.* **20**, 509; 1970). But in more recent years a definitive relation has been sought as proof that plate thickness increases as the square root of time. This relation follows naturally from the theory of conductive heat losses which lead to the cooling, contraction and basal freezing of oceanic lithosphere as it moves away from the zone of spreading.

A thermal model relating plate thickness, age (<120 Ma) and heat flow was summarised by Parsons and Sclater (*J. geophys. Res.* **82**, 803; 1977) who cited evidence from the North Pacific that q varies as $t^{-½}$ (q = heat flow, t = age). However, their data were limited to crust of age <20 Ma and >70 Ma because the reliability of heat flow and/or age control was inadequate for other times and places. In the main, uncertainties arose from the highly scattered heat flow values caused by hydrothermal disturbance in layer 1. Sclater and Crowe (*J. geophys. Res.* **84**, 1593; 1979) have taken an important step forward by measuring heat flow at 17 stations close to magnetic anomaly 13 (age = 35 Ma) on the Reykjanes ridge. By using piston cores and measuring thermal gradients across intervals down to 12 m in sediment they were able to isolate non-linear gradients above 5 m (sediments affected by recent changes in ocean bottom water temperature) from 'remarkably linear' gradients below 7.5 m. In the former case, anomalously high, non-equilibrium temperature gradients were attributed to recent changes in seawater temperature due to changes in the velocity and temperature of Norwegian Sea overflow water. The stable gradients below 7.5 m ranged from 69.9 to 102.2 mW m^{-2}, giving a mean heat flow for anomaly 13 of 82.9 ± 10.1 mW m^{-2}. The variations in these data are real in that they exceed the precision of measurement and an excellent inverse correlation, local to the survey area, was found between heat flow and depth of sediment overlying a seismic basement ridge. This suggested that either heat flow is refracted because of the contrasting thermal conductivity between layer 1 and layer 2 (that is, due to the insulating effect of the sediments) or that the interface between the layers is isothermal due to hydrothermal convection in the deeper layer: both explanations can account for the observed local variations in heat flow.

Sclater and Crowe have shown that reliable conductive heat flow data for oceanic lithosphere can be obtained by detailed and critical analysis of down-core temperature variations. Moreover, their results from the Reykjanes ridge fall on the $q/t^{-½}$ graph for Pacific data of other ages and provide further support for the thermal model of lithospheric thickening in proportion to the square root of age.

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A hundred years ago

Glow-worms

Shelley sings of a "glow-worm golden in a dell of dew," but last night, at 10 o'clock, while travelling on a bridle path among the bleak lonely mountains of Tynron, Dumfriesshire, bearing up against a high wind with cold rain, I espied three glow-worms shining among the grass and ferns. I had seen them in the same locality before, but scarcely expected to have noticed them in such ungenial weather when summer has with us scarcely yet begun. J. S.

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review article

The Loch Lomond Stadial in the British Isles

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During the last very cold period in the British Isles, which occurred more than 10,000 radiocarbon years ago, glaciers developed in many mountain areas, permafrost was extensive, frost break-up of rocks was severe (its effects including a pronounced coastal platform and cliff), downslope movement of debris was greatly accelerated, many rivers spread coarse debris over low ground, the vegetation was transformed and the fauna was greatly affected.

FOLLOWING the decay of the last ice sheet[1], oceanic polar water spread southwards again and reached the latitude of south-west Ireland[2]. The associated cold period in the British Isles is the Loch Lomond Stadial, approximately equivalent to the Younger Dryas of Scandinavia. The effects of the stadial were probably more pronounced and varied in the British Isles than anywhere else, due partly to varied relief but particularly to maritime location and the position of the maximal extent of polar water. Norway is similarly susceptible to oceanic temperature changes but was then mostly covered by the Scandinavian ice sheet. In continental countries now bordering the southern North Sea, the maritime influence would have been diminished due to location and because this part of the North Sea was then land (because of low world sea level). Comparison of pollen-bearing Younger Dryas sediments from many sites shows that very distinctive floras and sediments occur in the British Isles, but the stadial changes are less marked in the North European Plain and diminish southwards and eastwards of it[3].

Glaciers

Small cirque glaciers developed at various places in the mountains of Ireland and Wales. Their maximal limits are normally defined by fresh-looking end moraines; other end moraines frequently record subsequent intermittent retreat. In Ireland such moraines occur, for example, in the Wicklow, Mourne and Galty mountains, in the Nephin Beg range, in County Donegal and in western County Kerry[4]. In Wales they are present, for example, in the Brecknock Beacons, on Cader Idris and especially in Snowdonia[5–8].

The 64 glaciers that developed in the Lake District included many small cirque glaciers but there were also valley glaciers up to 5 km long (Fig. 1). The limits of the majority of the glaciers are marked by end moraines, the limiting moraine being often immediately succeeded by one to three other moraines. In a few places fluted moraines record the final direction of the ice movement and in many places hummocky moraine lies within the limits of the former glaciers. Despite their considerable number, the Lake District glaciers covered an area of only 55 km^2 and their combined volume was only 3.3 km^3. Small glaciers are said to have existed in the northern Pennines[9] and an isolated one developed on the Cheviot. In the Southern Uplands of Scotland, glaciers were most extensive in the centre around Broad Law but even here the largest was only a few kilometres long.

In total contrast a very large area of glacier ice accumulated in the western highlands of Scotland: its ice-shed altitude attained 850–900 m, numerous mountains (not shown in Fig. 2) rising above its surface. Over Rannoch Moor its thickness exceeded 400 m and in the southern Great Glen reached 600 m. One small part of this ice mass, the Loch Lomond Glacier, had a volume of ~80 km^3, thus far exceeding the combined volume of all the glaciers mentioned above[10]. The numerous other glaciers that formed in the Scottish Highlands and some of the islands included many small cirque glaciers as well as valley glaciers up to 10 km long. Some glaciers developed beneath rock walls lacking cirque form and others in broad, shallow valleys in plateau surfaces. This last type of accumulation resulted in an ice cap of 65 km^2 in the south-east Grampians and one of 360 km^2 in the central Grampians[11–20]. Yet glaciers were not ubiquitous in the Highlands. In the Monadhliath Mountains, where a large area of ground exceeds 600 m, there were only two; in the north-west Cairngorms only three out of seven cirques at 1,000 m altitude contained glaciers[19].

The landforms produced by the stadial glaciers in the Highlands are often clear and abundant. There are scores of end moraines, many of them small, but the longest extend continuously for 15 km or intermittently for 40 km, the highest has outer slopes 30–40 m high, and the broadest individual end moraine ridges are 200 m wide. These clear, often arcuate, end moraines record the maximal extent of many of the former glaciers: other clear end moraines often occur immediately behind them.

Many glaciers left extensive areas of hummocky moraine in the Scottish Highlands and some small ones deposited great quantities of boulders as continuous undulating spreads. Fluted moraines are especially common in north-west Scotland and Mull[12,16]. Hundreds of small (1–4 m deep) meltwater channels were formed in association with the large plateau ice cap of the central Grampians[14]. Outwash terraces occur at and near the limits of many of the former glaciers in the central and eastern Grampians but are small compared with a buried outwash fan in the western Forth valley[21] and with outwash masses at certain west coast localities (for example, by Lochs Etive, Linnhe and Shiel)[22,23]. Yet the morphological evidence is not prolific everywhere: the limit of the large west Highland ice mass is

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still not fully mapped partly due to the lack of clear landforms (Fig. 2).

Ice-dammed lakes

A lake held up by the Glen Moriston glacier (west of Loch Ness) occupied at least nine different levels, seven of them marked by shorelines. Excellent cross-valley moraines were formed at the ice margin during retreat. Sudden release of lake waters produced belts of rock swept free of drift that extend intermittently for several kilometres[17]. The 'parallel roads' of glens Roy, Gloy and Spean are mainly lake shorelines[24] formed by frost erosion and occur at five levels in Glen Roy[25]. The final Roy/Spean lake had an area of 73 km^2, a maximal depth of 200 m and a volume of 5 km^3. It has been suggested that catastrophic emptying of this lake in a few days, with peak discharge of 22,500 $m^3 s^{-1}$, caused the surface level of Loch Ness to rise 8.5 m and resulted in a massive spread of sand and gravel at the southern end of the loch[26].

Periglacial activity

The very cold climate of the stadial resulted in widespread and varied periglacial activity. In the Scottish Highlands especially, powerful frost wedging produced blockfields and extensive spreads of smaller debris that now mantle many summits and plateaus. Such debris often moved down slopes as sheets with steep, often crenulate (lobate) frontal margins typically 1–5 m high, the agency probably being interstitial ice. In many places formation may have begun during ice-sheet decay but the major lobes and sheets as now widely preserved in fossil form were produced during the stadial, this being in accord with their absence from ground then occupied by glaciers.

At the base of a few slopes in the north-west Cairngorms and the Lake District accumulations of coarse debris with steep fronts up to 10 m high seem to be fossilised valley-wall rock glaciers. Fossil rock glaciers with curving terminal ridges resembling end moraines occur in the north-west Cairngorms and Jura, having been supplied with abundant coarse debris from adjacent steep slopes[19,27]. Deposits associated with former perennial snow beds are much more common. Protalus ramparts occur in Snowdonia[6] and one on Fan Fawr in south Wales is 11 m high[7]. They also occur in the Lake District and at many locations in the Scottish Highlands, one in the Cairngorms having an outer slope 10–20 m high. By far the largest feature known is a complex rampart in north-west Scotland, which is 1 km long and up to 55 m high, its volume implying a 17 m retreat of the rock face that supplied it[28]. Some snow-bed deposits comprise groups of mounds of angular debris (for example, in the Lake District) and others consist of great quantities of boulders forming spreads and ill-defined heaps; one such deposit in the Cairngorms extends for 2 km. None of the above features is firmly dated although their distribution complements that of the stadial glaciers, implying approximate contemporaneity of formation.

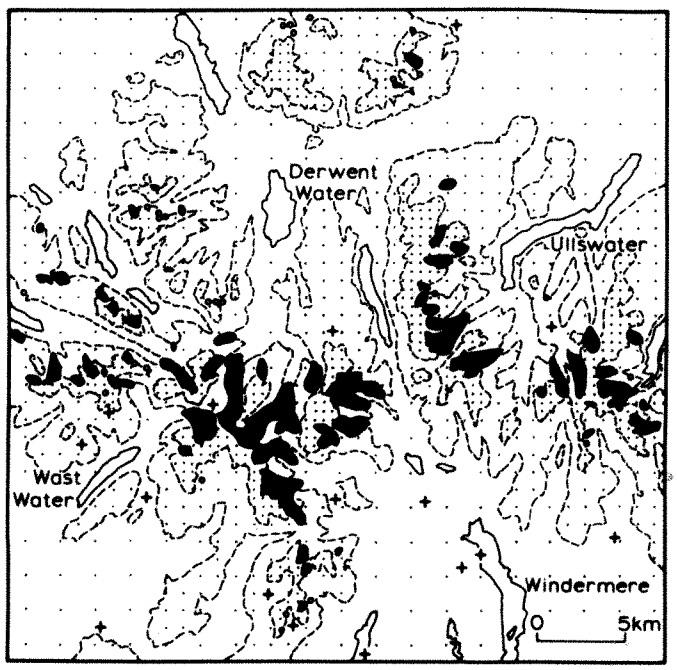


Fig. 1 Extent of stadial glaciers (solid) and locations of perennial snow beds (circles) in the Lake District. Lateglacial pollen sites shown by crosses. Altitude indicated by density of stipple, contours being at 300 and 600 m. Based on refs 103 and 110 and unpublished data.

Fossil screes are widespread: in Wales, for example, they occur especially on south- and west-facing slopes favoured by frequent freeze–thaw cycles. The near-surface layer of angular gravel, often ~0.5 m thick, is believed to have accumulated during the Loch Lomond Stadial[29,30]. Downslope movement of debris at low altitudes during the stadial is demonstrated in the Glasgow area, Dumfriesshire, Banffshire and the East Midlands by radiocarbon dates[31–34]. In Edinburgh a 2 m layer of stratified stony clay sandwiched between lake deposits[35,36] suggests stadial transfer of debris from Salisbury Crags by solifluction and snow meltwater over an average slope of 4–5°. Much further south, rapid frost break-up of the chalk occurred and trails of chalk rubble and mud extend for 2 km from coombes in the North Downs. These sheets of chalky slurry were carried by water released from melting snow and thawing of frozen ground. They often overlie an organic layer representing the Windermere Interstadial and their volume implies that at least some coombes were mainly formed during the stadial[37,38]. Soil instability and incomplete vegetation cover are indicated widely in the British Isles by minerogenic deposits in present and former lakes (although the minerogenic layer may not correspond exactly with the stadial as indicated by pollen analysis).

Occasional thin seams of silt in chalky stadial deposits point to wind action[37,38]. A radiocarbon date and pollen analyses show that part of the wind-blown Shirdley Hill Sand of Lancashire accumulated during the stadial[39]. In the Vale of York and vicinity wind-blown sand occurs extensively as slightly hummocky sheets or as low dunes, radiocarbon dates demonstrating mainly stadial deposition[40–42]. Small fossil wedges infilled with the sand suggest permafrost, as do the wedges related to the stadial scree deposits in central Wales[29,30] and frost cracks associated with a stadial gravel layer in Buckinghamshire[43]. In Wales organic sedimentation in features interpreted as fossil pingos began very early in the Flandrian[44–46], suggesting they ceased to form during the stadial, but this is not readily reconcilable with their thick infill (10 m) of clay. Pollen analysis suggests that some at least of the small (30–75 m diameter) pingos of south-east Ireland formed during the stadial[47]. Some of the fossil pingos on chalk near Cambridge are believed to have existed at this time, their restriction to locations where hydrological conditions were most suitable suggesting that permafrost was discontinuous[48]. Fossil frost wedges just within the Loch Lomond Advance limit at three western sites near sea level in Scotland point to widespread permafrost in that country[49].

Fluvial activity

Snowmelt and surface thawing of frozen ground together with direct precipitation caused spring floods and abundant deposition on low ground. In Edinburgh (Corstorphine Loch) an extensive alluvial fan is dated by Windermere Interstadial deposits below it and Flandrian deposits above it. The age of large fans at the mouths of small tributary valleys in Glen Roy is demonstrated by their relation to the former ice-dammed lake. The lower parts of numerous valleys cut in sandy raised beach deposits in Scotland are infilled with very early Flandrian marine deposits, implying their excavation during the stadial when subsurface drainage was prevented by permafrost[50]. At the mouths of three Longmynd valleys in Shropshire fans of angular

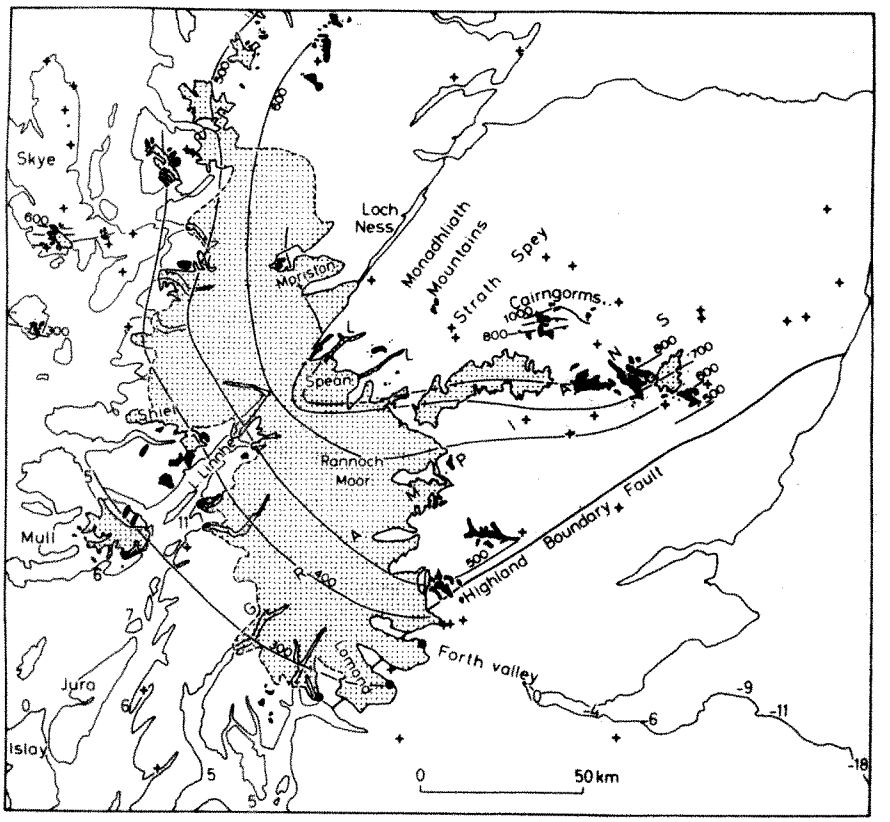


Fig. 2 Extent of stadial glaciers in part of the Scottish Highlands (stipple and solid) and regional firn-line altitudes (100 m interval): a dashed line is used where glacier limits are not yet mapped. Radiocarbon-dated marine shell sites related to glaciers shown by dots. Late-glacial pollen sites shown by crosses. Ice-dammed lakes of the Glen Roy area shaded and marked L. Representative altitudes for the Main Lateglacial Shoreline are indicated. Based on numerous sources including unpublished data provided by H. J. B. Birks, M. Robinson, D. G. Sutherland, K. S. R. Thompson, P. Thorpe and T. Wain-Hobson.

debris, one known to overlie Windermere Interstadial deposits, were partly laid down by stadial floods[51]. Even in the low-amplitude relief of Suffolk radiocarbon dates and other evidence indicate that sand and gravel deposits up to 400 m wide and 12 m thick accumulated in the Gipping valley during the stadial and very early Flandrian[52].

Flora and fauna

Open tundra vegetation prevailed during the stadial: there is only occasional macroscopic evidence suggesting the existence of tree birches, as at one site in Skye[53] and in the lower stadial deposits of a site in Lincolnshire[54], but dwarf birch was common. In pollen profiles annual pollen deposition rates are low and there is an increase in poorly preserved pollen. Pollen of Compositae, Cruciferae, Caryophyllaceae and Chenopodiaceae accord with open ground and disturbed soils, *Rumex* often being abundant and *Artemisia* especially characteristic. Large areas of fresh mineral soils are indicated by pollen of plants that favour base-rich conditions. The very low carbon content of stadial sediments in some lake sites in the Lake District and northern Scotland implies complete destruction of soils developed in the preceding interstadial, as well as low aquatic productivity[55–57]. The large numbers of moss spores in the sediments and, in northern Scotland, an increase (compared with underlying sediments) in the proportion of aerophile diatom taxa[58] are in accord with this evidence.

The crustacean *Lepidurus arcticus* has been found in stadial deposits in many parts of the British Isles. At Blelham Bog in the Lake District the arctic Cladocera *Chrydorus sphaericus* and *Acroperus harpae* increased in numbers[59]. Among beetles there was a preponderance of northern species, such as *Nebria nivalis* Pk, which is now found in Scotland only on the highest mountains, and *Bembidion dauricum*, now in Europe confined to arctic Norway and Swedish Lapland[60–64]. Vertebrates included reindeer, arctic lemming, Norway lemming, tundra vole and ptarmigan[65,66].

Marine activity

The stadial in some Scottish inshore waters is represented by thick deposits (such as 13 m) with foraminifera, ostracoda and/or dynoflagellate cysts indicating cold or very cold conditions[67,68]. These overlie interstadial 'Clyde beds', whose limited distribution above sea level is largely determined by marine erosion that occurred during the following cold period. This erosion, due to severe frost action combined with wave action, produced a platform often 20–30 m (but sometimes over 150 m) wide backed by a cliff up to 15 m high now seen on the Scottish coast between Loch Linnhe and the Firth of Clyde[69–71]. The shoreline (Main Lateglacial Shoreline) slopes owing to glacio-isostatic recovery, having a maximal altitude of ~11 m near Oban and passing below sea level in southern Kintyre and north-east Islay. Around Grangemouth in the Forth valley 28 km^2 of drift and, locally, bedrock were planated[50]. The shoreline is at −18 m, 9 km north of Berwick (Fig. 2), where the associated rock platform is probably 600 m wide[72,73]. The buried channel of the Tweed may well relate to this low sea level because it descends to ~−20 m and, significantly, lacks glacial till[74]. Pre- and post-stadial sea-level evidence from southern parts of England, Wales and Ireland[75–83] suggests a stadial sea level there between −35 and −50 m. The Main Lateglacial Shoreline may be represented by the sharp break of slope traced for 30 km at −35 to −40 m in Cardigan Bay[84] and by the pronounced erosional shoreline at −42 m identified off part of the coast of south-west England[85,86].

Climate

All the preceding evidence is intimately linked to climate. Suggested mean July temperatures for the stadial are 10 °C for southern England and 9 °C for northern England (beetle evidence)[63], 7.5 °C for the Lake District[9], 7 °C for the south-west Grampians[10] and 6 °C for the south-east Grampians[87] (sea level values based on glaciers). Since discontinuous permafrost indicates a mean annual temperature no higher than −1 °C, such figures imply a greater annual temperature range than at present, as (independently) do beetle assemblages; causes include greater differences than now between amounts of winter and summer solar radiation reaching the top of the atmosphere over the British Isles[88]: the smaller North Sea, probable freezing of the surface of this sea in winter, and an ice sheet over most of Scandinavia.

Glacier dimensions, altitudes and distributions imply that the principal snowfalls were associated with south to south-east air streams preceding warm or occluded fronts, although south-west air streams were important contributors of precipitation in western areas[10]. This suggests that many depressions followed more southerly tracks than prevail today[87], related to the junction of polar and warmer oceanic water masses at the latitude of south-west Ireland. The stormy climate resulted in extensive wind transfer of snow to glaciers and snow beds, especially by south-west winds, allowing widespread periglacial action on high ground thus largely denuded of snow. In the Scottish Highlands glacier firn-line altitudes (Fig. 2) imply that precipitation diminished inland from the west coast and northwards from the Highland Boundary Fault: in Strath Spey it may have been only 200–300 mm yr^{-1} (ref. 10). These precipitation inferences from glaciers in the Highlands accord broadly with proportions of *Artemisia* pollen in stadial pollen counts. Species of *Artemisia* favour well-drained ground and normally do not tolerate much snow. Percentages of *Artemisia* pollen increase eastwards into the Highlands from the west coast and northwards from the Highland Boundary Fault, culminating in Strath Spey, where at one site *Artemisia* constitutes 40–65% of total land pollen almost throughout the stadial[55,89–94].

Dating and climatic changes

Dates of ~10,800–10,300 yr BP have often been assigned to the stadial. Many radiocarbon dates are in accord but many others are not. Dates assigned to the beginning of the stadial are normally for the uppermost organic material underlying the stadial minerogenic layer in present or former lake sites. Dates may vary because of thickness of material sampled[95], contamination or other errors (several sequences show reversals) and because the initiation of minerogenic sedimentation would have been influenced by local factors (such as slope angles, and altitude) and geographic position. Due to the lag in vegetational response, and hence in the cessation of organic sedimentation, such radiocarbon dates will tend to be younger than the causative climatic change. Similarly, dates for the end of the stadial will tend to be too young, further factors here being soil removal during the stadial and slope instability. Coarse fluvial deposits continued to accumulate in the early Flandrian in Suffolk[52] and Shropshire[51] and are attributed to the very early Flandrian in the Isle of Man[96], while wind-blown sand continued to be deposited after the end of the stadial in the Vale of York[41]. In the Forth valley the highly micaceous minerogenic deposits of the Main Buried Beach, which ceased to accumulate at 9,600 yr BP[97], contrast with the overlying organic carse (mud-flat) deposits. Thus different processes that characterised the stadial operated over different periods of time and responded differently to climatic change.

Nine radiocarbon dates for material picked up or over-ridden by advancing stadial glaciers all lie between 11,800 and 11,300 yr BP[12,98–100]. Seven of these dates relate to five marine shell sites bordering the south-west Grampians (Fig. 2) and the other two are for a lake site in the Wicklow Mountains. They imply that glaciers reached their maximal extent later.

Coleoptera indicate that a decline in temperature began before 12,000 yr BP[34], Cladocera imply a deteriorating climate perhaps from ~11,800 BP[59], and some pollen evidence indicates irregularly declining temperatures from ~11,800 BP[57,101]. Since only a very few degrees drop in present summer temperatures would cause glaciers to form in Scotland[102], glaciers may have begun to develop here by 11,500 BP. Varied relief and precipitation and the southwards spread of polar water point to glacier initiation at different times in different places. The 400–450 varves of major Lake District lakes[103], which encompass both glacier advance and retreat (unpublished data) are far less numerous than those in Loch Ness[55], implying that glaciers existed for a much longer period in the Loch Ness catchment.

Retreat moraines show that some of the larger glaciers in the Lake District and central Southern Uplands withdrew actively (unpublished data). However, most Lake District glaciers and some Southern Uplands ones produced end moraines only at and near their furthest limits (in a zone 50–150 m wide). In the Scottish Highlands, except at ice-dammed lake sites and possibly at one other site, clear end moraines are restricted to the same situation, the end moraine zone being several hundred metres wide for some of the larger glaciers. It is tempting to suggest that all these end moraine zones are synchronous and that subsequently most glaciers decayed rapidly owing to the rapid rise in summer temperatures demonstrated by Coleoptera[104], this in turn being related to withdrawal of polar water (or to the latter having been rapidly over-ridden by warmer water). However, there is insufficient evidence to test this hypothesis.

Basal organic sediments from a site located behind a major end moraine at Callander have been dated as 10,670±85 yr BP[93]. Similar sediments from three sites on Rannoch Moor, in the heart area of the major stadial ice mass, range between 10,390±200 yr BP and 10,660±240 yr BP[105–108]. If valid these dates imply much earlier deglaciation than is normally assumed. It has been suggested that the dates may be too old due to the unique chemical and hence biological character of recently deglaciated terrain[109]. Yet this explanation does not apply to the Lake District evidence: here glaciers had withdrawn from the end-moraine belt before the end of the *Artemisia* pollen assemblage zone (ref. 103 and unpublished data) which, according to a radiocarbon date from Blelham Bog (located outside the glacier limits), ended before 10,650±170 yr BP[101]. This evidence suggests the attainment of glacier maxima ~10,800 yr BP, markedly out of phase with much other evidence. Many more radiocarbon dates are needed to resolve this apparent discrepancy.

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articles

General relativity, thermodynamics, and the Poincaré cycle

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An arbitrarily close return to a previous initial state of the Universe, such as predicted by the Poincaré recurrence theorem, cannot occur in a closed universe governed by general relativity. The significance of this result for cosmology and thermodynamics is discussed.

THE idea that history repeats itself—that every state of the Universe has occurred before and will occur again *ad infinitum*—is a concept which originated thousands of years ago and in the form of the Poincaré cycle and the 'cyclic' universe, still plays an important part in fundamental physics. This notion of an 'eternal return' will be considered here in the context of general relativity, and it will be shown that in a closed universe, no arbitrarily close (or exact) return to a previous state of the universe is possible, provided certain very general restrictions on the matter tensor and global causal structure hold. This result is in stark contrast to the situation in classical mechanics: Poincaré showed[1,2] that for almost all initial states, any mechanical system with a finite number of degrees of freedom, finite total and kinetic energy, and constrained to move within a finite box must return arbitrarily closely and infinitely often to almost every previous state of the system. The reason for 'no return' in general relativity and 'eternal return' in classical mechanics is that in general relativity, singularities intervene to prevent recurrence. General-relativistic closed universes are thought to begin and end in singularities of infinite curvature, and these singularities force time in general relativity to be linear rather than cyclic.

Poincaré recurrence has been reported[2–5] to be a major stumbling block in the definition of entropy, a function of the state of the system which never decreases, in terms of the fundamental microscopic variables of the system. Hitherto the increase of an 'entropy' defined by such variables has been obtained (not very successfully) by such tricks as 'coarse-graining'[2,6]; an *ad hoc* addition of randomness to the system in the form of a postulate of 'molecular chaos' or 'random phases'[2,3]; or by taking the thermodynamic limit[7,8]. The thermodynamic limit, which involves letting the volume of the system and the number of particles it contains go to infinity, is particularly unphysical as the infinite volume limit places one in the realm of cosmology, yet the limit ignores the most important cosmological force, gravity. The results of this paper may make such processes unnecessary, and there may be a strong connection between the increase of gravitational field entropy, which does not seem to require the above tricks, and the increase of matter entropy. The paper concludes with a discussion of the significance of the no-return theorem for cosmology.

To prove that no two states of the Universe can be identical or even arbitrarily close, we must first define the notion of 'close'. This can be done by regarding the set of all initial data as a Sobolev space W^s. The global topology of the space-time (M, g) is $S \times R$, where S is compact, if the space-time is globally hyperbolic. We choose some positive definite metric e_{ab} on M,

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and define a norm on W^s by

$$\|K^1_j\|_m \equiv \left[\sum_{p=0}^{m}\int_S (|D^p K^1_j|)^2\, d\sigma\right]^{1/2}$$

$$\|h, \chi\| \equiv \|h\| + \|\chi\| \qquad (1)$$

where $d\sigma$ is the volume element induced on S by e_{ab}, D^p is the generalised pth covariant derivative with respect to some chosen background metric $\bar{g}_{ab}$, and single verticle lines denote is the norm induced by e_{ab} (see ref. 9, p 233–235 for more details). Two tensors will be 'close' if they are 'close' in the norm of equation (1).

Assumptions in the no-return theorem

The no-return theorem will require four conditions on the matter tensor. The first condition, the time-like convergence condition (ref. 9, p 95), says roughly that gravitation is always an attractive force. The other three conditions, are here called (a), (b) and (c) (for precise definition see ref. 9, p 254–255). Briefly, (a) says that the development from a given set of initial data is locally unique; (b) says that this unique development is locally stable; and (c) says that the stress–energy tensor is a polynomial in the matter fields, their first derivatives, and the space-time metric. As discussed by Hawking and Ellis (ref. 9, p 255), these are physically reasonable conditions to impose on the matter fields, though strictly speaking they are not necessary conditions. Any other conditions which imply global Cauchy stability and uniqueness (as defined in ref. 9 Ch. 7) would be sufficient to prove the theorem below.

We also need two global conditions. The first, the requirement of unique development from Cauchy surfaces (ref. 9, p 205), says that laplacian determinism holds for the space-time. The second, the generic condition (ref. 9, p 101) says that every causal geodesic experiences a tidal force at least once in its history. As this would be expected to be false only for a 'measure-zero' set of solutions to the Einstein equations, the generic condition in the theorem below is analogous to the 'measure-zero' qualifications in the Poincaré recurrence theorem. A closed universe is defined to be a space-time in which the Cauchy surfaces are compact. A space-time that contains two disjoint space-like Cauchy surfaces which are isometric in their initial data is said to be time periodic. (Because if the evolution equations are well-posed, the development of the second Cauchy surface will be identical to that of the first, leading to a third identical surface, and so on.)

No-return theorem

Theorem: If a space-time (M, g) containing compact Cauchy surfaces is uniquely developed from initial data on any of its Cauchy surfaces, and if (M, g) also satisfies both the generic and the time-like convergence conditions, then the space-time cannot be time periodic. Furthermore, if the matter fields ψ and their first derivatives ψ' also satisfy conditions (a), (b) and (c), then for any neighbourhood U of any Cauchy surface S_1, a number $\varepsilon > 0$ exists such that $\|(h, \chi, \psi, \psi') - (h_1, \chi_1, \psi_1, \psi'_1)\|_{a+5} > \varepsilon$ for the initial data on any Cauchy surface S with $U \cap S$ empty. (ε Depends on e_{ab}, $\bar{g}_{ab}$ and U. The Cauchy surfaces S and S_1 are assumed space-like.)

The proof of this theorem will be published elsewhere. Note that the compactness of the Cauchy surfaces—the requirement of a closed universe—is an essential condition. If the word 'compact' is removed, then the theorem is false; it does not apply to open universes. For example, a static stop in asymptotically flat space satisfies all the conditions of the theorem except compactness, and this spacetime can be foliated by Cauchy hypersurfaces normal to the timelike killing vector field. The initial data is the same on each of these hypersurfaces.

Significance of no-return theorem

One might think that any field theory in euclidean space would have a non-recurrent property similar to the one demonstrated above, as a continuous field has an infinite number of degrees of freedom. From a physical point of view, this is not the case. It is not possible to make a precise measurement of the field variables at every point, and, in practice, a field restricted to a finite region S would be approximated by dividing up S into a finite number of subregions, and the field in each subregion replaced by its average value in that subregion. Evolution would be via the differential field equations, but one would compare the 'average' values.

Comparing initial data sets via the Sobolev norm is analogous to comparing the average values of the field variables (and their derivatives) at one time with the average values at another time. With the Sobolev norm, one essentially takes the absolute square average value of the initial data over the entire Cauchy surface rather than dividing up the Cauchy surface into subregions; the Sobolev average is a coarser average. The arbitrary metrics e_{ab} and $\bar{g}_{ab}$ in the Sobolev norm are analogous to an arbitrary coordinate system with respect to which averages are calculated. In the classical mechanics of fields in a finite box, one can define a similar norm $\|\,\| = [\int |\psi|^2\, d\sigma]^{1/2}$ on the classical field ψ. The sum $\sum_{i=1}^{n} \|\psi^{(i)}\|$ with $\psi^{(i)}$ being the ith derivative of ψ, is essentially the same as the Sobolev norm. This sum is a map f from the space of all values of the field and its first n derivatives into R. If the range of f is bounded—as it must be if the solutions ψ are stable—then the evolution of ψ must result in accumulation points in the range of f. An analogous accumulation is impossible in general relativity, as the above theorem shows. In general relativity the range of f is not bounded—singularities develop in space-time (this follows from the hypotheses of the above theorem and theorem 2 of ref. 9, p 266). This sort of singular behaviour is typical of many non-linear field theories[10,11], but in linear field theories such as electromagnetism the solutions are stable and are hence bounded. For example, the solutions to the Schrödinger equation in a finite box must recur[12,13] in the above average sense: for any initial value $\psi(t_0)$ for the probability distribution and any ε, there exists a time t for which $\|\psi(t) - \psi(t_0)\| < \varepsilon$.

In a finite universe without singularities there should be a finite number of physically distinguishable states (this should also be true if the matter in the universe is in the form of fields, for reasons stated above). I would expect that the states of such a universe would evolve in general in a quasi-ergodic way[14,15]. If the evolution of the physically distinguishable states is indeed quasi-ergodic, then in infinite time, every state would with high probability occur an infinite number of times.

Another way to model the evolution of such a finite-state always-existing universe, is by regarding the evolution as a finite discrete Markov chain[16] with stationary transition probabilities. That is, we will sample the state of the Universe at definite time intervals Δt, and we will assume that the state of the Universe at time t_j is determined by the previous state at time t_{j-1} and the probability matrix of going from this previous state to any other state. If the Universe has existed for an infinite time, then with probability one the states of the Universe form a closed set (ref. 16, p 384); that is, we may consider without loss of generality that the Markov chain representing the Universe is now irreducible. By theorem 4 of ref. 16 (p 392), it follows that with probability one, all states recur in the future.

Similar arguments will imply some form of recurrence in closed universes which avoid singularities by 'bouncing' at some small radius (such behaviour could, of course, occur only if some of the conditions in the no-return theorem are violated[17]). One could envisage the physical constants of the Universe, such as the elementary particle masses, the coupling constants, the specific entropy per baryon, and so forth, changing in some fashion at each bounce. These physical constants can be regarded as additional variables in the initial data. If the number of such constants is finite and if their range of variation is finite, then in a finite universe one would expect a finite number of physically distinguishable states. This implies accumulation points in the state space with an infinite number of bounces; with quasi-ergodic or markovian evolution, we would have recurrence of all states with high probability. Quantum-mechanical

considerations do not substantially alter this conclusion, as long as the number of physically distinguishable states is to be finite.

It is, of course, possible to avoid the recurrence conclusion by assuming that the range of variation of the physical constants is not bounded, or that the Universe, although closed, increases its radius at maximum expansion with each bounce. (Tolman[18], for example, argued that the monotone increase of entropy required a monotone increase of maximum radius at each bounce.) However, if the range of physical constant variation is not bounded, then a sequence of cycles would exist such that at least one physical constant would diverge in the limit. I would regard such a divergence as a singularity 'at infinity'. If the maximum radius increases monotonically with time, then with an infinite number of bounces in the past, the sequences of values of the maximum radius must have a limit point in the past. If its limiting value is non-zero, then one must have recurrence in the past, though this 'recurrence' may take the form of a gradual cessation of change, as in the Eddington–Lemaître universe[19]. If its limiting value is zero, then the singularity has not been really removed, it has just been placed at temporal infinity.

But the singularities in the single-cycle closed universe can also be regarded as at temporal infinity. In fact, both Milne[20] and Misner[21] have contended that because physical changes occur with diverging rapidity (as measured in proper time) near a singularity, the singularity should be regarded as occurring at temporal infinity as measured in physical time. Barrow and myself[22] have pointed out that extrinsic time[23], which is a naturally defined absolute time in closed universes[24], has just this property of placing the singularities at temporal infinity. In the extrinsic time scale, a closed universe has existed and will exist forever; the question of what preceded the big bang does not arise.

Because of Poincaré recurrence, it is impossible to define a monotonically-increasing entropy (or any monotonically-increasing function) for non-gravitational fields or systems of particles in terms of the phase space variables of the fields or particles. However, since Poincaré recurrence does not hold for gravitational fields, it is possible to define functions which increase monotonically with time—such functions are called Lyapunov functions[7,8]—on the phase space of the gravitational field. In fact, the extrinsic time, which is proportional to the trace of the extrinsic curvature of a leaf of a constant mean curvature Cauchy hypersurface foliation of space-time, is just such a function, as the extrinsic curvature is essentially the momentum of the gravitational field[25] and the extrinsic time increases monotonically in closed universes, ranging from $-\infty$ at the initial singularity to $+\infty$ at the final singularity[24]. In non-singular asymptotically flat space, a foliation of constant mean curvature Cauchy surfaces would have to have extrinsic time constant ($=0$). This supports the conclusion of classical mechanics that isolated systems cannot define a time direction.

Bohr[26] together with Prigogine and his coworkers[4,8] have suggested on the basis of Poincaré recurrence that thermodynamic concepts such as temperature and entropy are complementary to the phase space variables. In other words, they argue that a detailed microscopic description of the behaviour of a physical system would preclude the definition of thermodynamic variables for that system. If the gravitational field (or more precisely, the global structure of space-time) is taken into account, we see that it becomes possible in principle to define the thermodynamic variables and the phase space variables in a closed universe simultaneously; the notion of complementarity becomes unnecessary. This leads to speculation about the connection between quantum mechanics and the global structure of space-time: perhaps the impossibility of giving a detailed deterministic microscopic description of a quantum mechanical system is due to neglect of the global structure of space-time.

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Geochemical problems of the Antarctic dry areas

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The dry areas of the Antarctic are extreme deserts. Because of the cold they are not subjected to rainfall and geochemical separations can take place which would be destroyed by flowing water in the more temperate deserts. The distribution of soluble salts which accumulate on the surface and in the lakes is unusual and is explained on the basis of relative humidity separation mechanism. It is suggested that similar phenomena may take place on Mars.

ALTHOUGH much of the Antarctic continent is covered with ice and snow, there are some areas which are ice free. These so-called 'dry areas' are extreme deserts, characterised by extreme cold and aridity. They are also unique among deserts in being completely rainless. All precipitation falls as snow which is largely lost by sublimation. The salt geochemistry of water soluble salts which accumulate on the surface and concentrate in the lakes and ponds of these dry areas is described here. A relative humidity separation mechanism is proposed that can only operate in rainless deserts.

Although the salt and lake geochemistry of the McMurdo dry area has been studied for almost 20 years, the geochemistry of this region is far from being understood and there is a lot of

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apparently conflicting evidence. Even the origin of the salts in the lakes is in dispute. Since chloride is a minor constituent of Antarctic rocks and very little chemical weathering takes place, one might expect the chloride in the lakes (which are mostly chlorides of Ca, Mg and Na) to have originated from the sea. However, some lakes such as Lake Vanda, are essentially calcium chloride brines, a fact that is difficult to explain by normal geochemical models. Further, Jones and Faure[1] have presented isotopic evidence to suggest that most of the strontium in Lake Vanda does not have an oceanic origin. Other problems are that adjacent lakes frequently have very different chemical compositions which often do not seem to match that of their inflow streams. Furthermore, different layers of the same lake sometimes have different ratios of their ionic constituents. The bottoms of some chemically stratified lakes, which on other evidence must have been out of contact with the atmosphere for hundreds if not thousands of years, contain measurable amounts of tritium (half life = 12.3 yr) in their bottom waters. Glaciers which would be expected to have the same composition as the regional snowfall, because polar glaciers do very little cutting, frequently produce meltwater streams with very different chemical compositions to the snow which falls on their névés.

This article describes phenomena which can occur only in polar deserts and presents evidence in support of an hypothesis which could explain all the apparently anomalous data. First, it is necessary to describe the Antarctic dry areas in detail.

Geochemistry of the Antarctic dry areas

Why are the dry areas not ice-covered like the rest of the Antarctic continent? In this cold and arid region (mean annual temperatures of the McMurdo oasis region are approximately −20 °C) only a very small fraction of the snow that falls ever melts and almost all of it is lost directly by sublimation. The dry areas are snow free because ablation processes in total exceed all the accumulation processes. There must also be favourable topography to prevent ice from regions where accumulation processes exceed abalation from flowing into and inundating the dry area. The imaginary line which divides the region of net positive accumulation from the region where ablation exceeds accumulation is called the 'snow line'[2]. For a given region the net precipitation increases as altitude is increased. As one moves from the sea inland the snow line rises; presumably because total precipitation decreases. As one descends below the snow line the environment becomes more and more arid. Parts of the McMurdo dry valleys are so dry that $CaCl_2.6H_2O$ crystallises from ice-free saline ponds (such as the Don Juan Pond[3,4]). The fact that such a deliquescent salt can exist demonstrates that this area is among the most arid on the Earth.

If one digs into the loose 'soil' of a dry area in this cold and arid region, one passes through loose dry debris and can suddenly strike a very hard and impenetrable layer, which on examination proves to be debris as above but cemented with ice crystals. This phenomenon is equivalent to the water table of the more temperate regions of the world and is called here 'the frozen water table'. The depth to this frozen water table seems to be related to distance below snow line and is probably a balance between distallation of water downwards during brief periods of snow cover and evaporation outwards during periods of low relative humidity at the surface. Certainly as one goes to higher altitudes and hence nearer to the snow line, the depth to the frozen water table decreases. During the summer, cold saline water can be found moving downslope along the surface of the frozen water table.

Drainage basins

The dry areas of Antarctica frequently consist of a number of enclosed drainage basins. The lowest part of many of these is occupied by a lake, many of which are saline but some are relatively fresh. One of the most puzzling geochemical problems of the Antarctic dry areas is that these lakes have very different chemical compositions. Data on some are given in Table 1. The lakes seem to be of three main types; (1) Lakes which have derived their salts directly from seawater flooding. These are characterised by high Mg^{2+}/Ca^{2+} ratios. (2) 'Sodium chloride lakes' where the principal anion is chloride and the principal cation is sodium. These have a composition which suggests that they have been derived from the concentration of large quantities of glacial meltwater. This type of lake has a Ca^{2+}/Na^{+} ratio ~0.1. (3) 'Calcium chloride lakes' where the principal cations are calcium and magnesium and the principal anion chloride, the Ca^{2+}/Na^{+} ratio being >1. The calcium chloride lakes are generally relatively low in sulphate. The chemical composition of these lakes is perhaps the most difficult to understand.

Table 1 Chemical composition of water samples from the McMurdo dry valley area of Antarctica

	Ca^{2+}/Na^{+}
Inland snow*	0.13
Groundwater†	1.8
Canopus Pond	0.1
Lake Fryxell	0.4
Lake Hoare	0.09
Lake Joyce	0.03
Lake Miers (bottom)	5.0
Lake Vanda	
—upper water (13 m)	1.5
—lower water (61 m)	5.4
Don Juan Pond	10.2
'Amber Ice' at base of Meserve Glacier[13]	1.4
Seawater (for comparison)	0.04

* For example, Ice from Upper Taylor Glacier.
† For example, from surface of frozen water table east of Don Juan Pond.

Examples of lakes with salts of different origin are found in the lakes and ponds of the central Wright Valley. Canopus Pond and the upper waters of Lake Vanda have compositions of the sodium chloride type. However, the adjacent Don Juan Pond and the bottom waters of Lake Vanda have a very different composition, having a much greater proportion of calcium and magnesium chloride (see Table 1).

If the surface area of the glacier is insufficient to balance the ablation/precipitation budget, the glacier will advance further and further below the snow line until the total positive net accumulation above the snow line is balanced by the total ablation below the snow line. Usually the glacier has pushed sufficiently far below the snow line so that some summer melting takes place. In such cases, for a few days during the hottest part of the summer, a stream flows away from the glacier snout and feeds a lake which occupies the lowest point of that particular enclosed drainage basin or flows into the sea. In enclosed drainage basins the size of the lake is determined by that area needed to balance the evaporation/precipitation equation for that particular drainage area. If there is a net precipitation increase to the area the lake levels will rise and if there is a decrease in precipitation the lake levels will fall, provided that the temperature regime always remains similar.

Many of the lakes that occupy the lowest part of the various enclosed drainage basins are chemically stratified and these chemical concentration gradients may contain palaeoclimatic information.

Turning to the problem of the origin of chemical gradients in the saline lakes of the McMurdo oasis, an extreme example is Lake Vanda. The lake is 66 m deep, the lower 20 m being strongly density stratified with the specific gravity rising from 1.005 at a depth of 46 m to 1.10 at the bottom. It is interesting to speculate on the origin of the salt concentration gradient in the lake. Wilson[5] suggested that at some period in the past the climate was such that the Onyx River did not supply appreciable water to Lake Vanda. In these conditions the lake level would have dropped until only a few feet of concentrated calcium chloride remained. When the climate changed, the Onyx would

flow during the summer and fresh water would have flowed on top of this strong salt solution. Since then the salts would have been diffusing upwards. If such a model is assumed, it is possible to calculate the time when this climatic change occurred. On the above assumptions it is possible to calculate that Lake Vanda rose relatively suddenly 1,200 yr ago[5]. This same approach has been used to calculate diffusion ages for Lake Bonney[6,7].

When snow falls on a snow field or land surface it contains small quantities of salts, the principal cations being sodium and calcium and the principal anion being chloride with smaller amounts of Mg^{2+}, K^+ and SO_4^{2-} and other materials. The chemical composition of atmospheric precipitation has been the subject of much study (see refs 8–10). Although the chemicals in precipitation are generally believed to come from the ocean, the actual composition is not merely diluted seawater but has been modified by separation processes at the ocean surface. The actual composition varies as one moves away from the sea due, it is believed, to the existence of two types of aerosol particles in the atmosphere. The first type is large, falls out rapidly and has the composition of bulk seawater. Particles of the second type are very much smaller and have a composition very different from seawater. They have, for example, higher K^+/Na^+ and Ca^{2+}/Mg^{2+} ratios then bulk seawater. Both types of aerosols arise from the bursting of bubbles on the surface of the ocean[8,9]. In order to explain the distribution of salts in Antarctic soils and the composition of Antarctic lakes, the following hypothesis is presented.

Discussion

Over very long periods of time small quantities of snow fall on the land surface and on sublimation leave salts behind. These salts include Na^+, K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, Cl^-. It is proposed that fluctuations in relative humidity, due to the occasional snowfall and the summer-to-winter change, cause some of the more deliquescent salts to slowly percolate downwards through the soil. The more deliquescent will reach the frozen water table. At high altitudes, for example, on the top of the Asgard and Olympus Mountains and in the Alatna Valley, only the least deliquescent, for example sodium sulphate, can remain in the soil; the remaining salts, $CaCl_2$, $MgCl_2$ and much of NaCl, eventually reach the frozen water table where during the late summer they move down slope along the surface of the frozen water table. In the more arid situations at lower altitudes, the NaCl (77% equilibrium relative humidity at 0 °C, ref. 11) cannot reach the frozen water table and on the sides of the McMurdo dry valleys; for example, above Don Juan Pond and at similar altitudes along the sides of the Wright Valley, large quantities of NaCl can be found in the 'soils' and under boulders. At higher altitudes in the same region, almost pure Na_2SO_4 and $Na_2SO_4.2H_2O$ can be found in the same position (equilibrium relative humidity of sodium sulphate = 98% at 0 °C, ref. 11). The calcium chloride (equilibrium relative humidity = 44% at 0 °C, ref. 11) and magnesium chloride (equilibrium relative humidity = 38% at 0 °C, ref. 11) eventually move to the lowest part of the drainage system—in the case of the Wright Valley this is Lake Vanda and Don Juan Pond. Thus it is proposed that we have huge geochemical separation systems in the Wright Valley and other Antarctic dry areas, the salt fractionation being controlled by relative humidity and the deliquescent properties of the possible salts that can form from the anions and cations left by evaporated snow. Very saline groundwater can be detected flowing along the surface of the frozen water table in the late summer. For example, groundwater flowing along the top of the frozen water table in the south fork of the Wright Valley in late January 1974 had the following composition: 61,400 p.p.m. Cl^-, 11 p.p.m. SO_4^{2-}, 18,100 p.p.m. Ca^{2+}, 7,000 p.p.m. Mg^{2+}, 9,900 p.p.m. Na^+, 635 p.p.m. K^+ (sample collected by A.T.W. and analysed by A. Field). The groundwater is high in Ca^{2+} with respect to Na^+, and low in SO_4^{2-} with respect to Cl^- and has the chemical composition necessary to explain the origin of the calcium chloride type lakes discussed above.

In the above discussion, relative deliquescence is proposed as the primary mechanism for the separation of water soluble salts in the Antarctic dry areas. For completeness, freezing must also be considered. Eutectic temperature would at first seem to provide an alternative parameter that could regulate the compositition of the liquid phase, and such a mechanism will probably play some part in the initial separations. Evidence that relative deliquescence is the dominating mechanism is provided by the 'calcium chloride' type lakes and the 'groundwater' (see data above) which are not enriched in K^+ with respect to Na^+. This would be expected on the relative deliquescent model proposed (equilibrium relative humidity of 0 °C of NaCl = 77% and KCl = 88%[11]) whereas on an eutectic temperature model the reverse would be expected. When brines of the general composition expected in Antarctic soils are frozen[12], the K^+/Na^+ ratio remains constant until −8.2 °C, at which point sodium sulphate decahydrate forms. The K^+/Na^+ ratio continues to increase with falling temperatures until −22.9 °C at which point sodium chloride dihydrate forms. The ratio further increases until at −36 °C, sylvite (KCl) separates as does the dodecahydrate of magnesium chloride. On an eutectic feezing model, one would have expected potassium to follow magnesium and be enriched in the 'calcium chloride' type lakes and ponds. In fact, the reverse is true, leading one to conclude that it is the relative deliquescent mechanism rather than a freezing or a solubility mechanism which controls the separation of soluble salts in polar deserts.

The 'groundwater' described above can also flow into the sides of glaciers and explains the origins of the clear brown saline 'amber ice' often found in the basal layers of the local glaciers in the Antarctic dry areas (see, ref. 13). It also provides an explanation for the fact that some glacial meltwater streams have a higher Ca^{2+}/Na^+ ratio than the snow falling on their névés.

All the lakes in the Antarctic dry areas in practice have salts entering from both groundwater flow and meltwater from snow and glacier ice. Even glacial meltwater streams seem to have a significant contribution from groundwater salt, particularly early in the season. The fact that the composition of inflow streams varies throughout the summer and does not match the composition of the lake surface waters is an anomaly which is readily explained when the importance of the salt contribution by groundwater is realised. The interfingering of high Ca^{2+} groundwater to its appropriate density level can produce layers of very different Ca^{2+}/Na^+ in a lake. See, for example, the data of Bell[14] for Lake Miers. Layers of high Ca^{2+}/Na^+ ratio presumably contributed by groundwater can be seen at 17 m below the surface, and at the bottom.

Samples collected from the bottoms of Lake Bonney (east lobe) and Lake Vanda contain 14.9 ± 1.1 and 6.4 ± 0.4 tritium units respectively. Surface snow in this area contains about 100 tritium units. This was 'discovered' when I was attempting to provide the New Zealand Institute of Nuclear Science Tritium Dating Laboratory with tritium-free water for calibration purposes. It is generally believed that the bottom of the east lobe of Lake Bonney has been out of contact with the atmosphere for at least 60 yr[7] and the bottom of Lake Vanda for 1,200 yr[5]. As the half life of tritium is 12.3 yr, there should be essentially no tritium present in the bottom waters of either lake. I confirmed that the 'age' of the bottom waters of Lake Vanda is at least 1,000 yr by radiocarbon measurements. If the brine flowing along the top of the frozen water table enters a density-stratified lake it will interfinger to its appropriate density layer; and I propose that this is the mechanism whereby tritium has entered an apparently isolated geochemical system. Note that the inflow of saline groundwater into Antarctic lakes as proposed here would introduce small amounts of carbonate which had been in at least partial equilibrium with the atmosphere. This introduces complications for those attempting to use radiocarbon dating techniques to determine the times at which the lake levels, and hence climate, changed. The interfingering of saline groundwater into Antarctic lakes also provides an additional

mechanism for the formation of chemical stratification over and above that proposed by Wilson[5]. The use of diffusion calculations to determine the times at which lake levels changed must be made with caution with particualar attention being paid to how all the ionic ratios vary with depth.

The rather startling implication of this hypothesis is that much of the million tons of chloride contained in Lake Vanda has entered from groundwater flow. The amounts of Na^+ and SO_4^{2-} needed to bring the overall composition back to that of snow water are to be found in the soils on the lower sides of the valley (as NaCl) and high up on the Asgard and Olympus Ranges (as Na_2SO_4 and $Na_2SO_4.2H_2O$). Such a system would have taken a long time to develop and implies a great age to the Wright Valley geochemical system. A conclusion supported on other evidence[15].

Once the importance of saline groundwater is accepted one can appreciate how certain materials such as the strontium of Jones and Faure[1] can be leached from the glacial debris over which it flows. Thus, the isotopic ratio of the strontium in the sodium chloride type lakes could be of marine origin, whereas the calcium chloride type lakes where the salts are largely of groundwater origin could have components derived from the leaching of rock—which does seem to be the case[1].

The geochemical separation process described here can explain most of the apparently conflicting observations made during geomchemical studies in the Antarctic dry areas and the unusual geochemistry of the area. Such geochemical separation systems have developed in Antarctica and not in other arid parts of the world because the Antarctic dry areas are in a truly rainless desert. The infrequent but violent rains which occur even in the most arid of the non-polar deserts would destroy such a salt separation system. Although the geochemical situation described here is unusual on the Earth as a whole, being restricted to the rainless polar deserts, it may be expected to be found in other parts of the Solar System. For example, one could expect there to be soluble salts on the surface of Mars; and as Mars is also a rainless desert, one could expect simialar geochemical separations to those described here to have taken place.

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Embryonic development of identified neurones: differentiation from neuroblast to neurone

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Individually identified neurones are sufficiently large and accessible in grasshopper embryos to permit visualisation and impalement with intracellular microelectrodes from the time of their birth to their maturation. In this article part of the temporal pattern of differentiation from an identified neuroblast (precursor cell) to a group of identified neurones is described.

THE development of nerve cells involves the acquisition of specific neuronal phenotypes. Precursor cells proliferate and give rise to progeny which differentiate into neurones. During neuronal differentiation, processes grow out, arborise and reach their targets where synaptic connections are formed. The neurones acquire a responsiveness to neurotransmitters, an ability to make action potentials and a capacity for their own neurotransmitter synthesis, storage and release. The temporal sequence in which these and other phenotypes appear has not yet been determined for any group of nerve cells *in vivo*. A knowledge of the timing in any one instance would be a useful step in understanding the rules and mechanisms of neuronal development. For example, must the appearance of one specific phenotype precede the appearance of another?

It is possible to identify single unique neurones in the nervous systems of both invertebrates[1–3] and vertebrates[4]; they are usually identified on the basis of their characteristic location, morphology, physiology and biochemistry. Identified neurones are attractive for the study of neuronal development, as it is possible to follow the timetable of single cells from their birth to their maturation, thus avoiding the problems involved in examining the developmental average of a heterogeneous and asynchronous population.

We have been studying the embryonic central nervous system (CNS) of the grasshopper and have focused our attention on the development of the dorsal unpaired median (DUM) neurones because they represent a population of relatively large, identifiable and accessible embryonic cells. The adult CNS of the grasshopper consists of the brain and a chain of segmental ganglia running along the ventral surface of the thorax and abdomen. The DUM cells are a group of identified neurones (80–100) situated on the dorsal surface of the thoracic and abdominal ganglia, near the midline. The properties of the mature DUM neurones are well known from studies of adults[5–11]. The embryonic DUM neurones can be visualised and impaled with intracellular microelectrodes at the earliest stages of their development. Furthermore, the lineage of these neurones can be determined by serial examination of dissected preparations of increasing age, with interference contrast optics, as has been demonstrated for some neurones in the nematode[12,13]. We are now able to write down part of the developmental timetable of some of the identified DUM neurones of the thoracic ganglia in the embryo of the grasshopper. Preliminary accounts of this work have appeared[14,15].

The present study begins with the day 5 embryo, a stage at which the precursor cells (neuroblasts) for the segmental ganglia

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have already differentiated from the epidermis. The embryonic nervous system of the day 8 embryo is shown in Fig. 1*A*. At the centre of each body segment are the left and right plates of ventral neuroblasts (Fig. 1*A*iii) which give rise to the chain of segmental ganglia[16–20].

Each ventral neuroblast is ~30 μm in diameter (Fig. 1*A*iv). There are about 31 ventral neuroblasts in each left or right plate. Bate[20] found that the pattern and number of neuroblasts at early embryonic stages was at least as constant as that of the identified neurones of the adult segmental ganglia. Each neuroblast (NB) is a stem cell which will not in itself become a neurone. Rather, each NB maintains its large size while budding off a chain of smaller cells (Fig. 1*A*v). Each of these progeny divides into two cells which are postmitotic and differentiate into neurones. The neuroblasts eventually degenerate at precise times during embryogenesis[20].

Each segmental array of neuroblasts also contains a single unpaired neuroblast, called the median or dorsal unpaired median (DUM) neuroblast, at the posterior end of the segment and dorsal to the paired plates of ventral neuroblasts[16,18,20] (Fig. 1*A*iii, iv, v). As Wheeler[16] first described, the progeny of the single DUM neuroblast extend anteriorly across the dorsal surface of the developing ganglion, quite separate from the neuronal progeny of the ventral neuroblasts. Thus, neuronal differentiation in this lineage can be viewed by removing the embryo from the egg, removing the chain of segmental ganglia from the embryo, desheathing the dorsal surface of the ganglion, and viewing the dorsal surface under a ×40 water immersion lens of a compound microscope with Nomarski interference contrast optics.

The dorsal unpaired median neurones

In the adult grasshopper the somata (cell bodies) of most of the 3,000 neurones in each thoracic ganglion (pro-, meso- and metathoracic) lie on the ventral surface or lateral edge of the ganglion and the neuropil lies dorsal to them. These cells are paired, that is, each cell has a contralateral homologue that is its mirror image. The left and right plates of ventral neuroblasts give rise to these ventral paired neurones[16,18,20]. However, there is a group of 80–100 somata clustered along the midline on the dorsal surface of the ganglion. These neurones, called dorsal unpaired median neurones[7], have the following distinctive characteristics. The cells thus far examined in the adult are unpaired and have medium neurites which bifurcate into bilaterally symmetrical processes. The DUM neurones are the only neurones in the segmental ganglia whose somata generate overshooting action potentials. The soma spikes of the DUM neurones depend on both Na^+ and Ca^{2+} while the axon spikes are predominantly Na^+ dependent[11].

Whereas DUM neurones have certain characteristics in common, many if not all of them can be uniquely identified by their morphology. The largest DUM neurone in the grasshopper metathoracic ganglion (diameter ~40–60 μm) is a unique identified neurone with axons emerging bilaterally in nerve 5 and innervating the extensor tibiae (ETi) muscle in the femur of the leg (this neurone is called DUMETi (ref. 7)).

Only 6–8 of the DUM neurones have large-diameter somata (40–60 μm); most are somewhat smaller (15–30 μm in diameter). The somata of the DUM neurones stain with neutral red[8,9], a dye which selectively stains monoamine-containing neurones in the leech[21] and lobster[22,23]. Both the soma and axons of DUMETi have been shown to contain octopamine[9]. In fact, most if not all of the DUM neurones (particularly the large ones) contain octopamine[24].

Electrical and dye coupling

All cell types examined at early embryonic stages of the grasshopper are electrically coupled to each other; development involves a process of selective uncoupling. Dissected embryos were perfused with a simple saline (NaCl 125 mM; KCl 3 mM; $CaCl_2$ 10 mM; HEPES buffer 5 mM; adjusted to *p*H 7.4). Cells were impaled with intracellular microelectrodes, each with recording and current passing capability. Neuroblasts (~30 μm diameter) had resting potentials of −60 to −80 mV; non-neuronal cells (~10 μm diameter) often had recorded resting potentials of −40 to −60 mV, that may have been the result of damage during impalement. Records made by inserting two microelectrodes into the same ventral or dorsal neuroblast at

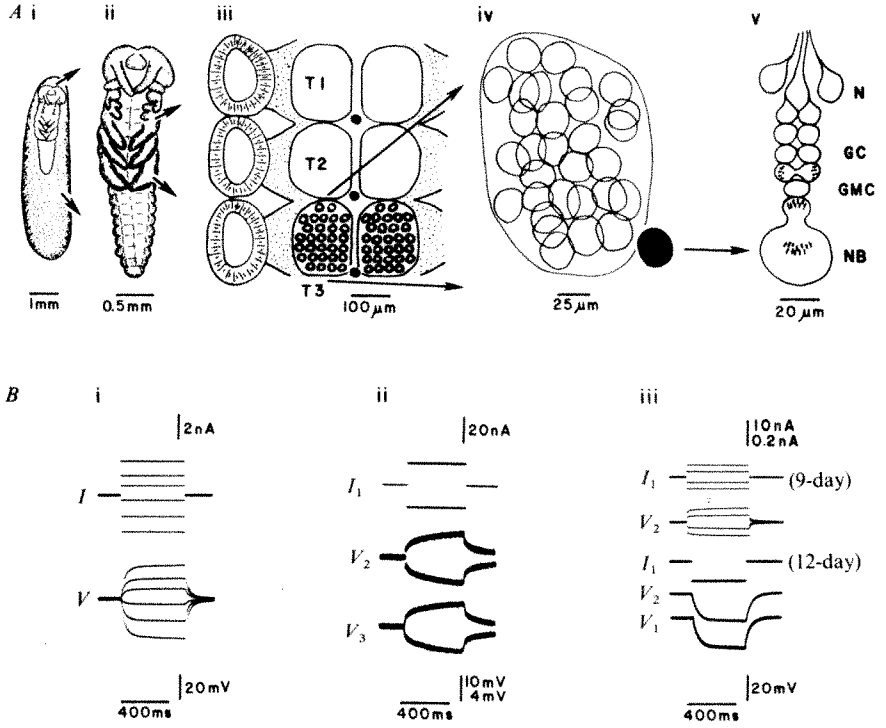


Fig. 1 *A*, Grasshopper embryo (*Schistocerca nitens*) at day 8; diagrams and camera lucida drawings of living specimens. Location of neuroblasts (precursor cells) and formation of neurones are shown (hatching occurs on day 20 at 35 °C[45]). (i) Embryo at day 8 viewed within the egg case. The embryo metabolises yolk, shown stippled. (ii) The day 8 embryo viewed from the ventral surface, showing the three prominent thoracic limb buds and the equivalent segmental appendages forming the mouth parts. (iii) Removal of the thoracic limb buds exposes the paired plates of ventral neuroblasts (enlarged ectodermal cells, open circles), and the single dorsal unpaired median (DUM) neuroblast (filled circles), also called the median (MNB) neuroblast, at the posterior margin of each segmental array of ventral neuroblasts. The contiguous chain of paired plates of neuroblasts gives rise to the chain of segmental ganglia (T1, pro; T2, meso-; T3, metathorax). (iv) Camera lucida drawing of the left hemiganglionic plate of neuroblasts in the metathoracic segment, also showing the dorsal unpaired median neuroblast, viewed with a ×40 water immersion lens and interference contrast optics. (v) A schematic drawing showing a neuroblast (in this case, the DUM neuroblast) as a stem cell; it maintains its large size while budding off a chain of ganglion mother cells (GMC) which each divide into two ganglion cells (GC) and then differentiate into neurones (N). *B*, Input resistance (R_{in}) and extent of electrical coupling of cells at different stages of development of the grasshopper embryo. (i) R_{in} for a ventral neuroblast at day 8 of development (~10 MΩ). Current (*I*) and voltage (*V*) electrodes in the same cell. (ii) Electrical coupling of one ventral neuroblast (I_1) to a second ventral neuroblast (V_2) and to an epithelial cell of the limb bud (V_3), in a day 8 embryo. (iii) Electrical coupling of DUM neuroblast (I_1) to nearby progeny cell (V_2) at day 9 (note large current); DUM neuroblast is still coupled to the rest of the embryo at this stage. Coupling of DUM neuroblast (I_1) to nearby progeny cell (V_2) at day 12 (note small current); DUM neuroblast becomes uncoupled from most of the rest of the embryo at day 10. R_{in} for the DUM neuroblast (V_1) and for the coupled network is ~200 MΩ.

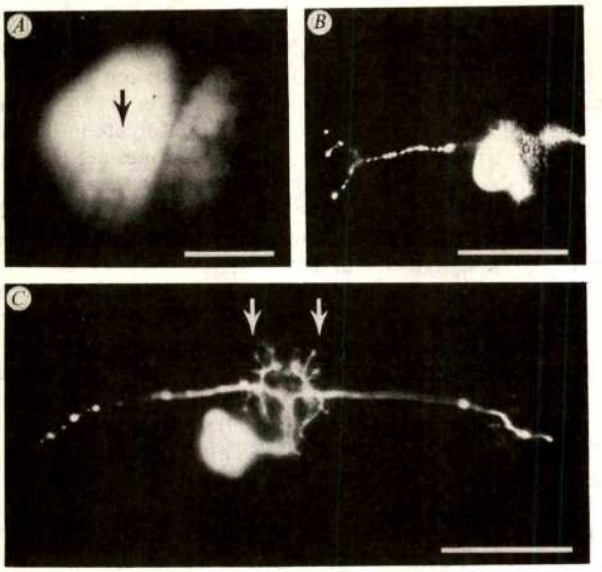


Fig. 2 Lucifer Yellow (450 MW) injections of a neuroblast and two identified neurones of grasshopper embryos. Fluorescence viewed with UV illumination. Scale bar, 100 μm. *A*, Injection of a ventral neuroblast (arrow) in the left hemiganglionic plate of neuroblasts of the metathoracic segment of an 8-day embryo. Dye spreads rapidly through other ventral neuroblasts and some of their progeny in the left plate, and within a few minutes through cells in the right plate. Note absence of spread to adjacent limb buds. *B*, *C*, Injection of identified neurones in the metathoracic ganglion of a 13-day embryo. Cell bodies appear unusually large because of intense fluorescence. The dye does not spread to other cells. Axons are truncated at points of exit from the ganglion. *B*, DUM 3, 4, 5: median neurite bifurcates, and left axon sends branches out nerves 3, 4 and 5 (top to bottom). *C*, DUMETi (DUM 5): axons extend out nerve 5 only. Arrows indicate beginnings of relatively symmetrical central arborisations.

easly stages (Fig. 1*B*i) show that the input resistance (R_{in}) is quite low (~10 MΩ), probably as a result of the extensive electrical coupling. The input resistance of a single ventral neuroblast was found to be about 150 MΩ after mechanically removing it from the network of neighbouring cells to which it was coupled, and impaling it with two electrodes. This is likely to be less than the true value because of possible damage during the isolation.

Ventral neuroblasts are electrically coupled to one another within a hemiganglion (Fig. 1*B*ii). Coupling also exists between left and right hemiganglia, between the DUM neuroblast and ventral neuroblasts, and between neuroblasts of a given segment and those of a segment either anterior or posterior to it. Furthermore, the neuroblasts are coupled to the smaller and morphologically distinct epithelial cells (termed cap cells) which lie just over them, and even to the more distant epithelial cells of the developing limb bud (Fig. 1*B*ii). Confirmation of the positions of the electrode tips and the identities of the coupled cells was achieved by injecting cells with the dyes Fast Green or Procion Rubine (2% dye in 0.25 M KCl). No evidence for rectification of electrical coupling was seen. In all such experiments, the coupling recorded between any two cells disappeared when either intracellular electrode was withdrawn from the cell. Ventral neuroblasts become electrically uncoupled from the limb buds at day 10, but remain electrically coupled to each other until they stop dividing and degenerate by day 15.

We examined the movement of low molecular weight tracers from the interior of one cell to the interior of another by injecting the dye Lucifer Yellow (450 MW), which has a high fluorescent quantum efficiency and rapid rate of diffusion. When iontophoresed into single cells (from electrodes filled with 5% dye in distilled H_2O), it will often spread to other cells to which the injected cell is electrically coupled[25]. When a single ventral neuroblast is filled with Lucifer Yellow in a day 8 embryo, the dye spreads to other ventral neuroblasts and some of their progeny in the hemiganglion within a few minutes (Fig. 2*A*). The fluorescence can be detected in the contralateral hemiganglion shortly thereafter, but spreads to anterior and posterior ganglia much more slowly. However, dye was not seen to spread to limb-bud epithelium, even though the neuroblasts are electrically coupled to the limb-bud cells. In control experiments, dye was not taken up by the cells when simply applied in the bath.

We next examined the electrical and dye coupling of the DUM neuroblast and its progeny. The DUM neuroblast begins dividing at day 6. At first, all of its progeny are electrically coupled to the DUM neuroblast (Fig. 1*B*iii). Until day 9, large currents injected into the DUM neuroblast produce small voltage changes in progeny cells (Fig. 1*B*iii, top), consistent with the extensive electrical coupling of the DUM neuroblast to the rest of the embryo. As early as day 10 (Fig. 1*B*iii, bottom, similar records from day 12) much smaller currents produce large voltage changes in progeny cells. Day 10 is the time at which the DUM neuroblast becomes electrically uncoupled from most embryonic cells other than its own progeny. Input resistance (R_{in}) for the coupled network of neuroblast and progeny (as well as the R_{in} of the DUM neuroblast) increases from <10 MΩ at day 9 to ~200 MΩ by day 12 (Fig. 1*B*iii). All progeny remain electrically coupled to the neuroblast until day 11, when the oldest progeny (identified DUM neurones described below) are first becoming electrically uncoupled. (Mature DUM neurones are not electrically coupled to one another.)

Lucifer Yellow injected into the DUM neuroblast at day 7 spreads throughout all of its progeny. However, at day 8, its

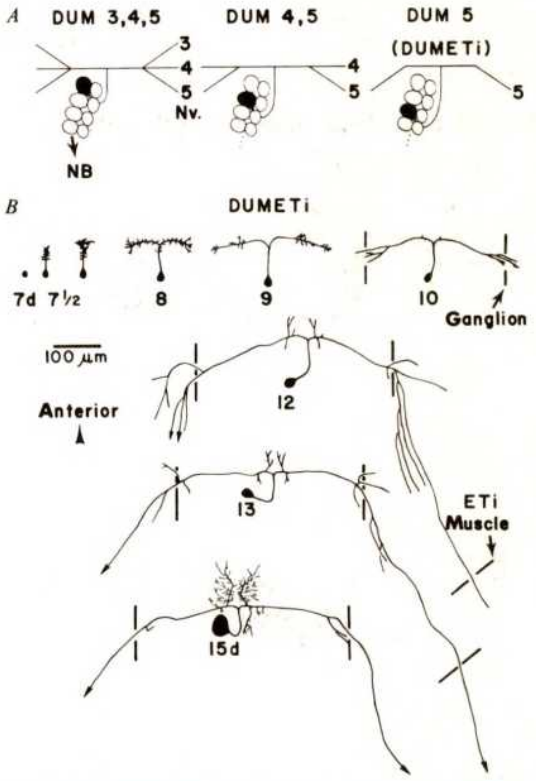


Fig. 3 *A*, DUM neuroblast gives rise to identified DUM neurones. The DUM neoroblast (NB), not shown here, gives rise to chains of progeny; some of the oldest of these progeny become identified DUM neurones. At days 12–13, we find two chains of progeny, generally two cells in width. We have examined the identities of the first three cells on the outside of the oldest chain of progeny ($n = 10$). The morphology of the DUM neurones is revealed by intracellular injection of Lucifer Yellow. In this schematic diagram, the most anterior cell on the outside bifurcates and bilaterally extends out peripheral nerves 3, 4, and 5; the second cell bilaterally extends out nerves 4 and 5; and the third cell extends out bilaterally only nerve 5 (this cell is DUMETi). *B*, Morphological development of the single identified DUM neurone, DUMETi ($n \geq 3$ for each time point). The DUM neuroblast begins dividing at about day 6. Many extra branches and growth cones are produced at the distal end, whether it be in the central nervous system or periphery, and these extra neurites later disappear as the correct branch for DUMETi continues to grow. 'Ganglion' marks the border of the metathoracic ganglion, and 'ETi muscle' marks the proximal boundary of the extensor tibiae muscle in the femur of the limb bud. Arrows mark points of axonal extension beyond the margins of the figure.

oldest progeny are dye-uncoupled. Dye does not spread into or out of these identified DUM neurones, although they are still electrically coupled to other cells (Fig. 2*B*, *C*).

DUM neuroblast gives rise to identified DUM neurones

The evidence that the DUM neuroblast gives rise to identified DUM neurones is described by considering day 12–13 embryos, although these cells can be followed from day 6 by serial examination of dissected preparations of increasing age. Viewing the desheathed dorsal surface of the metathoracic ganglion reveals chains of progeny extending anteriorly from the DUM neuroblast. Cells close to the neuroblast are young progeny which are the result of recent divisions (the neuroblast continues dividing until it degenerates on day 16), whereas those at the anterior (distal) end of the elongating chains are the oldest progeny. The DUM neuroblast is ~30 μm in diameter, the progeny at first are ~10 μm and quickly enlarge to ~15 μm in diameter. Several of the somata increase to a diameter of over 40 μm during embryogenesis. Some of the oldest progeny of the DUM neuroblast become identified as DUM neurones because within a few days they assume the characteristic location (dorsal midline), the characteristic morphology (median neurites which bifurcate and extend bilaterally, Figs 2, 3), the characteristic physiology (spiking somata, Fig. 5) and the characteristic histochemistry (somata which stain with Neutral Red[24]).

At days 12–13, we see two chains of progeny, each being generally two cells in width, with one chain extending anteriorly on the left side and the other chain on the right side of the midline. Initially the older chain is dorsal to the younger (day 7). Between days 7 and 11, the plane in which the two chains lie changes in orientation, from dorso-ventral to horizontal. When the orientation changes, there is a 50% probability ($n = 24$) that the dorsal (older) chain will assume either the left or right position in the horizontal plane. It can easily be distinguished, however, by the morphology of its cells throughout this period (C. M. Bate, C.S.G. and N.C.S., in preparation).

We have examined the identities of the first three cells on the outside of the oldest chain of progeny, and find the same identified neurone in the same position along the chain ($n = 10$) (Fig. 3*A*). The morphology of the DUM neurones is revealed by intracellular injection of Lucifer Yellow, and later visualisation in either living or fixed tissue, with UV illumination (Fig. 2*B*, *C*). (Tissue is fixed in 4% formaldehyde, dehydrated in an ethanol series, cleared in methyl benzoate and mounted in fluoromount.) The most anterior cell on the outside bifurcates and extends bilaterally out as peripheral nerves 3, 4 and 5; the second cell on the outside bifurcates and extends bilaterally out as peripheral nerves 4 and 5; and the third cell on the outside bifurcates and extends bilaterally out only peripheral nerve 5. The axons of this third cell run out nerve 5 to the extensor tibiae (ETi) muscle; this cell is DUMETi. In the mesothoracic ganglion, the first cell also bifurcates and extends bilaterally out as peripheral nerves 3, 4 and 5. Thus, from animal to animal, the cell in the same position along the chain, presumably having the same mitotic ancestry, develops into the same identified neurone.

Morphological development of identified DUM neurones

The outgrowth of processes by the single identified DUM neurone, DUMETi, was followed as a function of developmental age ($n \geq 3$ for each time point). Axonal outgrowth of DUMETi begins on about day 7 with the extension of a median neurite (Fig. 3*B*). At this time, the framework for the neuropil, nerve connectives and peripheral nerves already exists in the form of two longitudinal fibre tracts, two commissures in each segment, and lateral fibre tracts to the periphery. The distal end of the growing axon produces multiple branches with growth cones extending in different directions, often for over 20 μm in the wrong direction. As the correct branch continues to extend and send out branches at its distal tip, the extra incorrect neurites disappear, leaving a relatively naked proximal axon.

By day 8, the axon of DUMETi has bifurcated and both lateral axons appear fuzzy because of the presence of many fine branches. During day 9, the distal end of the axon grows out to the edge of the ganglion, and many of the proximal branches disappear. By day 10, the neurone sends axons out many of the available peripheral nerves and branches of those nerves, even though ultimately these extra branches will disappear and the axon of DUMETi will extend out only nerve 5 (and branch 5b1d) to the ETi muscle. By day 12, the distal axon reaches the ETi muscle. Three prominent features at this age are the occurrence of extra peripheral branches, the start of the central bilateral arborisations and the relatively naked central axon. By day 13, the axon has extended over the entire ETi muscle, but the cell body and arborisations are still relatively small.

After the axon of DUMETi reaches its target, there are three clear morphological changes: the extra peripheral neurites disappear, leaving behind only one axon in nerve 5; the central arborisations greatly increase in the extent and number of their

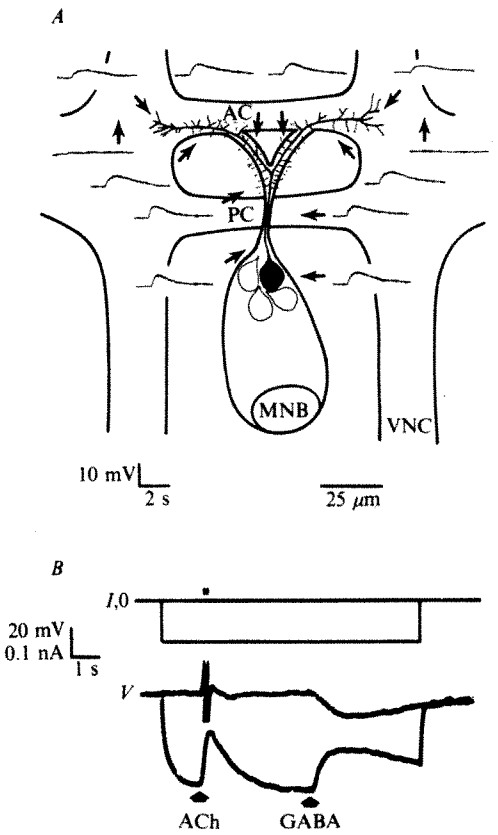


Fig. 4 Chemosensitivity of identified DUM neurones in the grasshopper embryo (the three oldest progeny as described above). *A*, Response to iontophoretic application of ACh in a day 8 embryo. The diagram shows the embryonic neuropil of a single segmental ganglion (T3), as viewed from the dorsal surface (anterior at top). The DUM neuroblast (NB) is shown with its packet of progeny; those most anterior (oldest) send their axons anteriorly in a median bundle of processes which cross the posterior commissure (PC) and bifurcate near the anterior commissure (AC). The extent of branching of an individual neurone was determined by intracellular injection of Lucifer Yellow. The longitudinal connectives extend into the ventral nerve cords (VNC) and are seen on either side. The lateral neuropil extends (beyond the margin of the figure) into three fibre tracts which become peripheral nerves 3, 4 and 5. The response to application of ACh at the points indicated by the arrows were recorded by an intracellular electrode in the cell body. Processes are sensitive to ACh over their whole length. Small vertical or lateral displacements of the iontophoretic electrode abolished the response. *B*, Responses to sequential iontophoretic application of ACh and GABA from a double-barrelled micropipette, recorded with an intracellular electrode in a day 13 embryo. At a resting potential of −55 mV, ACh depolarises the cell to threshold, eliciting overshooting action potentials, while GABA hyperpolarises the cell. When the cell is hyperpolarised by injected current, the response to ACh is larger, but remains subthreshold; the cell is then depolarised by GABA.

fine branches; and the cell body greatly enlarges. A similar initial overproduction of distal branches and eventual disappearance of these extra branches was found also for DUM 3, 4, 5 and DUM 4, 5.

Onset of chemosensitivity

At day 7, neither the DUM neuroblast nor its progeny show responses to bath application of acetylcholine (ACh) or γ-aminobutyric acid (GABA) at 10^{-3} M. In contrast, by 13 days the oldest identified DUM neurones (described above) are sensitive to bath application and iontophoresis of both of these agents. This latter result is illustrated in Fig. 4*B*, which shows the intracellularly recorded responses of a developing DUM neurone to iontophoretic application of ACh and GABA from a double-barrelled electrode. These responses involve a membrane conductance increase (not illustrated). The observed reversal potential for GABA is −70 mV, while the extrapolated reversal potential for ACh lies above +20 mV. The ACh response is abolished by removal of Na^+ (replacement with choline); the GABA response is abolished by removal of Cl^- (replacement with isethionate). The ACh response is reduced by the nicotinic antagonists curare (10^{-4} M), hexamethonium (10^{-3} M) and decamethonium (10^{-3} M); nicotine is an agonist. However, α-bungarotoxin does not significantly reduce the response at 10^{-6} M. The ACh response is unaffected by the muscarinic antagonists atropine (10^{-6} M) and quinuclidinyl benzilate (QNB, 10^{-7} M); pilocarpine has no effect (10^{-3} M). The GABA response is rapidly blocked by picrotoxin (10^{-4} M); muscimol is an agonist.

The oldest DUM neurones first become sensitive to ACh and GABA at day 8 of development, and seem to become sensitive to both neurotransmitters at the same time. Young progeny in close proximity to the DUM neuroblast show no response to iontophoretic application of ACh. In contrast, the oldest progeny at the distal end of the chain, which are still electrically coupled (Fig. 1*B*iii) and already possess neurites (Fig. 3*B*), are depolarised by iontophoretic application of ACh onto their somata. (No desensitisation is seen in response to prolonged application of these agents.)

The processes of the oldest DUM neurone are also depolarised by iontophoretic application of ACh on day 8. Processes seem to be sensitive over their entire length. It seems that chemosensitivity is distributed over the whole surface of the cell, at or soon after the time that it first appears. Identified DUM neurones thus develop chemosensitivity to ACh and GABA while still electrically coupled, and may be sensitive to other neurotransmitters as well. The reversal potential and ionic dependence of the response to ACh and GABA appear similar at days 8, 13 and 18 of development.

Onset of electrical excitability

We have examined the onset of electrical excitability in the oldest DUM progeny by observing the responses of cells to depolarising current pulses injected through an intracellular recording electrode. They are generally incapable of producing action potentials at day 11. They become electrically excitable between days 11 and 12 (Fig. 5*A*), while they are becoming electrically uncoupled. It seemed possible that voltage-dependent channels were not revealed while the cells were electrically coupled because of the long time constant of the coupled network of cells, preventing depolarisation to threshold before channel inactivation occurred. This possibility was tested by exposing the cells to veratridine 10^{-5} g ml^{-1}, which specifically depolarises cells possessing voltage-dependent Na^+ channels[26]. The treatment has no effect on the resting potentials of any of the ventral neuroblasts, the DUM neuroblast, or its progeny up to day 11. In contrast, the oldest DUM progeny are depolarised by the treatment by day 12 (we thank A. Willard for suggesting this approach).

Between days 11 and 12, the oldest DUM neurones first produce small action potentials which fail to overshoot the zero

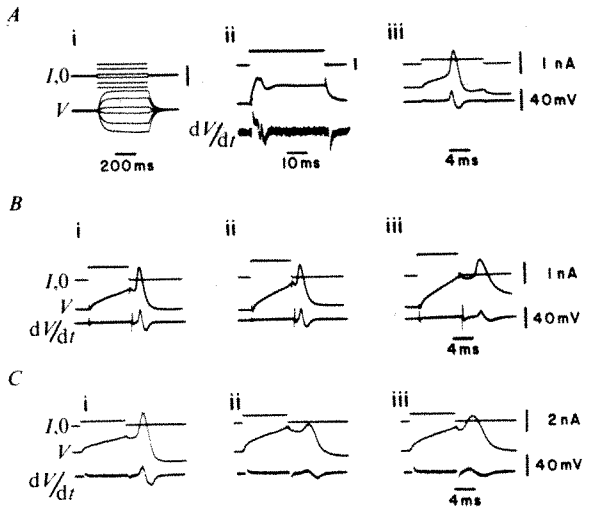


Fig. 5 Electrical excitability of the oldest DUM neurones in the grasshopper embryo. *A*, Responses in the oldest DUM progeny between days 11 and 12. (i) The DUM neuroblast is impaled with one microelectrode to inject current, and an electrically coupled progeny is impaled with a second microelectrode to record voltage. These cells have resting potentials of −50 to −70 mV and appear electrically inexcitable. Depolarisation and hyperpolarisation of the neuroblast and its progeny by 50 mV reveals a constant membrane resistance. (ii) As the cells become uncoupled they develop neurite action potentials in which the inward current is carried predominantly by Na^+ (data not shown). The early embryonic neurites bifurcate at a T-junction, and in many cases the first embryonic axon spikes have the adult characteristic of appearing double-peaked; each peak in the adult and presumably in the embryo corresponds to a separate site of initiation along one of the two lateral axons. (iii) Current injected into a DUM neurone at a later time yields an overshooting action potential that appears to arise in the cell body. *B*, Ionic dependence of the somatic action potential in a day 12 embryo. The overshoot of the spike seen in normal saline (i) is not greatly affected by replacement of Na^+ with Tris (ii) or addition of 30 mM Co^{2+} (iii). *C*, Ionic dependence of the action potential of a DUM neurone in a day 18 embryo. The overshoot (i) is reduced by either removal of Na^+ (ii) or addition of Co^{2+} (iii). In both day 12 and day 18 action potentials, simultaneous removal of Na^+ and addition of Co^{2+} (not shown here) abolishes the action potential.

potential and often have a characteristic double-peaked appearance (Fig. 5*A*). These action potentials are likely to be produced in the neurites, because this response has been shown in adult DUM neurones to be the sum of action potentials arising from two symmetrical neurites distal to the point of bifurcation[10]. These action potentials depend largely on an inward Na^+ current, because they are rapidly blocked by removal of Na^+ or addition of 10^{-9} g ml^{-1} tetrodotoxin (TTX). These cells and those in older embryos are depolarised by 25 mV or more when exposed to veratridine. This depolarisation is blocked by prior removal of Na^+ or addition of TTX.

Within a day, the oldest DUM neurones are capable of producing overshooting action potentials, 2–4 ms in duration, that appear to arise in the cell body ($R_{in} \cong 300$ MΩ). These action potentials depend on Na^+ and Ca^{2+} for their inward current. Removal of Na^+ or addition of 30 mM Co^{2+} (to block Ca^{2+} channels) has little effect on the overshoot (Fig. 5*B*), although simultaneous application of both treatments abolishes the action potential. However, by 18 days, blockage of either inward current reduces the overshoot in these same neurones (Fig. 5*C*). It is known that in at least one adult DUM neurone, DUMETi, the blockage of either inward current alone is sufficient to abolish the action potential[11]. The basis for these developmental changes is not yet known.

Development of later phenotypes

The transmitter of the DUM neurones, octopamine, begins to appear in the thoracic ganglion on day 13, after the axons of the oldest DUM neurones reach their peripheral target[24]. Shortly thereafter, the somata of the oldest DUM neurones begin to stain with Neutral Red.

The first spontaneous synaptic activity, from unidentified synaptic input, is recorded in DUM neurones at 15 days, 1 week after the appearance of chemosensitivity. The source of these synaptic inputs is not yet known.

Temporal sequence of development

There is a distinct order in the acquisition of particular phenotypes by the identified DUM neurones that are the oldest progeny of the DUM neuroblast (Fig. 6). This developmental sequence occurs first in the oldest progeny; younger progeny may be as much as several days behind in their differentiation.

Some of the phenotypes initially express change during the course of further embryonic development whereas others do not. There are changes in the morphology of identified neurones: the initial fine branches and the extra peripheral neurites all eventually disappear. Similarly an initial overproduction and later disappearance of many central branches around the site of the main arborisation has been observed in an identified neurone in the brain of the grasshopper (D. Bentley and A. Raymond, personal communication). In other systems, gap junctions[27] and profiles of chemical synapses[28] appear transiently on cell bodies shortly before the outgrowth of their processes. In the embryonic DUM cells, there appears to be no change in the ionic basis of the responses to ACh and GABA, as indicated by the ion substitution experiments and the reversal potential determinations. In contrast, there is a change in the ionic requirements for electrical excitability of the cell body, consistent with, although not qualitatively as great as, the changes reported for the development of excitability in other neurones[29–33].

The initially extensive electrical coupling and eventual uncoupling are consistent with observations in other embryos[34–36]. The outgrowth of processes and the appearance of chemosensitivity of the identified DUM neurones begin after their terminal mitoses but while they are still extensively electrically coupled. The observation that cells become dye uncoupled before they become electrically uncoupled suggests that there may be some progressive restriction on the molecular size of substances that can pass freely between cell interiors. Further work will be needed to determine the mechanisms involved in these changes and the role of uncoupling in further differentiation.

How general is the sequence of appearance of neuronal phenotypes observed in the DUM neurones? Studies of the development of amphibian spinal cord have shown that Rohon–Beard neurones become electrically excitable a substantial time after their final DNA synthesis[30,37]. They are already depolarised by GABA at this time, but it is not known how early chemosensitivity first appears[38]. The development of electrical excitability of the neurites of embryonic amphibian neurones seems to precede the cell body by a small time interval[33]. The identity of the neurotransmitter of Rohon–Beard cells is not known. The known portion of the developmental sequence for these cells is compatible with the sequence for DUM neurones.

Neurotransmitter synthesis is a relatively early phenotype for vertebrate superior cervical ganglion cells[39,40], whereas it is a relatively late phenotype for invertebrate DUM neurones. However, vertebrate ciliary ganglion neurones show a late large increase in neurotransmitter synthetic enzyme activity after their axons have reached and synapsed on their targets[41]. Neurotransmitter synthesis may differentiate earlier in other neurones in grasshopper embryos, since high levels of ACh are already present by day 5, before differentiation of central neurones[24]. Sanes and Hildebrand[42] found that antennal sensory cells in the pupae of the moth *Manduca* were capable of synthesising and storing ACh before the axons arrived at their targets in the central nervous system. If the onset of neurotransmitter synthesis requires particular environmental cues[43,44], then these cues may exert their influence at different points in the lives of these different types of cells. Alternatively, different types of neurones may simply be reading out different intrinsic programmes.

Further work will be needed before any causal relationship in the sequence of phenotypes of the embryonic DUM neurones can be established or ruled out. However, it is worth noting that several events are closely linked temporally. The outgrowth of processes and the onset of chemosensitivity occur at about the same time. Cells become electrically uncoupled and electrically excitable at about the same time. Neurotransmitter storage is detected just after cell processes have reached their targets. Cell body enlargement, Neutral Red staining, the disappearance of extra peripheral neurites, and the expansion of central arborisations occur at this time. It may now be possible to manipulate one phenotype and examine the consequences for the later expression of others.

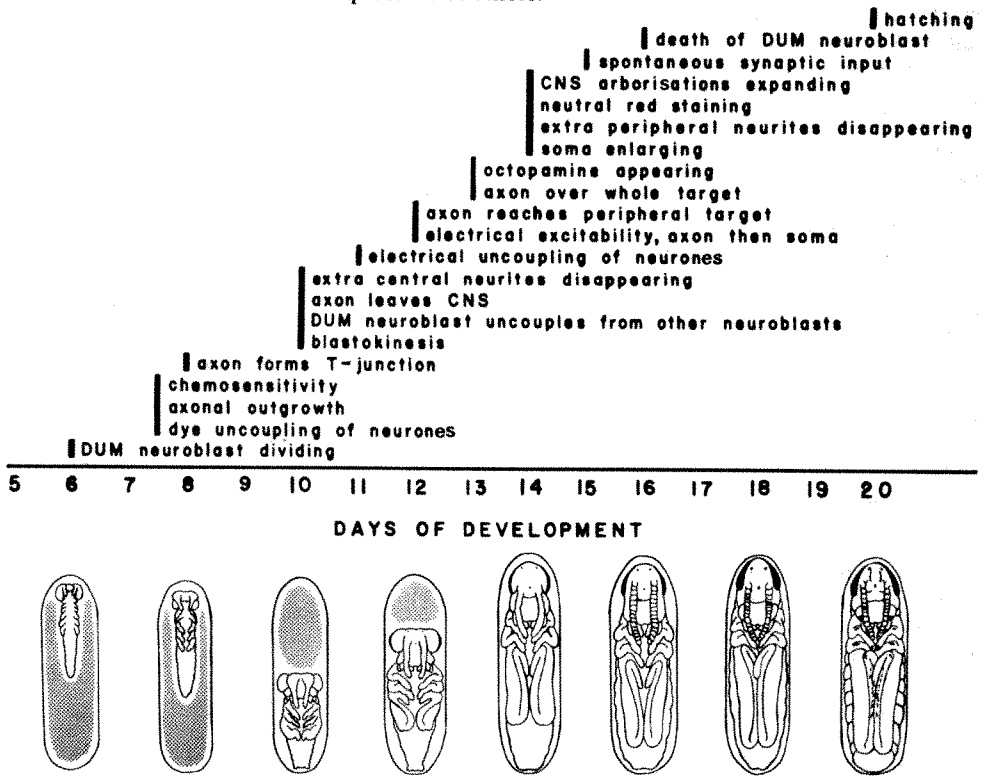


Fig. 6 The developmental timetable for the oldest DUM neurones of the grasshopper embryo *in vivo* [DUM 3, 4, 5; DUM 4, 5; and DUM 5 (DUMETi)]. The egg is laid at day zero, and at 35 °C hatching occurs at day 20. The time of onset or first appearance of a phenotype in these neurones is indicated by a vertical bar. The morphology of the embryo, viewed from the ventral surface in the egg case, is illustrated below as traced from camera lucida projections. Note that the embryo makes a major rotation (blastokinesis) at day 10, and thus from day 10 onward the embryo is viewed from the opposite side of the egg case.

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letters

Accretion on massive black holes in galactic nuclei

STELLAR collisions and/or tidal break-up of stars by a massive black hole[1–3] accompanied by subsequent accretion of the released gas onto the hole play a crucial part in most black hole models of quasars and active galactic nuclei. It is usually assumed that an accretion disk forms around the hole due to the large orbital momentum of a disrupting star. However, we show here that the accretion mostly has disk characteristics only when $M < M_{cr}$ and becomes quasi-spherical when $M \gg M_{cr}$.

The appearance of the critical mass M_{cr} is determined by the fact that the reservoir of the accreting matter is replenished discretely. Moreover, the time scale between two stellar break-ups τ_d (determined by the consumption rate) does not generally coincide with the evolution time scale, τ_{evol}, of the first star remnant. If $\tau_{evol} < \tau_d$, the accretion of this remnant ceases before the next star can be disrupted. Hence an accretion disk will form producing a rather discrete picture in time. Whenever $\tau_{evol} \gg \tau_d$ many other stars will be disrupted during the evolution of the first star's disk debris, producing remnants orientated randomly in space. Collisions between these remnants will lead to the more or less spherical shape of the resulting gas cloud, and its accretion onto the black hole will proceed in this nearly spherical region.

On considering quantitative details we first obtain the evolution time scale τ_{evol} for the disk remnant of a disrupted star (called 'disk' here). After tidal break-up of a first star (which may be accompanied by its thermal flare[4] the gas remnant expands and after one or few revolutions around the hole, that is the time scale $\tau_{form} \approx 3\times10^2(M_h/10^6M_\odot)$ yr forms an elliptical disk with axis depending on both energy and angular momentum of the orbiting star. If they are conserved after the disruption of the star, the minimal and maximum distances of the disk boundary are related by

$$\frac{r_{min}}{r_{max}} = \frac{a(1-e)}{a(1+e)} \approx \frac{1}{2}(1-e) \qquad (1)$$

Here $e \approx 1$ is the eccentricity of the disk; r_{min} is of the order of the tidal radius $R_t = 2.2r_*(M_h/m_*)^{1/3} \approx 1.5\times10^{13}M_6^{1/3}$ cm for a black hole of mass $M_h = 10^6M_6M_\odot$; $r_{max} = (4/3)r_*M_h/m_* \approx 10^{17}M_6$ cm; $m_* \approx 1M_\odot$ and $r_* \approx 1R_\odot$ are the typical stellar mass and radius. Therefore, $1-e^2 = 6\times10^{-4}M_6^{-2/3}$, and the semi-axis of the disk are

$$a = \tfrac{1}{2}r_{max} \approx 5\times10^{16}\mathrm{M}_6 \text{ cm}$$
$$b = a(1-e^2)^{1/2} \approx 1.2\times10^{15}M_6^{2/3} \text{ cm} \qquad (2)$$

Viscous friction causes the disk to accrete gradually on to the hole. This accretion proceeds in the optically thick manner. The viscosity in the disk is determined mainly by turbulent transfer processes, so we may write $\nu = v_t l$, where v_t is the velocity of turbulent motions, and l is the free path length. The value of l is approximately equal to the disk's half-thickness $H = rv_s/v_\varphi$ (v_s is the sound velocity and $v_\varphi = (GM/r)^{1/2}$ is the keplerian velocity). Assuming $v_t \approx v_s$, one obtains $\nu \approx rv_s^2/v_\varphi$ which by a factor ~1 coincides with the Ichimaru's theory of magnetohydrodynamic turbulence in the accreting disk[5]:

$$\nu = \left(\frac{2}{\pi}\right)^{3/2}\left(\frac{kT}{m}\right)^{1/2}H$$
$$H = 1.18r\left(\frac{GM}{r}\right)^{-1/2}\left(\frac{2kT}{m}\right)^{1/2} \qquad (3)$$

(m is the ion mass). The rate of viscous dissipation per unit

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volume is $\varepsilon_\nu = \frac{1}{2}\nu\rho r^2(\partial\Omega/\partial r)^2 \sim \Sigma(kT/m)^{1/2}\Omega^2(\rho, \Sigma = 2H\rho$, and Ω being volume density, surface density, and angular velocity respectively). The maximum rate of viscous dissipation is reached in the outer domain of the disk near the radius

$$R_m = 1.2\times10^{13}M_6^{1/3}\dot{M}_{-6}^{2/3}\ \text{cm} \quad (4)$$

($\dot{M}_{-6} = \dot{M}/10^{-6}M_\odot\ \text{yr}^{-1}$) from when the bound–free and free–free absorption dominate the electron scattering opacity. In this region ($r \geqslant R_m$) the balance between the rate of viscous dissipation and the rate of radiative loss yields the temperature of plasma in the disk

$$T = 4.4\times10^4M_6^{1/4}\dot{M}_{-6}^{3/10}r_{13}^{-3/4}\ \text{K} \quad (5)$$

From equations (3) and (5) we find the viscosity coefficient

$$\nu = 8.5\times10^{15}M_6^{-1/4}\dot{M}_{-6}^{3/10}r_{13}^{3/4}\ \text{cm}^2\ \text{s}^{-1} \quad (6)$$

Knowing the main parameters of the steady state disk (including the dependence of ν on r) enables us to simulate the secular evolution of the viscous disk following the model of Lynden-Bell and Pringle[6]. The mass of the disk diminishes as it accretes on to the black hole according to the law $m = m_i(1 + t/\tau_{evol})^{-2/5}$ where $m_i \approx 1M_\odot$ is the initial disk mass, R_i is its characteristic size, and

$$\tau_{evol} = \frac{32}{75}\frac{R_m^{3/4}R_i^{5/4}}{\nu(R_m)} \approx 5.4\times10^5M_6^{1.84}\ \text{yr} \quad (7)$$

is the evolution time scale. In equation (7) we use $R_i = (ab)^{1/2}$ to account for the elliptical disk form and adopt $\dot{M} = m/\tau_{evol}$ in equation (4). A more approximate, but essentially the same estimate for

$$\tau_{evol} \approx \Sigma/\dot{\Sigma} \approx \Omega\left|\frac{\partial\Omega}{\partial r}\right|^{-1}\frac{r}{\nu}\Bigg|_{r=R_i} \approx 1.8\times10^6M_6^{1.84}\ \text{yr} \quad (8)$$

is obtained from time-dependent equations for disk accretion.

Now, comparing τ_{evol} from equation (7) with the time scale τ_d between successive tidal disruptions of two stars[1]

$$\tau_d \approx 5\times10^2M_6^{-4/3}v_{200}n_7^{-1}\ \text{yr} \quad (9)$$

we obtain for the critical black hole mass

$$M_{cr} \approx 1.1\times10^5n_7^{-0.32}v_{200}^{0.32}M_\odot \quad (10)$$

For conditions similar to those in the nucleus of our Galaxy (star density $n \approx 10^7\ \text{pc}^{-3}$, velocity dispersion $v = 200\ \text{km s}^{-1}$) we find $M_{cr} \sim 10^5M_\odot$, and for a dense globular cluster ($n = 5\times10^4\ \text{pc}^{-3}$, $v = 20\ \text{km s}^{-1}$)$M_{cr} \sim 3\times10^5M_\odot$ is obtained.

At $M < M_{cr}$ the disk evolves faster than the next star enters the Roche lobe of the black hole. Therefore when the black hole mass is small enough, the accretion takes place in the disk regime and ceases before the next star breaks up. As follows from equation (7), at $t \leqslant \tau_{evol}$ the accretion rate exceeds Eddington critical rate $\dot{M}_{Edd} \approx 2\times10^{-2}M_6$ ($M_\odot\ \text{yr}^{-1}$) if the black hole mass $M_h < 4\times10^4M_\odot$. For smaller masses the fact that the accretion rate $\dot{M}$ is proportional to $t^{-7/5}$ is very critical during the time interval $t_s \approx 7M_4^{2.03}$ $\tau_{evol} = 0.8\times10^3M_4^{-0.19}$ yr. However, the accretion keeps its disk character as the luminosity of the hole $L \leqslant (0.6-1.0)L_{Edd}$ (ref. 7). Mass outflow from the disk is possible during t_s, but the value of t_s is negligible compared with τ_d (equation (9)) if $M_4 \leqslant 1$. As for the smaller black hole masses, we believe that at $M_h < 10^4M_\odot$ the tidal disruption of stars is not the main fuel for black holes in galactic nuclei.

When $M \approx M_{cr}$, the characteristic time scales τ_{evol} and τ_d are of the same order of magnitude. In this case and especially when $M > M_{cr}$, star–disk collisions shorten the time scale τ_d. The accumulation of star remnants around the black hole leads to the spherisation of the resulting cloud and to the subsequent quasi-spherical accretion. So when $M \gg M_{cr}$, we may assume that the accretion will be quasi-steady with the rate equal to that of stellar consumptions, analogous to the case of accretion on to a compact object in close binary systems where the accretion rate is controlled by matter outflow rate from the normal star.

We have assumed, in accordance with most models, that the accreting gas is fuelled by tidally disrupted stars. However, there are three other possible sources of this gas: (1) gas loss by stars during their evolution; (2) star collisions; (3) accretion of intergalactic gas.

As for the source (1), the present rate of gas loss by stars in the galactic nuclei is of the order of $M^{-1}\dot{M} \sim 10^{-12}-10^{-11}\ \text{yr}^{-1}$ (refs 8, 9). This gas probably has a large angular momentum and forms a disk which supplies the black hole with gas at a rate less than 10^{-4}–$10^{-5}M_\odot\ \text{yr}^{-1}$ when the total mass of stars in the nucleus is 10^7–$10^8M_\odot$. The real accretion rate seems to be much smaller as part of gas transforms into young stars, and another part is expelled from the nucleus by supernovae explosions. (As an example, the nucleus of our Galaxy shows evidence both of increased number of young stars and of supernova remnants.) It is easily seen that at $M_h \ll 10^4$–$10^5M_\odot$, gas lost by stars may become the main fuel for the black hole, but the accretion remains in a disk regime.

In contrast, when the black hole mass is large enough ($M_h > 2\times10^7n_7^{1/2}r_c^{3/4}M_\odot$, the core radius, r_c, (pc)) the breakup of stars by collisions begins to dominate the tidal disruptions. In this case the evolution time for a disk formed (which may be estimated analogous to equation (7)) seems to be much larger than the time interval between successive collisions, so that the accretion remains quasi-spherical.

Only when the main source of the accreting gas is the flow of intergalactic gas which has a very large angular momentum relative to the galactic centre, is the accretion expected to have a disk character irrespective of the black hole mass. However, the intergalactic reservoir of gas may, in principle, be distinguished from the intragalactic reservoir as in nuclei of galaxies belonging to a close pair or group, the activity is expected to be simultaneous (coincidence scheme)[10]. It is possible that some point radio sources in the nuclei of elliptical and spiral galaxies which are members of the same groups or pairs[11,12] show evidence for the accretion of intergalactic gas or flow of outlying clouds.

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Observation of large 2.2 μm polarisation in 3C345

INFRARED polarisation observations of the quasars 3C345 and 3C273 are reported here. The highly polarised and variable optical emission of BL Lacertae objects is correlated with similar high polarisation and variability in the IR, and is clearly the product of non-thermal processes. IR polarisation by dust, or by a combination of dust and non-thermal processes, is also found in Seyfert galaxies[1–5]. These observations suggest that the measurement of IR polarisation in quasars be used to investigate the often dominant IR emission in these sources. However, so far only one measurement of polarisation at wavelengths as long as 2.2 μm in a quasar, 3C273, has been reported[4].

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A clear distinction exists between the variable, steep slope QSOs which often show strong and variable optical polarisation, and the less variable QSOs with lower spectral index which do not[6,7]. The former resemble the BL Lac objects in the steepness of their optical spectra and in their optical polarisation. 3C345 is such a quasar with variable visual and IR flux[8] and highly variable visual polarisation[9,10]. 3C273 may vary somewhat on a long time scale, but this slow (if present at all) variation and the small optical and IR polarisation suggest that 3C273 is a member of the latter group. As these two quasars seem to show different phenomena, we have observed them in order to compare their IR polarisation.

Linear polarisation at 2.2 μm was measured with a chopper-coupled polarimeter (to be described elsewhere[11]) at the Kitt Peak National Observatory 4-m telescope. The sensitivity to polarisation is a function of both the intensity and polarisation of the sources; as a rough guide, in one hour of integration time 1% polarisation can be measured on a source with K magnitude of 11 at the 4-m telescope. Instrumental polarisations were measured to be 0.42% (J, 1.25 μm), 0.38% (H, 1.6 μm) and 0.12% (K, 2.2 μm). The data were corrected for this effect by subtraction of the appropriate Stokes parameters. A further correction was made for the polariser efficiency determined by measurement of the transmission of crossed polarisers.

The position angle was calibrated by observing the polarisation of VI Cyg 12 and computing the difference between the measured angle, as determined by the arbitrarily positioned polariser, and the angle at K of $\theta = 113°$ determined by Dyck and Jones[12]. The K polarisation that we found for VI Cyg 12, $P_K = 1.39 \pm 0.04\%$, is in reasonable agreement with the Dyck and Jones measurement of $P_K = 1.22 \pm 0.12\%$.

We first observed 3C345 on 5 May 1977 (Table 1). Very large 2.2 μm polarisation of $20.6 \pm 7.2\%$ was noticed at that time, but because the signal-to-noise ratio did not give a completely convincing result, we felt that the measurement required corroboration. This became possible in April 1978 (Table 1), and this time 3C345 was observed on three nights. The 2.2 μm polarisation was again very large. The magnitudes of the polarisations are close to those found in the previous year and the orientations also seems similar. However, this agreement may well be coincidental as the IR polarisation may be variable as is the visual. Further observations are required to establish IR variability.

Remarkably, the IR polarisation of 3C345 is much greater than the visual. The visual measurement that was made on 25 April (at the same time as the two IR measurements of that night) was noisy because of sky background but established an upper limit to the B (0.44 μm) polarisation of 6%. The measurements of 26 and 27 April are consistent with this result. They also show that despite being much smaller in magnitude, the visual polarisation had approximately the same orientation as the IR on the previous nights.

In extragalactic sources in which polarisation has been measured, the IR polarisation is either equal to (for example, BL Lac)[1] or less than (for example, MK421, NGC4151)[2] the visual polarisation. High IR polarisation accompanied by much smaller visual polarisation is a as yet a phenomenon unique to 3C345 amongst extragalactic objects.

Table 1 Polarisation measurements of 3C345 and 3C273

Object	Date	λ (μm)	$P \pm \delta P$	$\theta \pm \delta\theta$
3C345	5 May 1977	2.2	20.6 ± 7.2	32 ± 10
	23 April 1978	2.2	23.8 ± 6.4	67.0 ± 7.5
	24 April 1978	2.2	21.9 ± 4.2	65.4 ± 5.5
	25 April 1978	0.44 (B)	< 6	—
		1.6	9.7 ± 6.1	—
		2.2	32.6 ± 6.5	65.0 ± 5.6
	26 April 1978	0.44 (B)	4.5 ± 0.7	68 ± 9
	27 April 1978	0.44 (B)	2.2 ± 0.7	43 ± 19
3C273	5 May 1977	2.2	0.07 ± 0.23	—

We have observed 3C273 intermittently since 1975, and observed it again in April 1978. In April 1978 we observed the source with the new instrument and obtained a much lower limit on the polarisation than in our earlier studies. The formal measurement (Table 1) of $P_K = 0.07 \pm 0.23\%$ corresponds to an upper limit (2σ) of 0.46%. This is consistent with the possible detection of $P_K = 0.33 \pm 0.14\%$ of Kemp *et al.*[4]. Thus 3C273 does not seem to be an object with active IR polarisation.

Optical polarisation of 3C345 has been studied for more than a decade. These data and the radio polarisation have been reviewed by Kinman[10]. Between 1967 and 1974 the optical polarisation varied between 1% and 12% and was accompanied by position angle fluctuations of 20°–110°. Kinman suggests that the position angle shows some regularity, lying between 80° and 110° during 'outbursts' of high flux, and this direction may be related to the position angle of super-relativistic expansion, $105° \pm 5°$. Stockman *et al.* have also suggested an alignment of the optical and radio components[13]. During our observations, however, the apparently parallel visual and IR polarisation directions were not aligned with the expansion direction.

The spectrum and variability of 3C345, and now the polarisation, suggest that the IR emission is non-thermal. Polarisation by scattering off dust seems unlikely to give the observed wavelength dependence. It would probably be difficult for any scattering mechanism to account for the large IR polarisation unless there is very great asymmetry. The synchrotron mechanism can give large visual and IR polarisation, although the peculiar wavelength dependence remains to be explained.

Any explanation for the wavelength dependence of the IR polarisation based on Faraday rotation is unlikely for several reasons. First, in optically thick sources, the polarisation tends to decrease towards longer wavelengths, opposite to the sense found in 3C345 (ref. 14). Second, in this mechanism the 2.2 μm and visual polarisation parallelism would be unaccounted for. And third, Faraday rotation at IR wavelengths would require larger fields and densities than are generally encountered in similar sources.

It may be more plausible to look for a dilution mechanism because the approximate parallelism of the IR and visual polarisations suggest that the IR and optical sources are related. However, spectra of 3C345 taken by Wampler[15] do not show emission lines in the B passband strong enough to dilute continuum polarisation. Wampler also showed that the absence of a Balmer break indicates that thermal continuum emission is unlikely to be strong in 3C345.

An explanation may lie in the models of optical polarisation of Kinman *et al.*[9]. They suggest that three sources contribute to the polarisation. Two of these, one a slowly and one a rapidly varying component, are assumed to be polarised by approximately 17%, but in unrelated directions. Then as intensity variations occur, the sum of the polarisations combines to give the observed 2–11% variations. The IR polarisation that we observed is close to Kinman *et al.*'s value of 17% for the components. The observed wavelength dependence of polarisation would result if, for example, the slowly varying component extends from the visual to the IR, and the rapidly varying component, perhaps with flatter slope, interferes with the visual, but not the IR polarisation. Some support for a multicomponent model is found from the observed dual component structure in the radio. The agreement of the two measurements separated by a year implies that the IR polarised component is only slowly varying.

The high degree of IR polarisation places 3C345 among the 'violent variables' shown by Nordsieck[16] to occupy the upper right portion of a plot of polarisation against spectral index. The polarisation requires a high degree of order in the magnetic field. From Nordsieck's equations we find his parameter δ describing the 'squash' of the magnetic field (where $P(H, \chi) = P_I[H_x^2/(1+\delta^2) + H_y^2/(1-\delta^2)]^{1/2}$ is the probability that an element of volume has a field H perpendicular to the line of sight in direction χ) is $\delta \approx 0.5$ (with spectral index 1.2). Similarly the ratio of a directed field to an isotropic field (Nordsieck's case IIa)

is $\varepsilon/H \approx 0.4$. Evidently considerable long-range order exists in the source.

The detection of strong IR polarisation in a quasistellar source presents another point of similarity between quasars and BL Lac objects. IR polarisation measurements can evidently provide insights into the structure and emission mechanisms of quasi-stellar objects, especially the optically active ones which often also have steep slopes. IR and visual polarisation activity seems to be correlated in quasars as it is in BL Lac objects. However, as an example with greater polarisation in the IR than in the visible has now been found, it may be useful not to preselect these objects by optical polarisation criteria alone; non-thermal sources that may be partially obscured by other visual emission may appear in the IR.

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High-pressure $(Mg, Fe)_2SiO_4$ phases in the Tenham chondritic meteorite

THE high-pressure phase transformations of olivine $(Mg, Fe)_2SiO_4$ are of fundamental importance to the dynamics of the mantle[1–3]. Experiments[4] have demonstrated that at high pressure olivines transform to a solid solution series of (Mg, Fe) spinel (γ phase), while in olivines with Mg/Mg+Fe ratios greater than 0.85, 'modified' spinels with an orthorhombic structure (β phase) are formed[5]. The β phase in pure Mg_2SiO_4 is considered to have a stability field at pressures intermediate between those of olivine and the γ spinel phase, although the possibility that it forms as a metastable quench product from γ spinel has not been entirely ruled out. A central issue is the mechanism and hence the kinetics of these transformations but no such experimental work has been carried out. Details of the mechanisms involved may be inferred from experimental results, but available natural samples of high-pressure $(Mg, Fe)_2SiO_4$ polymorphs are confined to shock-produced

Table 1 Electron microprobe analysis of purple ringwoodite and olivine from the Tenham chondrite

	Ringwoodite (wt %)	Olivine (wt %)
Na_2O	0.00	0.00
MgO	39.24	39.73
Al_2O_3	0.00	0.00
SiO_2	38.66	38.67
K_2O	0.00	0.00
CaO	0.00	0.00
TiO_2	0.00	0.00
Cr_2O_3	0.00	0.00
MnO	0.24	0.47
FeO	22.75	22.83
NiO	0.00	0.00
Total	100.89	101.70
Formula	$Mg_{1.51}Fe_{0.49}Si_{1.0}O_4$	$Mg_{1.52}Fe_{0.49}Si_{1.0}O_4$

Analyses by S. O. Agrell.

phases in chondritic meteorites (for example, Tenham, Coorara, Catherwood and Coolamon[6–10]). Binns *et al.*[6] first reported the X-ray diffraction evidence for the existence of natural γ spinel in the Tenham chondrite where it occurs as small ($\approx 100\ \mu m$), purple grains within shock-produced veins. The natural γ spinel is termed ringwoodite. Shock-wave experiments by Jeanloz and Ahrens[11] have failed to produce a spinel phase, and recently Jeanloz[12] has claimed, from X-ray powder diffraction, IR and optical data that ringwoodite is a complex aggregate of goethite, majorite garnet and γ iron, mis-identified as the spinel phase by previous workers. Here we present definitive evidence from electron-probe microanalysis, transmission electron microscopy and diffraction on the true nature of the purple grains (termed ringwoodite) in the Tenham chondrite.

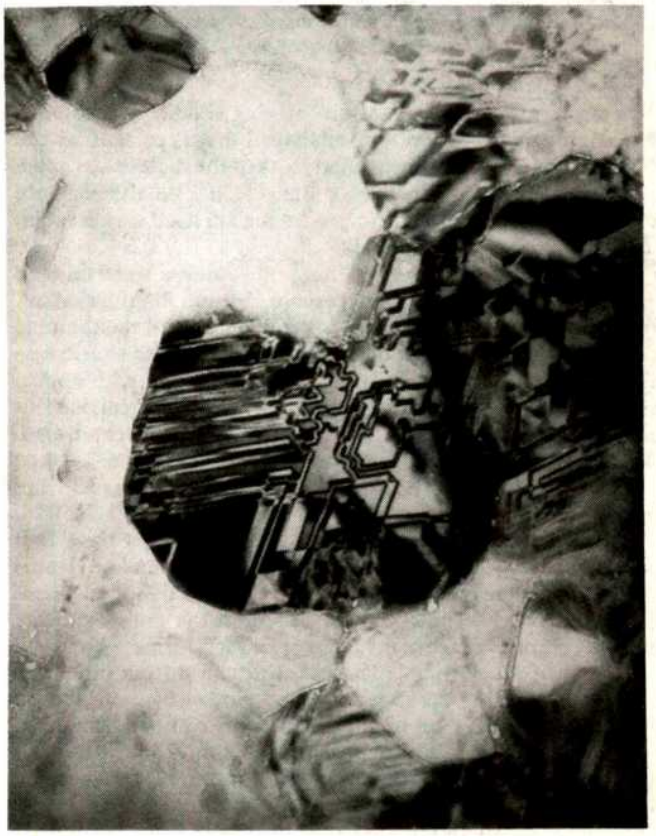


Fig. 1 Transmission electron micrograph of one crystal from a polycrystalline intergrowth formed by the transformation of olivine to γ spinel. The antiphase and twin boundaries within the crystal are due to the inversion of γ spinel to the β 'modified' spinel phase. Other crystals in this section are tilted out of contrast (magnification, ×140,800).

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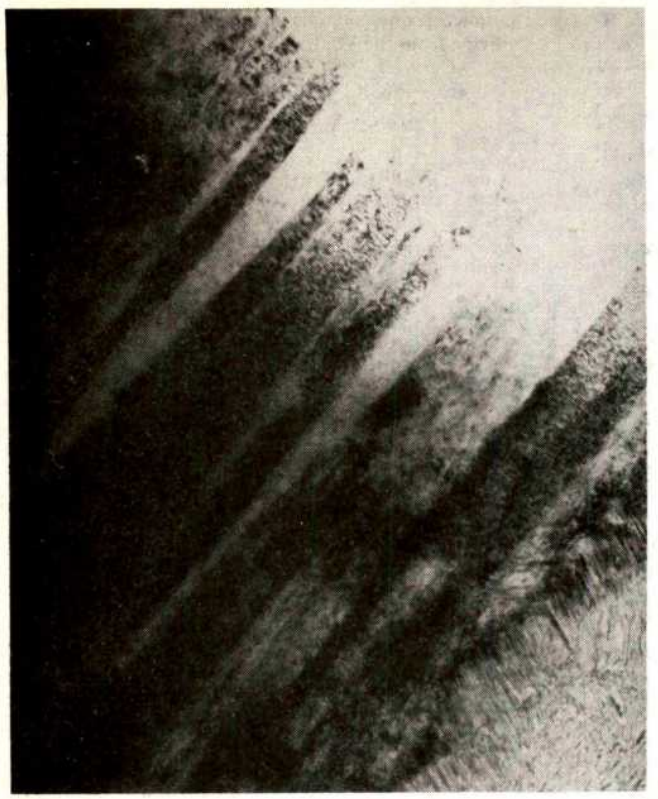


Fig. 2. Transmission electron micrograph showing the second type of morphology produced by the olivine to γ spinel transformation. The twin-related plates lie on {111} planes of the γ spinel structure. Subsequent inversion to the β 'modified' spinel phase has produced the very fine antiphase domains within the plates (magnification ×60,200).

Electron microprobe analysis of ringwoodite indicates a composition identical, within analytical error, to that of the olivine in the relatively unshocked parts of the meteorite (Table 1). This agrees with the results of Binns *et al.*[6]. Analyses do not vary significantly from grain to grain which in itself suggests that a complex aggregate is unlikely.

Optically thin sections containing ringwoodite were thinned for transmission electron microscopy by ion bombardment. Observations were recorded after various stages of the thinning process and a complete traverse made through the shock vein into the ringwoodite. Correlation of thinned areas with optical micrographs ensured that TEM observations were carried out on the purple grains where the composition has been confirmed. Electron petrographical observations of the microstructure and phase assemblages of the shock vein will be reported in full elsewhere.

Transmission electron microscopy and diffraction show that the purple ringwoodite consists of a polycrystalline spinel aggregate partially inverted to the β 'modified' spinel phase (Figs 1 and 2). The microstructures within the grains indicate that the β phase is an inversion product formed by rapid quenching of the γ spinel. The transformation from olivine to the original γ spinel has apparently taken place by two different mechanisms leading to spinel grains with distinctly different morphologies: first, as equant subregular intergrown crystals (grain size $\approx$1,000 Å) (Fig. 1) and second as twin-related parallel plates on (111) planes of the spinel structure (Fig. 2). The former morphology indicates an independent nucleation and growth mechanism while the latter suggests a cooperative shear mechanism with some of the characteristics of a martensitic transformation. As the transformation from olivine to spinel involves a change in the close packing of oxygen atoms, the mechanism may be dependent on the orientation of the original olivine crystal with respect to the shock-wave front. No previous observations of possible mechanisms have been made, although Sung and Burns[3,13] have emphasised nucleation and growth in attributing deep-focus earthquake genesis to this transformation. It is questionable whether the possibility of a martensitic mechanism would enable the necessary overpressures to be generated for an implosive transformation.

The subsequent inversion of γ spinel to the orthorhombic β 'modified' spinel phase on quenching requires no change in the oxygen packing and involves a redistribution of the cations[5,14]. Thus the original γ spinel grain boundaries and the twin-related oxygen packing distribution (Fig. 2) are preserved. The relationship between the two structures introduces a very large number of translational and orientational variants leading to a complex anti-phase and twin domain structure within the grains (Figs 1 and 2). Electron diffraction patterns show splitting of the main spinel reflections due to the formation of twin domains of lower symmetry. Weak superlattice reflections are also observed which are consistent with the formation of the $a \times \sqrt{2}a \times a/\sqrt{2}$ body-centred orthorhombic cell of the β phase (where $a \approx$ 8.2 Å, the lattice parameter of the γ spinel). The fine scale of the microstructure within the grains indicates that the nucleation of the β phase has occurred during a relatively rapid quench. There is no evidence in the microstructures or the diffraction patterns to suggest that the γ spinel phase was formed from olivine through any intermediate stage. The similarity between the β phase and γ spinel results in very similar X-ray powder patterns, that is predominantly that of spinel, with some extra lines. IR spectra will also indicate some similarities to spinel although with a significantly lower structural symmetry. Jeanloz' recent X ray and IR data[12] are consistent with our observations that the purple grains are a γ spinel–β phase intergrowth.

The quench texture of the β phase again raises the question of its thermodynamic stability. Direct experimental evidence can be obtained by either studying the transformation *in situ* at high pressures, or more easily by observing the microstructures preserved in quenched experiments. Such TEM studies have yet to be carried out. Note, however, that the γ–β transformation has many of the characteristics of the formation of metastable transitional phases in non-equilibrium processes. A direct transformation from γ spinel back to olivine on pressure and temperature reduction involves a major structural change with a large activation energy. Metastable phases formed as kinetically more favourable alternatives are invariably modifications of the parent phase and involve minor structural reorganisation and a reduction in translational symmetry[15]. The β phase has these characteristics.

The purple grains in the Tenham chondrite described here represent the first reported natural occurrence of β-$(Mg, Fe)_2SiO_4$. Following Ringwood and Major[4], we suggest that this β phase in nature be termed Wadsleyite.

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New phyllosilicate types in a carbonaceous chondrite matrix

CARBONACEOUS chondrites provide valuable information as they are the least altered examples of early Solar System material[1]. The matrix constitutes a major proportion of carbonaceous chondrites. Despite many past attempts, unambiguous identification of the minerals in the matrix has not been totally successful[2]. This is mainly due to the extremely fine-grained nature of the matrix phases. Recently, progress in the characterisation of these phases has been made by electron diffraction studies[3,4]. We present here the direct observation, by high resolution imaging, of phases in carbonaceous chondrite matrices. We used ion-thinned sections from the Murchison C2(M) meteorite for transmission electron microscopy. The Murchison matrix contains both ordered and disordered intergrowths of serpentine-like and brucite-like layers. Such mixed-layer structures are new types of layer silicates.

For an unknown mineral, electron diffraction patterns from a number of principal crystallographic zones are a sufficient (although not necessary) means of identification. However, selected area diffraction patterns of phyllosilicates from the Murchison matrix are of poor quality because of the small sizes of individual grains. Good images, on the other hand, can be obtained. Interpretation of these images is based on previous investigations of Murchison[5], and on images from related high-resolution transmission electron microscopy (HRTEM) studies of terrestrial phyllosilicates[6–9]. Phyllosilicate structure types may be identified by their characteristic (001) basal spacings when viewed parallel to their sheets[10]. For example, serpentines show a basal spacing of 7.3 Å, while micas and talcs have a layer repeat of 10 Å (ref. 11).

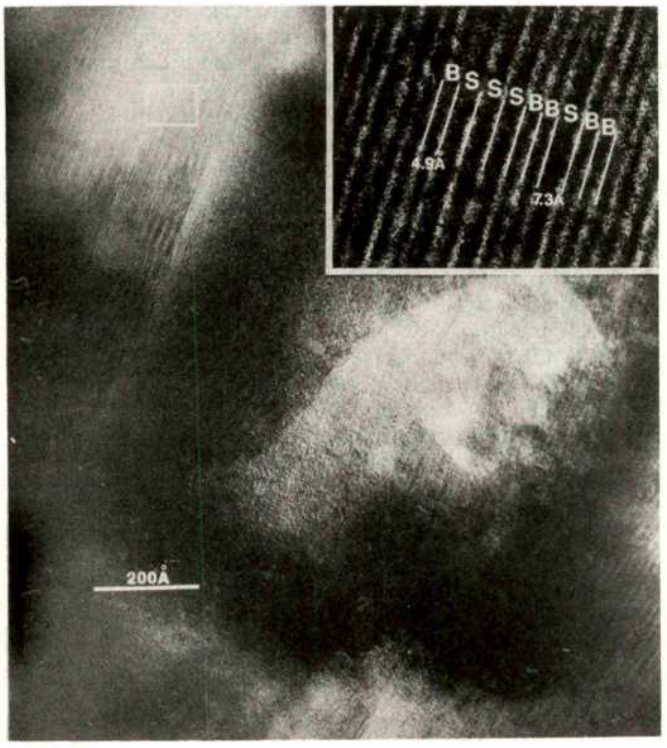


Fig. 1 A HRTEM image showing basal fringes in several mixed layer silicate crystals within the matrix of the Murchison carbonaceous chondrite. The inset is an enlargement of a portion of the same area which reveals a disordered arrangement of serpentine- (S) and brucite-type (B) layers.

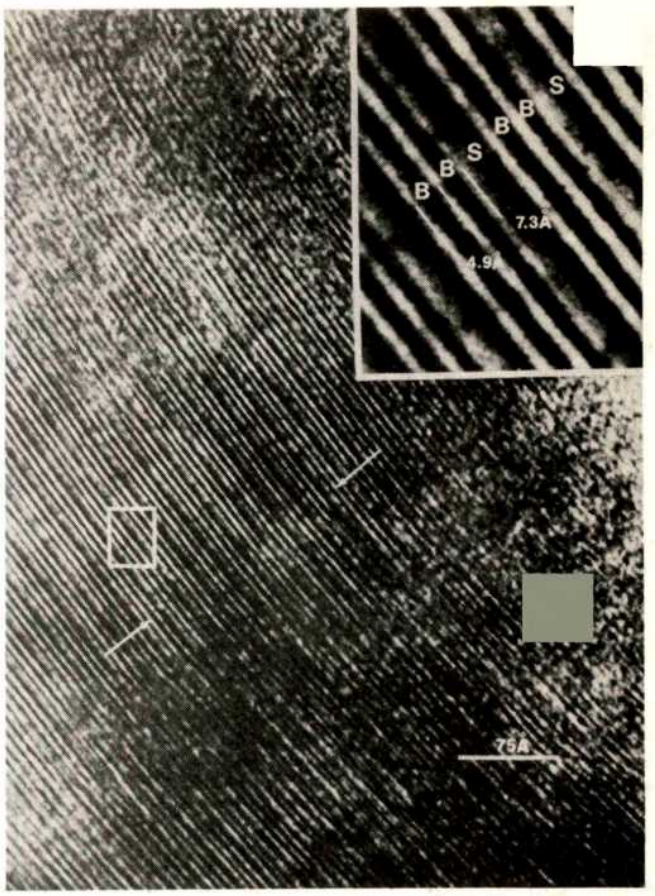


Fig. 2 A HRTEM image of layer silicate ordering in the Murchison matrix. An ordered sequence of 10 SBB units is indicated between the arrows. The inset is an enlargement of the sequence shown in the white rectangle.

The matrix material in Murchison consists of amorphous material[17], and crystalline regions ranging in width from a few thousand to less than one hundred Ångströms. Discrete crystals occur surrounded by amorphous material; crystalline material also occurs as intergrowths in sub-parallel orientations, or as apparently aggregated, but randomly orientated groups of crystals. A considerable fraction (~50%) of the crystalline phases are phyllosilicates.

The extremely small grain size and character of the matrix crystals have also been confirmed by selected area microdiffraction using high-resolution scanning transmission electron microscopy (STEM). (Using a 20-μm limiting aperture in a STEM (Vacuum Generators HB5) equipped with a field-ion emission gun, the effective beam size is 10 Å. Selected area microdiffraction patterns of many phyllosilicate orientations were obtained with beam movement of <100 Å across the Murchison matrix.) Interpretation of microdiffraction patterns having periodicities of ~10 Å or greater is uncertain (J. M. Cowley, personal communication), but is sufficient to confirm the observations obtained by HRTEM imaging.

Figure 1 is a HRTEM photograph of part of the Murchison C2 carbonaceous chondrite showing typical phyllosilicate basal fringes in several crystals that are orientated in sub-parallel directions. The inset is a magnified portion of the same area, showing a disordered arrangement of 7.3 and 4.9 Å fringes representing layers of different types. (Fringe spacings are measured with a relative error of ~10%.) Fringe spacings of ~10 Å have also been observed in other portions of the Murchison matrix[12].

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Basal fringe spacings of 7.3 Å are typical of minerals from the serpentine group. This group contains minerals ranging in composition from the pure Mg rich end member, serpentine, to the Fe, Mg, Al-silicates of the septechlorite series[11]. Here we refer to the 7.3 Å fringes as representing serpentine layers in order to imply a structural type, rather than a compositional variety. The sheet hydroxides brucite, $Mg(OH)_2$, and gibbsite, $Al(OH)_3$, have basal spacings of 4.9 Å (ref. 11). However, the low total aluminium content of the Murchison matrix[5] excludes gibbsite as a likely species in these layered intergrowths. Interpretation of the images as resulting from alternating layers of serpentine (S) and brucite (B) is consistent with compositional data[5] and the morphology of phyllosilicates at high resolution[6-10]. Serpentine–brucite associations are also known to occur in terrestrial rocks[13] and are consistent with the optical and X-ray studies of Fuchs *et al.*[5].

As far as we know coherently intergrown serpentine- and brucite-type layers have not been described previously. Our observations of such intergrowths are a consequence of improvements in instrumental resolution and techniques as applied to meteoritic phyllosilicates. Assuming that our interpretation of the phyllosilicate phases is correct, it is possible to define several types of intergrowths. For example, we have observed (1) disordered sequences of S and B (Fig. 1), and (2) ordered sequences of ...SBBSBB... (Fig. 2). The origin and stability ranges, if any, of such new types of intergrowths are yet to be determined. Such information may prove to be genetically significant and potentially can provide information about the origins of carbonaceous chondrite matrices. Future experimental studies could well be directed towards establishing pressure–temperature stability ranges for such mixed layer assemblages.

A remarkable feature of the phases shown in Fig. 2 is the nature and extent of the phyllosilicate ordering. Repeats of serpentine and brucite occur continuously for more than 300 Å (...SBBSBB...). Following the procedures of Veblen and Buseck[14], calculations for ordered SBB sequences in the Murchison matrix indicate that 20 repeating units have a probability of 1.57×10^{-15} of occurring randomly.

Experimental work on the serpentine system[15] provides a limited, but, nevertheless, useful guide to the conditions of phyllosilicate formation in the Murchison matrix. Intimate mixtures of brucite-like and serpentine-like layers and their cylindrical morphology[6,10] suggest that the 7.3 Å fringes arise from a Mg-rich serpentine such as chrysotile. An excess of Mg and relatively low silica activity at the time of matrix formation could possibly promote the growth of brucite layers. Alternatively, moderate silica activity and slightly lower temperatures might encourage the growth of brucite and iron-rich serpentines (septechlorites).

The data of Hemley *et al.*[15] suggest that chrysotile and brucite can exist stably between 250 and 375 °C at 1 kbar water pressure. The effects of Fe and Al in the serpentine system are competitive: Fe reduces the temperature range slightly, while the upper stability limit for serpentine is raised by the presence of Al[13]. Specific compositional data are not yet available for the Murchison serpentine–brucite layers. However, indications of compositional variations from Mg-rich serpentine have been obtained in other C2 carbonaceous chondrites[16] and suggest that Fe and Al occur in the layered intergrowths.

A more pervasive influence on the serpentine–brucite stability range may be pressure. The effect of pressure on serpentine stability is not well known. However, at the pressures assumed for the presolar nebula (10^{-2}–10^{-3} atmospheres[5]), the chrysotile–brucite stability field would occur at considerably lower temperatures. Thus, 250–375 °C is a probable upper temperature limit for some of the phyllosilicates in the Murchison matrix.

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An experimental measurement of cation diffusion in almandine garnet

COMPOSITIONAL zoning in minerals is a disequilibrium phenomenon which is in principle potentially useful for interpreting the thermal history of the host rock. Almandine-rich garnets from low and medium grade regionally metamorphosed rocks are commonly zoned[1-8]. The manganese content typically varies from core to rim, being high in the centre and low at the rim, producing a bell-shaped profile, whereas Fe is antipathetic to Mn. With the exception of the diffusion models of Anderson and Buckley[9], such zoning has generally been explained in terms of fractionation-depletion processes[2-4] where diffusion in the growing mineral is assumed to be negligible. However, in garnets from high grade rocks[8,10,11], zoning is usually weak, absent or reversed, and recent authors[8,12,13] have favoured the homogenisation of pre-existing or developing zoning by volume diffusion. To evaluate the importance of cation diffusion to garnet zoning, Fe–Mn interdiffusion coefficients have been measured over a restricted temperature range.

Interdiffusion experiments were performed between almandine-rich garnet (100–400 μm in diameter) of approximate composition $(Mg_{0.93}Fe^{2+}_{2.07})Al_2Si_3O_{12}$ and a sinter of $Mn_3Al_2Si_3O_{12}$. Pseudobinary couples comprising garnet and sinter (1:5 weight ratio) were packed in iron crucibles, and sealed in alumina tubes with alumina cement. One atmosphere diffusion runs were performed at temperatures of 822, 915, 934 and 1,002 °C for periods for 300–501 h.

Several garnets of approximately the same size were recovered from each experiment and mounted together for analysis. Only light grinding for a few seconds on 600 grade SiC paper was necessary to reach the centres of the crystals. Provided that the interdiffusion region does not account for more

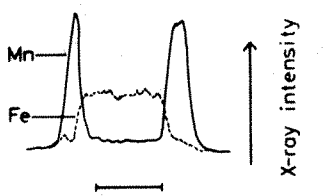


Fig. 1 Fe–Mn interdiffusion in almandine garnet (specimen 117: 915 °C, 501 h) variation of Fe and Mn X-ray intensity along traverse through the centre of a euhedral crystal. Scale bar, 80 μm.

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than 20% of the garnet radius (r), the measured diffusion profile will not be significantly distorted if the exposed face is not more than $0.3r$ from the true centre. Samples were polished with diamond paste, coated with carbon, and examined by Cambridge Instruments Geoscan Microprobe.

The interdiffusion regions were analysed with a 'stepping interval' of 2 μm. Operating and data correction procedures are described in detail elsewhere[14]. As it was not possible to identify how close the exposed face was to the crystal centre, errors from misalignment were minimised by selecting only the largest crystals with the smallest interdiffusion regions (always less than $0.3r$). Interdiffusion coefficients ($\tilde{D}$) were calculated by the Boltzmann–Matano method[15], and in common with other systems where only one member of the diffusion couple is available for analysis[16], the Matano interface was assumed to coincide with the crystal rim. The maximum error in $\tilde{D}$ resulting from misalignment is thought to be ±15%, and this has been incorporated in the final uncertainty of the data.

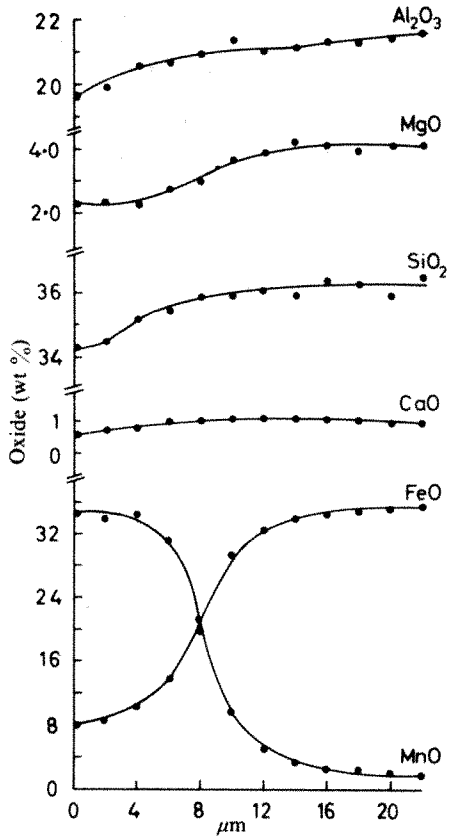


Fig. 2 Typical profiles of Al, Mg, Si, Ca, Fe and Mn in the interdiffusion region of garnet specimen 117 (915 °C, 501 h).

Although the experiments were performed outside the stability field of the garnet, there was no sign of breakdown, or subsequent surface growth. Measurable diffusion profiles were obtained at temperatures above 900 °C. The interdiffusing elements (Fe and Mn) exhibit complementary profiles (Fig. 1) and a detailed analysis is presented in Fig. 2. The shape of the Fe and Mn composition profiles warrants some comment. The flattening of the curves near the interface is probably due to the use of a sinter[16,17] rather than single crystal spessartine. The intersection of the Fe and Mn profiles some 8 μm inside the crystal is of no significance as the cross-over point should not necessarily coincide with either the Matano interface or the original interface[18,19]. It is possible that some interdiffusion of Mn–Mg and Mn–Ca has occurred but the effect on the location of the Matano interface is considered negligible. To a good approximation, the description of the system as an Fe–Mn pseudobinary is valid.

Results for $\tilde{D}$ (Fe–Mn), based on a minimum of eight crystals, are shown in Fig. 3. The composition-dependence of the interdiffusion coefficients may be described by

$$\tilde{D} = D^*_{Mn} \exp(\beta X_{Mn}) \quad (1)$$

where D^*_{Mn} is the tracer diffusion coefficient of Mn in Mn-free garnet, X_{Mn} the content of Mn in wt% (to a maximum of 33.1 wt% for the complete replacement of Fe by Mn) and β the composition factor. Over the temperature range 915–1,002 °C, D^*_{Mn} increased from 3.7×10^{-18} m^2 s^{-1} to 6.6×10^{-18} m^2 s^{-1}, whilst $\beta = 0.09\pm0.01$.

The temperature-dependence of the interdiffusion process may be obtained from an Arrhenius plot of the form

$$\tilde{D}_{Mn} = D_0 \exp(-Q/RT) \quad (2)$$

where $\tilde{D}_{Mn}$ is the interdiffusion coefficient at constant composition, D_0 the pre-exponential factor, and Q the activation energy (in kJ mol^{-1}). Results at 10 and 15 wt% Mn may be described by $\tilde{D}_{10} = 1.07\times10^{-13}\exp[-94(\pm16)\,\text{kJ}/RT]$ m^2 s^{-1}, and $\tilde{D}_{15} = 2.01\times10^{-14}\exp[-72(\pm25)\,\text{kJ}/RT]$ m^2 s^{-1} respectively.

There is good agreement between the present $\tilde{D}$(Fe–Mn) results and data from a recent petrological study. Hensen[20] examined the reaction of garnet (of composition Mg/(Mg+Fe^{2+}) ≃ 0.28) and cordierite at 1,000 °C and 10 kbar, and found that some garnet grains did not homogenise in 305 h. With grains of 10–40 μm diameter, diffusion distances of 5–20 μm would be required to produce uniform compositions. Assuming a diffusion distance, x, of 15(±5) μm, in a time, t, of 305 h, then in this case $\tilde{D}$(Fe–Mg) may be estimated from an approximate solution of the diffusion equations[15]

$$x^2 \simeq KDt \quad (3)$$

where $K \simeq 4$ and is a constant[21]. The calculated interdiffusion coefficient of $5(\pm3)\times10^{-17}$ m^2 s^{-1} compares well with the value of 2.3×10^{-17} m^2 s^{-1} obtained here for $\tilde{D}$(Fe–Mn) at 15 wt% Mn. By defining $\tilde{D}$[Fe–(Mn, Mg)] as the interdiffusion coefficient of Fe with either Mn or Mg, an estimate for this parameter may be obtained by combining the present data for $\tilde{D}$(Fe–Mn) and the result deduced from Hensen for $\tilde{D}$(Fe–Mg). At compositions of ~13 wt% (Mn, Mg) in silicate garnets $\tilde{D}$[Fe–(Mn, Mg)] = $6.77\times10^{-12}\exp[-132(\pm45)\text{kJ}/RT]$ m^2 s^{-1}. Unfortunately, experimental data are available for only a restricted temperature range, and it is dangerous to extrapolate diffusion coefficients to temperatures outside the range of measurement since the diffusion regime and Q may change[22]. A direct assessment of diffusion rates at significantly lower temperatures must await more sophisticated experiments.

Evidence for volume diffusion in natural garnets has recently been cited[8,12,13], but in general, the interpretation of zoning is not easy. In a survey of garnets from west central Massachusetts, Tracy, Robinson and Thompson[7] found they were unable to distinguish fully between garnet zoning due to continuous prograde, or retrograde reactions, or exchange with Fe–Mn–Mg inclusions, or diffusion within the garnet. Other authors[8,12] have suggested that all garnets develop zoning by a fractionation-depletion process during growth, which is subsequently homogenised by diffusion, particularly in the highest grades. From

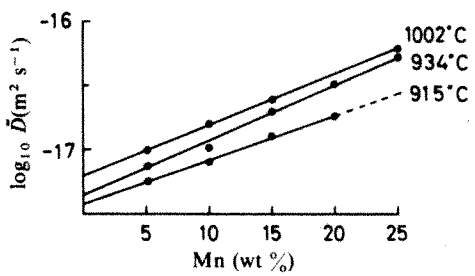


Fig. 3 Composition-dependence of $\tilde{D}$ (Fe–Mn) in almandine garnet at one atmosphere for the temperature range 915–1,002 °C.

estimates of apparent diffusion distances (x), and temperatures of the metamorphic zones, Yardley[8] calculated Q for diffusion in garnet to be 293–418 kJ mol^{-1}. In principle this is a reasonable approach but x may be hard to assess. In the highest grades, homogenised garnets may unequivocally be attributed to volume diffusion if the earlier zoning is recorded, for example, in the composition of inclusions[12]. However, in the lower grade rocks effective diffusion distances may be ambiguous since garnets from a single sample often exhibit a range of zoning profiles[13,23]. It is possible, therefore, that Yardley underestimated x for his samples 146 and 149, and by using plausible alternatives, activation energies as low as 154 kJ mol^{-1} are obtained.

The present experiments confirm that diffusion does occur in garnets, that $\tilde{D}$[Fe–(Mn, Mg)] is typically 10^{-17} m^2 s^{-1} at temperatures around 950 °C, and activation energies are probably in excess of 100 kJ mol^{-1}. In environments of geological interest interdiffusion rates will be sensitive to (T, P), composition gradients, bulk composition and possibly shearing stress[12], or the presence of a fluid, and thus the detailed thermal history may be difficult to elucidate.

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Palaeo-stress estimates in the Moine Thrust Zone, Eriboll, Scotland

STRESSES in the Earth's crust and mantle are of great interest to geologists and geophysicists, particularly the stresses associated with the formation of major tectonic structures. The measurement of quartz microstructural features reported here indicate the stresses during mylonitisation in the Moine Thrust Zone.

Recent studies[1–5] have demonstrated that deformed and syntectonically recrystallised minerals in tectonites may be used to estimate the palaeo-stresses operative during their last deformation. The technique arises from the observation that in large-strain steady-state dislocation creep or hotworking of materials, a stable microstructure develops at low to moderate strains and subsequently remains approximately constant. Similar observations have been recorded for naturally deformed quartzites[7].

The elements of the microstructure that are stress dependent are the dislocation density ρ, sub-grain size d and the size of the recrystallised grains D. The following dependencies apply for metals[6], ceramics[8] and minerals[9–11]:

$$\sigma = k\mu b\rho^{1/2} \qquad \sigma = \frac{l\mu b}{d} \qquad \sigma = md^{-0.7}$$

where σ is the differential stress, k, l and m are constants, μ the temperature compensated shear modulus and b the magnitude of the Burgers vector. In principle it is possible to make quantitative stress estimates if k, l and m have been determined experimentally for the mineral in question and if care is taken[12]. The approximate values of l and m for quartz can be obtained from the observation[13] that at 900 °C and a stress of ~100 MPa (1 kbar) the sub-grains in a deformed quartzite were approximately 5 μm in diameter and the size of recrystallised grains was 20 μm. Goetze[9] has proposed a value of 3 for k. However, there is the possibility that ρ, d and D may have slight temperature dependencies[12], especially D in the case of minerals[3]. This must be resolved before accurate estimates can be made because there are normally large differences between the temperatures of experimental runs and those of crustal deformation. Temperature effects will lead to an overestimation of stresses and the values quoted below probably represent upper limits.

I have measured the variation in ρ, d and D in two mylonite zones in quartz-rich rocks in the Moine Thrust Zone at Eriboll, in North-West Scotland. The first was a narrow, 10-cm thick mylonite band in the Pipe Rock in the imbricate zone at Ben Heilam. A decreasing strain gradient exists away from the mylonite band with only slightly deformed Pipe Rock at ~25 cm from the mylonite band. The second zone was associated with the upper thrust plane (the Moine Thrust) of the Moine Thrust Zone. This out crops in Alt Odhrsgaraidh. These mylonites gradually grade into the overlying Moine schists (see Soper and Wilkinson[14]). A sequence of specimens was collected from both localities. The first appearance of recrystallised grains around the old quartz grain margins was taken as the outer limit of the deformation producing the mylonites. Some low-strain deformation must exist beyond this limit to have produced the recrystallisation. However, it is probable that sub-grain sizes may not have equilibrated to the applied stress at these low strains and they were not measured.

The grain size was determined from optical micrographs of petrological thin sections. Dislocation density and sub-grain sizes were measured (diameters in the case of equidimensional sub-grains or widths if elongated) from electron micrographs taken from foils prepared from the above thin sections. All electron microscopy was performed in an AE1 EM7 high voltage transmission electron microscope operating at 1,000 kV.

The sub-grain size and the grain size, the mean of at least 100 measurements, in both sequences of specimens decreased into the mylonites and then remained constant. In the Pipe Rock, d decreased from 13.3 to 3.7 μm (see Fig. 1) and D from 24.6 to 14.6 μm. The corresponding decreases in the Moine mylonite zone were; d from 13.6 to 2.6 μm and D from 42.9 to 18.6 μm. Wherever possible, only sub-grains away from grain boundaries were measured because of a likely stress intensification in their vicinity. It was observed, in both mylonites, that the presence of mica led to a dramatic reduction in grain size and consequently grain size analyses were limited to mica-free areas. Unfortunately electron microscopy revealed that such areas, in optical micrographs, often contained small mica-grains. The dislocation densities varied erratically in both groups of samples from 2×10^7 to 1×10^8 intersections cm^{-2} (averaging 5×10^7). This observation supports previous findings that care must be taken when using ρ for palaeostress estimates because of the ease with which it can be reset by small strains after the main deformation. The sub-grains have a greater stability as they are low energy structures containing geometrically necessary dislocations and were the most reliable palaeo-stress indicator in the specimens studied. The presence of mica adversely affected grain size estimates.

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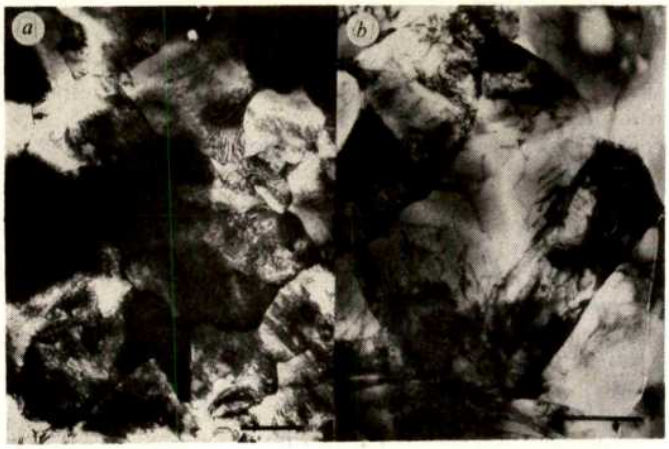


Fig. 1 Electron micrographs showing the decrease in sub-grain size in deformed quartz grains: *a* outside (scale bar, 10 μm); *b* inside (scale bar, 2 μm) a shear zone in the Pipe Rock.

The sub-grain sizes in both areas indicate a stress intensification by a factor between 3 and 4, into the mylonite zones. They indicate a similar low stress, 37 MPa (370 bar) at the edge of each deformation zone. The stresses increase to 128 MPa (1.28 kbar) in the Pipe Rock mylonites and to 180 MPa (1.8 kbar) in the Moine mylonites. The grain sizes indicate a smaller variation, for example, from 50 MPa (500 bar) to 100 MPa (1 kbar) for the Moine mylonites, but as stated previously little reliance can be placed on these values. The average dislocation density indicates a stress of ~35 MPa (350 bar) which is similar to the low stresses indicated by the sub-grains.

Shear zones and thrusts represent zones of inhomogeneous deformation and can be likened to shear band structures in metals, ceramics, polymers and soils[15,16]. These bands mark zones of strain softening which may arise from mechanical or thermal effects. It has been suggested that mylonites also indicate zones of strain softening[17]. In the case of those studied there is no evidence for any marked shear heating and it is concluded that a mechanical softening mechanism was operating. I would expect the mylonite zones to initiate from local stress notches in the same way as shear bands initiate. Notches were not identified but would be expected as the Pipe Rock is inhomogeneous and in the case of the Moine Thrust there are marked changes in rock type. Therefore the propagation of the mylonite zone can be regarded as being similar to that of a shear band or a planar discontinuity in a cohesive material[16]. In such a model, which would apply to narrow zones, the stress gradient recorded by the sub-grains would be that at the tip of the propagating discontinuity. It would cause deformation in the surrounding material with that above and below frozen in after propagation. The softening due to the mylonite zone which develops leads to a relaxation of the localised stress concentration and also concentrates the deformation into the zone. Growth of the zone will occur if the flow rate in the mylonite cannot accommodate the overall imposed regional rate. The stresses required to produce the mylonite during the initiation and during the growth of a shear zone or ductile fault should be similar for a given rock type and deformation temperature. After the development of the required width of mylonite to accommodate the regional strain rate the adjacent country rock should play little further part in the deformation and the above gradients are preserved. Therefore the high stress estimates are likely to be those associated with the mylonite development and the low estimates should approximate to the regional background stresses to which the former relax. I suggest that the difference between the two represents the overall stress variation associated with the formation of the Moine Thrust Zone in the area studied. Note that the dislocation densities indicate a stress close to the relaxed stress. This may indicate that, in this area, the dislocation densities have been reset to the regional stress after the mylonite formation.

In conclusion, I suggest that the quartz mylonites in the Moine Thrust Zone, at Eriboll, were produced by a localised stress of ~150 MPa (shear stress of 75 MPa or 750 bar) with the regional stress being ~35 MPa (shear stress of 17.5 MPa or 175 bar) which is the likely stress during thrusting after the mylonites formed.

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Recent thrusting in the Appalachians

NUMEROUS orogenic episodes from late Precambrian to early Permian have shaped the Appalachian Mountains. Its last orogeny, the Allegheny orogeny, began before the end of the Lower Carboniferous and ended after the early Permian[1]. Most of the Palaeozoic mountain belts of Europe and other continents were worn down long ago. It is still being discussed whether the Appalachians were constantly uplifted in post-Palaeozoic times or whether there was a Cenozoic rejuvenation of uplift[2–4]. The composition of the Mesozoic and Cenozoic Atlantic coastal sediments and their gentle dip away from the Appalachians imply that the mountains stood as a high broad arch despite two hundred millions of years of erosion[1]. Geological evidence has been found and is reported here of thrusting during the past 12 yr at many locations in the Appalachian Plateau and Valley and Ridge province. Five sites have been selected in Tennessee, West Virginia, and Pennsylvania to show considerable horizontal shortening and associated vertical uplift that actually holds up the mountain ranges. The recent faulting and folding have been quantitatively studied and absolutely dated by offsets of boreholes that have been drilled for blasting operations in road-cuts.

Along the interstate highway 40 between Harriman and Rockwood, Tennessee folded and thrusted Pennsylvanian shales and sandstones are exposed striking N 45° E and dipping 10° towards south-east or north-west (Fig. 1). In this position the highway trends west-north-west and cuts across a syncline more than 15 km wide in the transition zone between the Valley and Ridge province and the Cumberland plateau following to the west.

West (10.5 km) of exit 338 (Westel road) on interstate highway 40 east, a thrust fault of supposedly Alleghenian age has recently been reactivated by an offset of 25 cm horizontal throw and 16 cm vertical movement (Fig. 2). The thrust plane strikes and dips N 42° E/32° SE and the hanging wall block is overthrusted in the direction of N 80° W, being parallel to the

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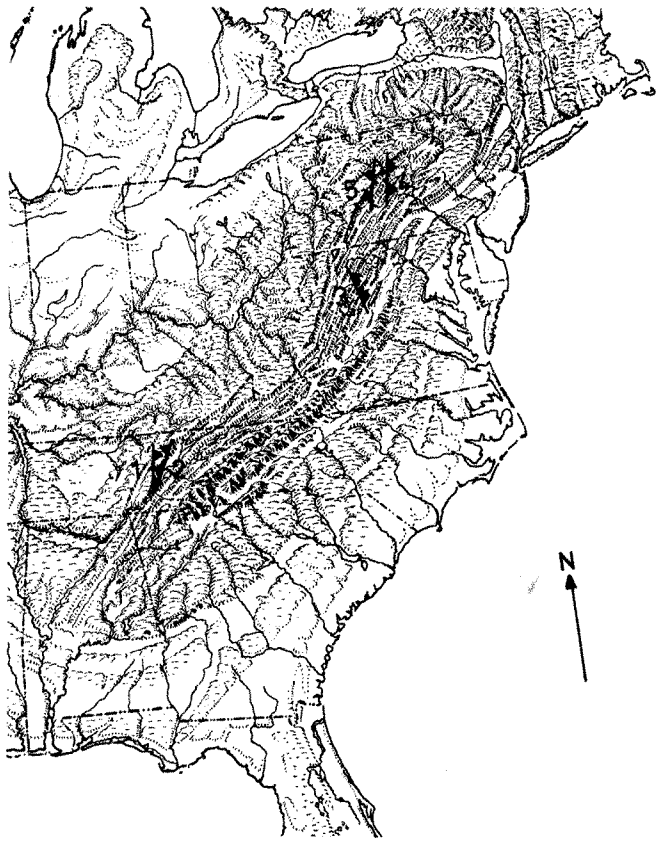


Fig. 1 Sites of recent thrusting in the Appalachian Plateau and Valley and Ridge province. Barbs on hanging wall blocks. Eastern part of US landform map by E. Raisz. Numbers of sites refer to Table 1.

orientation of the road-cut walls as can be seen by the displacements of the boreholes. Likewise, the direction of maximum horizontal stress that caused the thrusting seems to be N 80 °W-orientated as no strain–slip of the rocks towards the open road-cut during the past 12 yr could be observed. The boreholes were drilled in summer 1966. The same thrust throw was manifested in April 1974 as in August 1978. Thus, for the period 1966–74 an annual average thrust-slip rate of 3.8 cm can be calculated. From 1974 to 1978 no distinct offset could be observed.

Four kilometres east of exit 338 on highway 40 east, boreholes were drilled during the summer of 1968. Here, on the eastern flank of the aforementioned syncline, Pennsylvanian sandstones and shales strike and dip N 70° E/20° NW (Fig. 3). Highway 40 now is N 40° W-orientated and the displacements of the drill-holes at both sides of the road indicate thrust faulting along bedding planes, particularly at the transition from sandstones to shales. The direction of thrusting, however, is no longer strictly parallel to the road-cut walls. The boreholes are obliquely offset to the road on both sides of the highway revealing an east to E 40° S thrust direction of the hanging wall layers. The maximum horizontal throw recorded is 20 cm and the corresponding vertical movement is 7.5 cm. Some drill-holes are offset twice along different thrust (bedding) planes.

Considering the two exposures west and east of exit 338 on interstate highway 40 it is concluded that a recent stress field with a maximum compressive stress directed west-north-west has horizontally shortened an area of 14.5 km length by at least 45 cm and has induced vertical uplift of 7.5 cm to 16 cm during the past 12 or 10 yr, respectively (Fig. 4).

West (16 km) of Franklin, West Virginia and 3 km south-west of Oak Flat, West Virginia dark siltstones and shales of presumably middle Devonian age are exposed along highway 33 (Fig. 1). The sediments strike and dip N 50° E/15° SE. Boreholes were drilled there for blasting operations between June and August 1973. Five years later the total horizontal displacements of the drill-holes along two bedding planes was about 50 cm. The hanging wall sediments were shifted to W 15° S. As the direction of overthrusting almost corresponds with the strike of sediments no vertical uplift occurred. This considerable offset during such a short time-span seems to require other means for displacement in addition to tectonic stress. But the direction of thrusting is again parallel to the road-cut, although the inclination of the sediments towards the highway could have induced gravitational sliding at the north-western road-cut wall. A west-south-west-directed maximum horizontal stress has been suggested as the cause of the recent displacement of the boreholes whose major throw may have occurred shortly after the blasting. A major strain release by thrust-slip movements (soon after the blasting), is also indicated by the observations at site 1 (Fig. 1, Table 1).

Exposures of Devonian quartzites have been studied on highway 22, 14.5 km north-west of Millerstown, Pennsylvania. The layers are almost horizontal (N 8° E/5° SE) being close to the centre of a syncline within the Pennsylvanian Valley and Ridge province. According to borehole offsets only a minor thrusting in the direction of N 80° W has been observed. The horizontal throw amounts to 1.5 cm and vertical movements were calculated to be 0.1 cm. I do not know when the drilling and blasting for the construction of the road-cut occurred, but according to the state of the highway and the freshness of the exposed rocks along the road sides it seems that blasting was conducted no more than 10 yr ago.

The highway 22 continues northwestwards and intersects narrow folds of the eastern Allegheny plateau. About 6 km north-west of Port Matilda, Pennsylvania the highway 22/322 cuts across Mississippian and Pennsylvanian red beds of sandstones, siltstones and shales that strike and dip N60° E/20° NW. Many displacements of drill-holes indicate overthrusting along bedding planes in the direction of E 50° S. There are numerous displacements of minor throw within the sandstone sequences and there are a few thrusts of major throw along the sandstone/shale contact planes, where friction is reduced. Thus, the maximum thrusting with a horizontal throw of 14 cm and a vertical uplift of 5 cm has been measured of a sandstone overriding a shale. Another thrust-fault was recorded with a horizontal throw of 5 cm and a vertical uplift of 1.8 cm. Along both sides of the highway 322, from 6 to 8 km north-west of Port Matilda, Pennsylvania, most of the 20°-NW-dipping thrust-faults indicate a movement of the hanging wall blocks in the direction of E 50° S. But some borehole offsets at the south-west

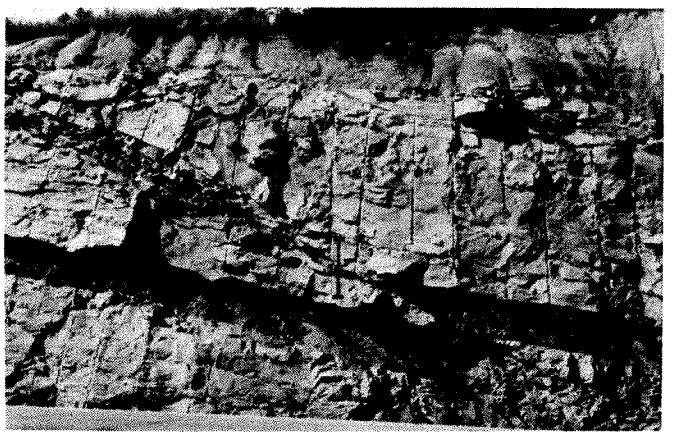


Fig. 2 Thrust-fault in Pennsylvanian sandstones intercalated by a dark shale layer, located between Rockwood and Harriman, Tennessee, 10.5 km west of exit 338 on interstate highway 40 east. Borehole offsets occurred after blasting in 1966. Displacements of 25 cm horizontal throw and 16 cm vertical uplift have been recorded in 1974. Direction of thrust motion is N 80° W and corresponds to the orientation of maximum horizontal stress. Scale bar, 1.83 m (6 ft).

Table 1 Sites of recent thrusting in the Appalachian Plateau and Valley and Ridge province

Location	Direction of thrusting and inferred direction of σH_{max}	Inclination of thrust plane	Horizontal throw	Vertical throw	Period of recent thrusting
1*. Rockwood–Harriman, Tennessee Interstate 40 east, 10.5 km west exit 338	N 80° W	32° SE	25 cm	16 cm	1966–74
2*. Rockwood–Harriman, Tennessee interstate 40 east, 4 km east exit 338	East to E 40° S	20° NW	20 cm	7.5 cm	1968–78
3*. 3 km south-west Oak Flat, West Virginia, highway 33	W 15° S	0°	50 cm		1973–78
4*. 14.5 km north-west Millerstown, Pennsylvania, highway 22	N 80° W	5° SE	1,5 cm	0.1 cm	about 10 yr
5*. 6–8 km north-west Port Matilda, Pennsylvania highway 322	E 50° S N70° E	20° NW	14 cm 5 cm	5 cm 1.8 cm	about 10 yr

* Numbers correspond to sites in Fig. 1.

road side wall show strain–slip movements in direction of N 70° E towards the open road-cut. A recent maximum horizontal compressive stress which caused the thrusting is suggested to be north-west directed rather than west-north-west, as can be deduced from the strain–slip movements.

The drill-hole offsets were considered to have been caused mainly by blasting, as at site 1 where measurements have been repeated no displacement was observed during the past four years. However, there is evidence of updip thrusting after the blast operations and not of gravity controlled movements. The thrusting at sites 1 and 2 occurred in exactly the opposite directions due to the corresponding orientation of maximum horizontal stress (σH_{max}) at both locations. The change in the orientation of the road-cut at site 2 (N 40° W) in respect to the direction of the road excavation at site 1 (N 80° W) is not reflected by the orientation of the borehole displacements.

The energy released by the explosions is probably insufficient to displace rock masses of more than 50,000 m^3 (site 5) updip on planes as one block.

Borehole offsets have been observed in southeastern Connecticut after blasting operations in 1970 [5]. Measurements at one location have been repeated between 1970 and 1978 revealing that the initial displacement of 23 mm was followed by a period of creep at an average rate of 2.8 mm yr^{-1}. It is suggested that this permanent creep results from stresses acting on the Appalachians at present, rather than from palaeo-stresses that would have created one episodic rock movement during or shortly after the blasting. As for site 1 it is suggested that thrusting will continue if σH_{max} reaches the critical value that was achieved some time between the blasting of 1966 and 1974.

If the borehole offsets on highway 322 between Port Matilda and Philipsburg, Pennsylvania and those on highway 22 between Lewistown and Millerstown, Pennsylvania are about 10 years old, we may apply the calculated amounts of annual uplift from the sites where recent thrusting has been studied to the Pennsylvanian Valley and Ridge province in general. Vertical movements of at least 2 cm yr^{-1} can be inferred from the structural investigations on the highway 322 north-west of Port Matilda, Pennsylvania and of 0.1 cm on the highway 22 west-north-west of Millerstown, Pennsylvania. However, it has been frequently observed that recent thrusting is enhanced in 20°–30° inclined sediments and that thrusting decreases in more gentle or steeper dipping layers.

The crests of the main ridges of the Valley and Ridge province north-west of Harrisburg, Pennsylvania show small remnants of an old peneplain surface formed by an ancient Susquehanna river-system when the region was near base level. The Susquehanna river is now superimposed on the rejuvenated Valley and Ridge province that was regionally uplifted by about 300 m in relation to the present valley floor. If a continuous uplift of 2 cm yr^{-1} is inferred for the recent and past vertical movements in that area, the present elevation of the ridge crests above the ancient peneplain will be achieved during a period of 15,000 yr only. However, at this point of investigations of recent thrusting and folding in the Appalachians we have to consider the thrust–slip rates as local phenomena.



Fig. 3 Recent thrusting after drilling and blasting of boreholes in 1968 occurred along a bedding plane of Pennsylvanian sandstones, 4 km east of exit 338 on interstate highway 40 in eastern Tennessee. Scale bar, 1.83 m (6 ft).

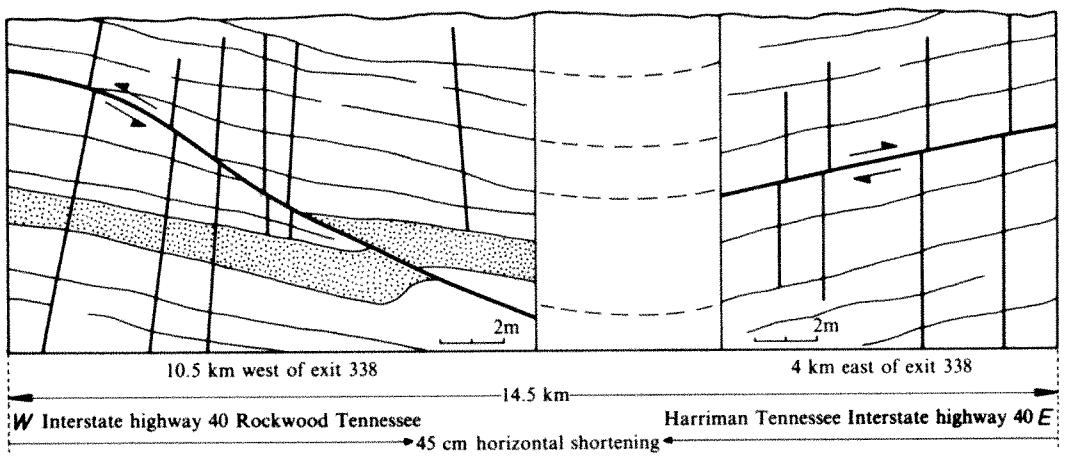


Fig. 4 Recent thrusting and folding of a syncline in Pennsylvanian sandstones and shales (stippled), between Rockwood, Tennessee and Harriman, Tennessee. More or less vertical lines indicate boreholes that are offset at a recently reactivated thrust-fault (left) and along a bedding plane (right).

The rates of relative vertical movements as determined from levelling data of the eastern US reveal that the Appalachians rise relative to the interior platform and to the Atlantic coast at rates of up to 0.6 cm yr^{-1} (ref. 6). This is already two orders of magnitude larger than the estimated average uplift from the early Cretaceous to the present. Brown and Oliver[6] have published relevelling profiles with small peaks of increased vertical motion superimposed on the broader uplift of the Appalachians. Two of their profiles traverse close by the sites where recent thrusting was recorded. A peak of prominent uplift at Tyrone, Pennsylvania is located 22 km south of the Port Matilda, Pennsylvania site. Another, but less pronounced peak of vertical motion between Somerset, Kentucky and Harriman, Tennessee is established about 100 km north of the Rockwood–Harriman, Tennessee site in the continuation of the geological strike.

An apparent uplift at the Blue Ridge–Piedmont boundary relative to the Atlantic coastal plain and the Valley and Ridge province is indicated by precise relevelling along a line from Morristown, Tennessee to Newton, North Carolina and along another traverse in Georgia[7].

The areas of investigation and generally the central and southern Appalachians are associated with only a moderate seismicity[8]. Nevertheless, the Appalachian mountain belt is contoured by a cluster of epicentres in relation to its foreland and hinterland. The Atlantic coast area is seismically active only in Virginia and South Carolina, the latter area being part of the Charleston–Cumberland seismic zone as an onshore extension of the Atlantic Blake transform fault[9]. The Rockwood–Harriman, Tennessee site of recent thrusting in eastern Tennessee is located at the north-western termination of the Charleston–Cumberland seismic zone. There, the present maximum horizontal north-west–south-east compression (σH_{max}) inferred from thrusting does not correspond to the north-east–south-west-orientation of $\sigma 1$ derived from composite fault plane solutions of earthquakes that occurred in the same seismic zone further to the south-east at Lake Jocassee, South Carolina[10] (the seismic data have been revised since instrument polarities were reversed).

The results of structural investigations in the Rockwood–Harriman, Tennessee area contrast also to the *in situ* stress directions of a north-east σH_{max} and a north-west σH_{min} obtained from hydrofracturing[11]. Other σH_{max} directions determined from hydrofracturing in the western Appalachians from West Virginia to New York State approximately correspond to those derived from recent thrusting at the West Virginia and Pennsylvania sites. Both areas are associated with little seismicity despite prominent recent tectonic events. Some horizontal *in situ* stresses have been determined in the Appalachian Piedmont from Georgia to Pennsylvania by the overcoring technique[12]. All sites but one reveal σH_{max} to be north-east directed. The Appalachians are probably governed by two different stress regimes, one in the west with σH_{max} orientated north-west to east-west and another in the eastern Appalachians with σH_{max} predominantly north–east directed. It is assumed that absolute stresses of 50–150 bar as determined by *in situ* overcoring in the Piedmont may be enough to cause recent thrusting like that in the Appalachian Plateau or Valley and Ridge province.

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Direct measurements of secondary currents at a river inflexion point

SECONDARY currents are defined as currents which occur in a plane normal to the local axis of primary flow. They may develop in pipes or open channels as a result of non-equal distribution of boundary shear stress or by skewing of cross-stream vorticity into a long stream direction[1–3]. Secondary currents distort the distributions of primary isovels and boundary shear stress from those found in simple flows and have important effects on sedimentary processes of erosion and deposition[4,5]. Direct measurements of secondary currents in river bends were reported recently[6]. Further data have been obtained for the reach around the inflexion point between bends (Fig. 1) and are reported here.

At a river bend a strong skew-induced secondary flow cell carries surface water towards the outer bank and bed water towards the inner bank. Measurements made in rivers indicate that a small cell of reverse rotation can exist close to the outer bank in a bend, if that bank is steep[6].

This cell is generated locally by interaction between the skew-induced cell and the outer bank[6,7]. It is not connected with the reverse circulation of a relic cell from an upstream bend[8–10]. Few measurements have been made in the reach between opposite bends, however, to ascertain how the secondary current pattern from the upstream bend decays, to be replaced by that associated with the downstream bend. Some observations have been made in laboratory flumes[8–10]. Flume data collected by Toebes and Sooky[10] suggest that the skew-induced cell for the downstream bend tends to displace the relic cell laterally towards the outer bank in the downstream bend. Wilson hypothesised that this might be the case on theoretical grounds[11]. In the flume study the decaying cell does not ever disappear completely, so that a remnant cell persists through the downstream bend.

The secondary flow pattern at the inflexion point shows two approximately symmetrical cells which converge at the surface and diverge at the bed. Chacinski and Francis observed a rather different system in their flume study[8,9]. Here the skew-induced cell for the second bend originates near the bed and develops upwards, displacing the decaying cell as it does so. This leads to a

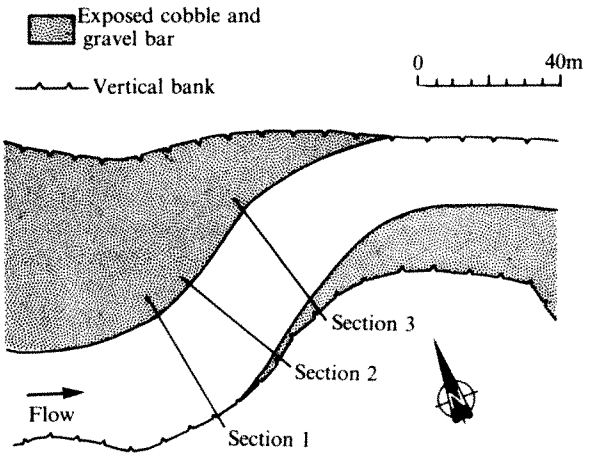


Fig. 1 River Severn at Penstrowed, Powys. Discharge about 13 $m^3 s^{-1}$.

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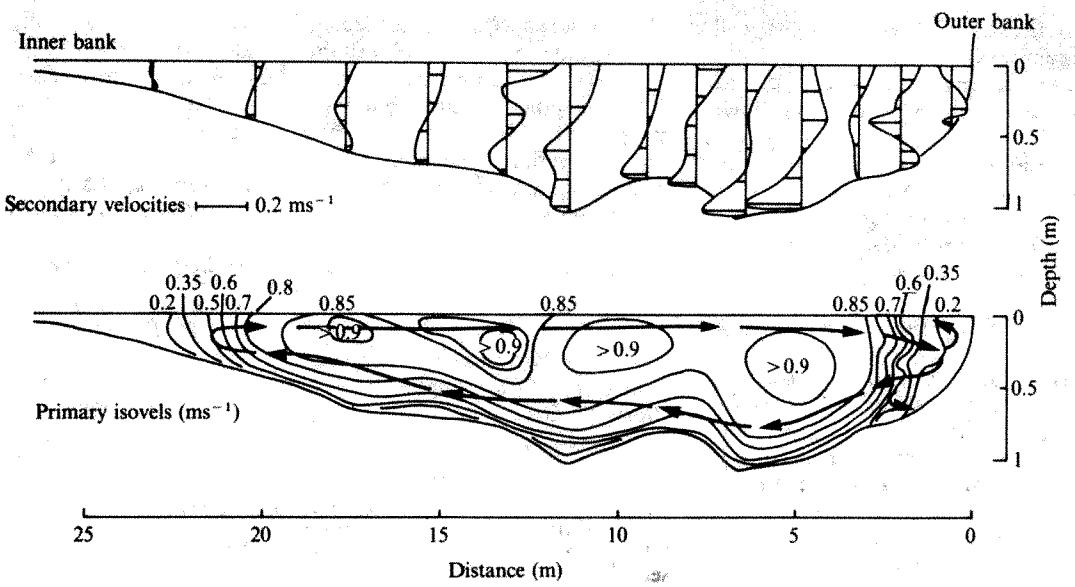


Fig. 2 Primary and secondary velocities at section 1, exit of upstream bend. 19 November 1977, discharge 13 $m^3 s^{-1}$.

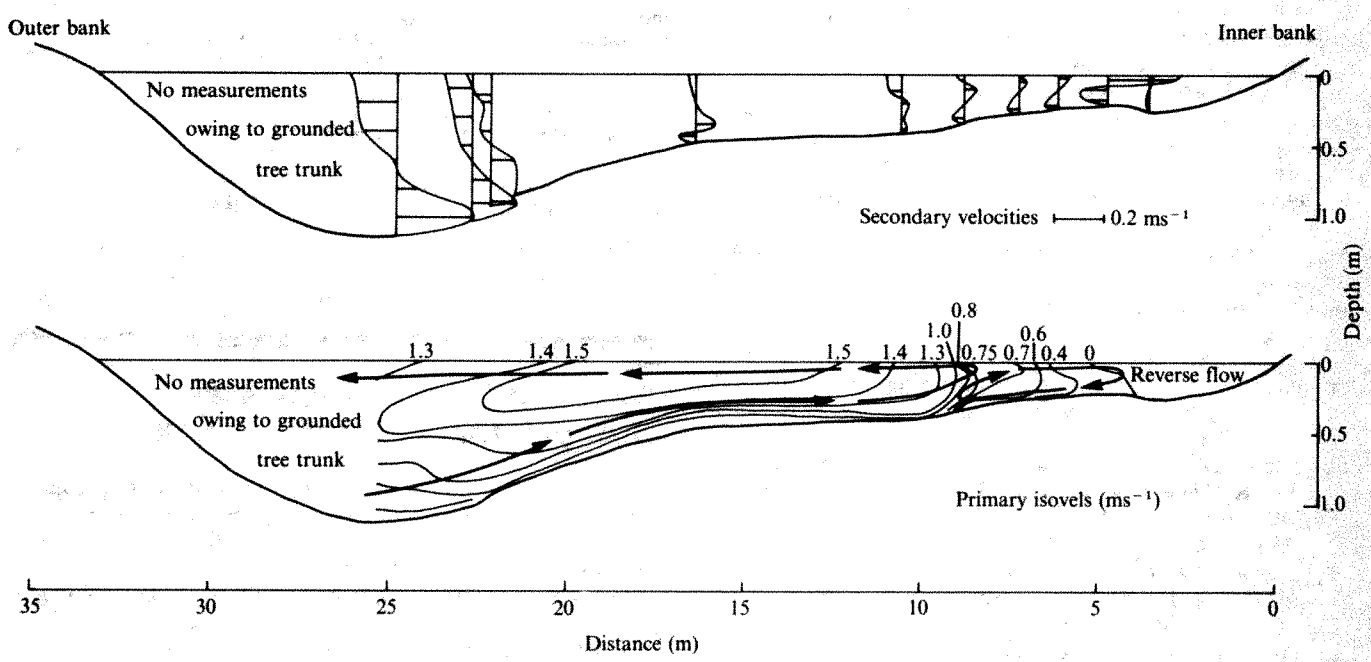


Fig. 3 Primary and secondary velocities at section 3, entrance to downstream bend. 18 November 1977, discharge 17 $m^3 s^{-1}$.

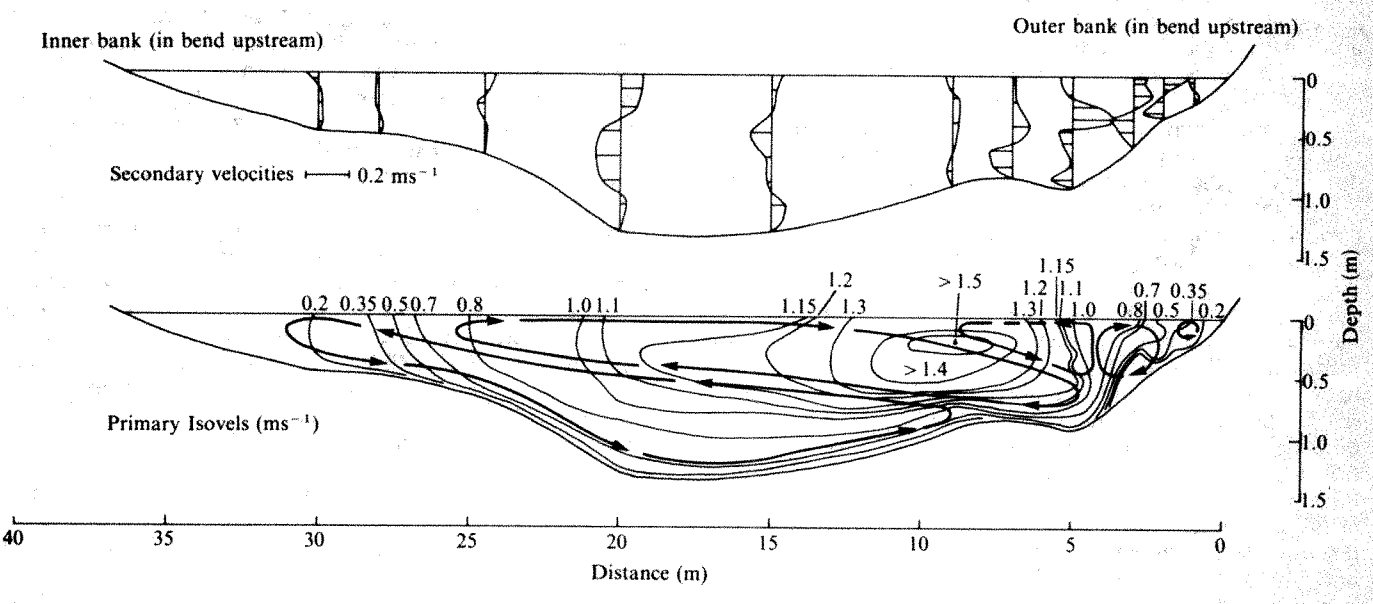


Fig. 4 Primary and secondary velocities at section 2, inflexion point between bends. 17 November 1977, discharge 22 $m^3 s^{-1}$.

system of two stacked cells in the second bend, the upper cell being a remnant of the upstream flow.

The explanation of these apparently contradictory results probably lies in the cross-sectional shape of the flume channels used in the two experiments. In Toebes and Sooky's case the width to depth, or aspect ratio was about 1.5. However, Chancinski and Francis used much higher aspect ratios up to 40 and in some cases 80. As the latter values are more typical of the aspect ratios found in natural streams, it should be expected that the observations of Chacinski and Francis should be more directly applicable to rivers.

Rozovskii[12] used the equations of motion to produce equations describing the development and decay of skew-induced secondary flow. On the basis of these equations, he suggests patterns like those observed by Chacinski and Francis for connecting bends of different directions[12].

To investigate the pattern of secondary flow between bends in a river, data were collected at Penstrowed, Powys, on the River Severn, using an electromagnetic flow meter. This instrument measures velocity in two perpendicular directions simultaneously with an accuracy of $10\,mm\,s^{-1}$ (refs 13–15). Measurements were made at three sections across the river between two meander bends. The meter was positioned to measure long and cross-stream velocity. These are not necessarily the primary and secondary velocities but a theory previously developed allows us to calcaulate these components[6]. Although the measurements were made on three consecutive days discharge was not constant. However, the change in discharge did not produce a marked change in the geometry of flow through the reach and so it is still possible to compare qualitatively the patterns of flow at the three sections. Section 1 was located at the exit of the upstream bend, section 2 at the inflexion point, and section 3 at the entrance to the downstream bend (Fig. 1).

The secondary flow pattern at the bend exit (Fig. 2) is dominated by the skew-induced cell from the upstream bend. There seems to be a rather weak outer bank cell near the right hand bank. This is not surprising as secondary currents are known to persist for considerable distances downstream from single bends[16]. The data for section 3 (Fig. 3) at the entrance to the downstream bend indicate that this is not the case for a series of bends. Here a skew-induced cell of opposite rotation to that in the upstream bend dominates the secondary flow pattern. Section 2 (Fig. 4) at the inflexion point, seems to show a two cell system with vertical stacking in the central part of the channel. The upper cell is the relic skew-induced cell from the upstream bend, whilst the lower cell is the developing skew-induced cell for the downstream bend. The right-hand margin of the system is complex, but there seem to be outer bank cells resulting from interaction of the skew-induced cells with the bank[6].

The river measurements are similar to those made by Chacinski and Francis in a laboratory flume of high width-to-depth ratio. A significant difference is that the skew-induced circulation for the downstream bend in the River Severn was fully developed at the bend entrance, whilst in the flume experiments a remnant of the upstream circulation persisted well into the downstream bend. The reason for this difference may lie in the difference in bed topography between the river and the flume. In the river the cross-section at a bend is highly asymmetrical with the deepest point close to the outer bank. At the inflexion point the channel is almost symmetrical, often with a pronounced medial bar. The switching of thalweg from right to left banks between sections 1 and 3 reflects this. It is the curving of streamlines resulting from this effect of bed topography which is initially responsible for the generation of the new skew-induced cell close to the bed. At the bend entrance downstream, plan form curvature reinforces this effect, leading to full development of the skew-induced secondary flow early in the bend. Clearly this would not be the case in the laboratory experiments carried out in flume channels with uniform rectangular cross-sections[8–10]. In a channel with a uniform cross-section, plan shape curvature alone is responsible for generating the new skew-induced cell and consequently that cell does not fully displace the relic cell until well into the downstream bend.

Direct measurements of secondary currents at a river inflexion point confirm that the system of cell development proposed by Chacinski and Francis can operate in rivers as well as uniform flume channels. An important difference is that the bed topography in a natural stream seems to result in early and more complete replacement of remnant cells from an upstream bend.

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Aneuploidy in mouse fetuses after paternal exposure to X rays

ANEUPLOIDY can result either from non-disjunction, giving trisomy and monosomy, or from chromosome damage leading to loss of chromosomes (monosomy) only. It is known that chromosome loss can be induced after irradiating post-meiotic male germ cells[1], but apart from findings on radiation-induced non-disjunction in oocytes from mice[2–5], there is no evidence that paternal exposure to X rays induces chromosomal mal-segregation during male meiosis to produce monosomic and trisomic offspring. Szemere and Chandley[6] did observe significantly more mal-segregated X and Y chromosomes in spermatocytes II after 100 and 200 rad had been given during preleptotene and pachytene, but failed to recover aneuploid fetuses in progeny from irradiated males. They suggested that only a few aneuploid spermatocytes would be transmitted to the next generation. We have now shown that such induced aneuploidy is indeed transmitted and can be detected in a monosomic and trisomic condition in F_1 fetuses on day 9.5 of gestation.

Twenty 8–10-wk-old random-bred NMRI/Han male mice were given whole-body irradiation of 20 or 200 rad (10 males in each group) and were mated from day 2 to day 36 after irradiation to untreated females of the same age and strain. Non-irradiated males served as matched controls. The radiation source was a Philips–Müller tube with tungsten anode and beryllium window; tube voltage was 150 kV and current 8 mA, Cu filter was 1 mm and the exposure rate was 55 rad min^{-1}. The animals were housed under controlled conditions, darkness lasting from 6 pm to 6 am, tapwater and Altromin pellets (RH 1324) were supplied *ad libitum*. The females were checked every morning (day 0.5) for vaginal plugs and those found to be positive were isolated and kept separately until day 9.5 *post coitum*.

The pregnant females were killed by cervical dislocation, their implantation sites removed and placed into TC 199 culture

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Table 1 Aneuploidy in 9.5-d-old mouse fetuses from strain NMRI/Han after paternal irradiation

Dose of X rays given to males (rad)	No. of fetuses (aneuploid/total) karyotyped after G-banding from days 2–8	9–15	16–22	23–29	30–36	Overall (2–36)	
0						0/211 0.0%	
20	0/75	0/33	2/30	0/42	1/36	3/216† 1.4%	1.7%*
200	3/155	0/71	1/20	2/67	1/72	7/385‡ 1.8%	

Matings were from day 2 to day 36 after X-ray exposure. The difference between the control and the irradiated series is statistically significant; there is no significant difference between the 20 rad and 200 rad series.

*Irradiating meiotic and post-meiotic stages $P < 0.05$ (Fisher's exact test).

†Irradiating meiotic stages only, $P < 0.05$ ($F_{2;6}0.05 = 5{,}14 < 5{,}92$) after 20 rad.

‡Irradiating meiotic stages only, $P < 0.05$ ($F_{2;8}0.05 = 4{,}46 < 5{,}04$) after 200 rad both compared to the control.

medium containing 17% fetal calf serum, L-glutamine 2 μM, penicillin 1,000 IU ml^{-1}, streptomycin 10 mg ml^{-1} (reagents from Seromed) and 0.004% colchicine. The embryo proper or the egg membranes only were cultured for 2 h and prepared cytologically according to the method of Evans *et al.*[7]. Chromosomes from at least 7-d-old air-dried slides were stained for G-banding and metaphases were analysed from coded slides. Only one karyotype was prepared from the best 'banded' metaphase with optimal spreading of chromosomes from each embryo or egg membrane. As many extra karyotypes as possible, however, were compared when the first metaphase was abnormal. This procedure of analysis certainly detects those anomalies present in all cells, but would miss some aneuploidies developing only in a mosaic condition.

It has been suggested[6] that triploid mouse fetuses might arise from irradiation of early spermatocytes. We observed four such fetuses with a karyotype of 60,XXY—three in one litter after 200 rad and one fetus after 20 rad. All triploids were generated, however, from fathers exposed 10 days before mating—after the second meiotic division. This finding, together with the fact that three triploids were derived from one litter and the observation of a high spontaneous background of digynic triploidy in the NMRI/Han mouse strain[8], suggested that X-ray exposure did not enhance polyploidy in our study.

Overall, 10 of 601 fetuses derived from irradiated fathers were monosomic or trisomic, but no aneuploidy was found among 211 karyotyped fetuses from non-irradiated fathers (Table 1). All the aneuploid fetuses were generated from different fathers and mothers. Our data from the untreated fetuses confirm that aneuploidy is rare in post-implantation mouse embryos not exposed to mutagens.

The identification of the odd chromosome in all 10 aneuploid fetuses provides further information on the survival of monosomics and trisomics during mouse embryogenesis, although chromosomal mosaicism cannot be excluded in our fetuses and in those of other studies[9]. The results shown below are in agreement with the conclusion reached after the studies of robertsonian translocations in the tobacco mouse[9]—that autosomal monosomics are more prone than trisomics to an early elimination during pre-and peri-implantation development.

The following aneuploidies were identified, with the day of mating after irradiation given in parentheses: First group after 20 rad with 41,XX, +11 (21); 41,XY, +2 (21) and 41,XX, +11 (31). Second group after 200 rad with 39,X0 (4); 39,XY, −8 (5); 41,XX, +14 (7); 41,XY, +13 (16); 39,X0 (25); 41,XYY (28); 40,X0, +13 (32). The latter karyotype with 40,X0, +13 is obviously the result of two events: (1) a sex-chromosome loss either by non-disjunction or due to loss after breakage, and (2) due to non-disjunction of chromosome 13. No 41,XXY karyotype was found in our study, in agreement with the observation of only one XXY male among more than 5,000 offspring studied genetically after paternal irradiation[1].

Non-disjunction was induced in mouse oocytes after exposure to equally low doses (10–30 rad) during the preovulatory phase[2,3]. Aneuploidy was assessed in these studies in oocytes, that is, at a stage long before selection against chromosomally imbalanced fetuses could act. Our observation of 1.4% aneuploid fetuses 9.5 days *post coitum* after paternal exposure to 20 rad would mean that mouse spermatogenesis is at least as sensitive to radiation-induced non-disjunction as oogenesis. Aneuploidy was not significantly enhanced over the effect after 20 rad with the still higher dose of 200 rad. Either some kind of saturation was already reached at the cellular targets for non-disjunction, or the enhanced effects after 200 rad were modified secondarily by, for example, the elimination during spermatogenesis or embryogenesis. [Note that we did not observe any structural anomaly in the 9.5-d-old fetuses after 20 rad, but found balanced and imbalanced translocations after exposure to 200 rad.]

In 6 of the 10 observed aneuploid fetuses the mal-segregation can be allocated with certainty to meiosis I or II. Both primary and secondary non-disjunction were also found to be involved in generating some cases of Down's syndrome[10,11]. The other four fetuses, however, were derived from sperm irradiated in the vas deferens (39,X0 and 39,XY, −8) or the epididymis (41,XX, +14), or irradiated as mid-spermatids (41,XY, +13). A chromosome loss most probably may explain the X0 karyotype and the monosomy 8, although mitotic non-disjunction during early cleavage cannot be ruled out and should have produced a mosaic condition. This suggestion is not unlikely, because some fetuses with mosaic aneuploidy were found after maternal and paternal irradiation[5,6]. Mitotic non-disjunction during early cleavages could also explain our two trisomic fetuses (trisomy 13 and 14), generated 7 and 16 d after irradiation. Either we failed to detect the respective monosomic cell lines, or these hypodiploid cells were selectively eliminated earlier—a reasonable suggestion, as monosomics are usually eliminated during the early mouse embryogenesis[9].

A study of human spontaneous abortions showed that occupational X-ray exposure in man can increase the risk of chromosomally abnormal conceptions[12]. Our finding of fetal aneuploidy after paternal irradiation, affecting both autosomes and sex chromosomes, even after exposure to rather low doses, may have implications for the human situation and may lead to a better understanding of the mechanisms involved in non-disjunction.

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Vitamin D and its analogues as a new class of plant growth substances affecting rhizogenesis

SEVERAL substances actively promote adventitious root formation in cuttings: they include indoleacetic acid (IAA), indolebutyric acid (IBA) and α-naphthylacetic acid. Others, which themselves do not or only slightly promote root formation, can act synergistically with one or another of the former to enhance their activity[1]. Root formation in cuttings is exploited in the propagation of new varieties of plants, such as polyploids or those with disease resistance. While screening steroids for their effect on rooting in *Populus* spp., we have found a synergistic effect of cortisol on IBA-promoted root initiation[2]. We now report a similar effect of vitamin D and its analogues, and suggest an endogenous function for this group of compounds.

Vitamin D_2 and vitamin D_3 are produced by UV irradiation of the respective provitamins ergosterol and 7-dehydrocholesterol—transformations which can be effected *in vitro* or *in vivo* in animal tissue. The thermal and photochemical pathway involves the sequence: ergosterol, previtamin D_2 and vitamin D_2. Tachysterol and lumisterol, once thought to be intermediates, are now considered to be isomerisation side products[3] (Fig. 1).

To test for their effects on plant growth, ergosterol, dihydrotachysterol and vitamin D_2 were applied alone and in the presence of IBA, to *Populus tremula* (aspen) herbaceous cuttings. Dihydrotachysterol, a reduction product of tachysterol, was used because the latter is not readily available and is highly susceptible to aerial oxidation. Later work (results not given) has shown that dihydrotachysterol and tachysterol have similar activities.

The results (Fig. 2*a*) show that ergosterol alone has no significant effect at concentrations of 10–50 mg l^{-1} but that with dihydrotachysterol or vitamin D_2 alone, the number of adventitious roots produced increases by about 60% and 100%, respectively, the maximum effect occurring at 10 mg l^{-1}. IBA alone at 10 mg l^{-1} promoted rooting as expected, by about 200% (Fig. 2*b*). Ergosterol in the same concentration range of 10–50 mg l^{-1}, applied with IBA at 10 mg l^{-1}, caused no greater root formation than did the IBA alone, whereas the same concentration range of dihydrotachysterol and vitamin D_2 applied with IBA at 10 mg l^{-1}, caused a pronounced increase in root formation. Thus, in the concentration range 10–50 mg l^{-1} there was a significant enhancement of root formation (compare this with the similar, but less pronounced, synergistic effect obtained with cortisol and IBA in comparable conditions[2]) and this effect was dependent on concentration. A similar enhancement of root formation has also been observed with woody cuttings from *Populus tremula* and *Populus nigra* and with cuttings of varieties of *Phaseolus vulgaris*.

Vitamin D_3 has been isolated from *Dactylis glomerata* and various species of grasses[4] and the presence of vitamin D or its analogues has been suspected in other plant tissues said to have anti-rachitic activity[5]. However, so far no function has been proposed for them in plants. Using the same rooting test, we have found that vitamin D_2 and vitamin D_3 have similar activities (results not shown). Concerning structure/effect relation-

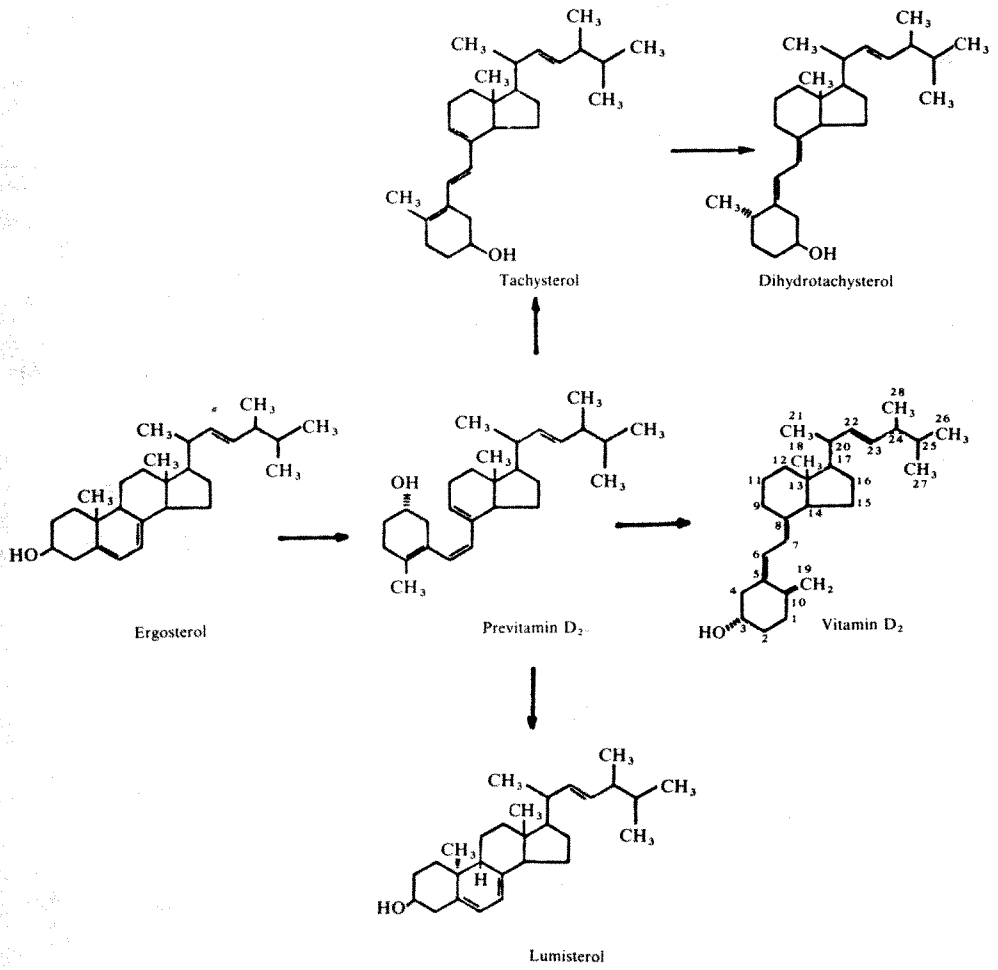


Fig. 1 Thermal and photochemical transformations of ergosterol.

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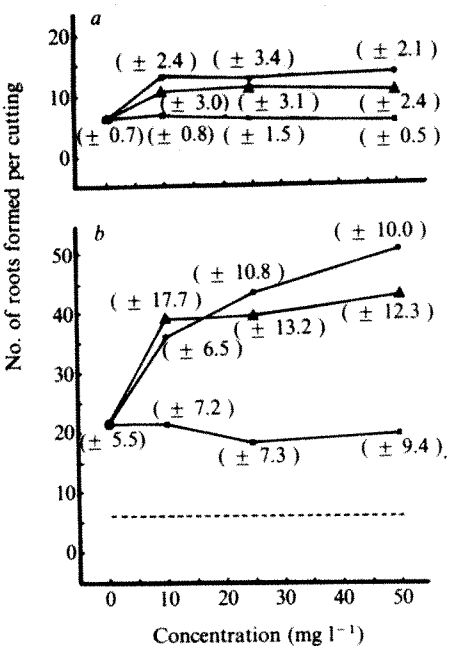


Fig. 2 The *Populus tremula* seedlings were prepared as previously described[2] and solutions of the test substances were applied to the cuttings for 24 h followed by a change to distilled water. The adventitious roots were counted after 18 d of culture, by which time the number of roots formed was essentially constant, and the results expressed as the mean of at least four culture series (s.d. in parentheses). *a*, Without IBA; *b*, with 10 mg l^{-1} IBA. ●, Vitamin D_2; ▲, dihydrotachysterol; ×, ergosterol; – – –, H_2O control.

ships, we can say that the opening of the steroid ring B of ergosterol to give a seco-steroid is an important step, but changes in the alkyl side chain at C-17 have little or no effect.

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Effects of visual experience on the maturation of the efferent system to the corpus callosum

IN normal adult cats the first and second visual areas (V_1 and V_2) in the two cerebral hemispheres are interconnected through the corpus callosum (CC) by neurones (callosal neurones) which have a characteristic laminar location and are restricted to the region of the boundary between cytoarchitectonic areas 17 (V_1) and 18 (V_2)[1–4]. Callosal neurones acquire this distribution postnatally by disappearing (dying, or eliminating their callosal connections) from most of areas 17 and 18 and decreasing in number in layer VI (refs 5, 6). Here we show that although kittens deprived of pattern vision by binocular lid suture still develop an apparently normal distribution of callosal neurones, monocularly deprived or enucleated, or bilaterally squinted kittens retain to adulthood some callosal neurones in parts of area 17 where such neurones are not normally found. Thus, visual experience can influence the selection of callosal neurones although this selection is probably influenced by more than one factor.

The distribution of callosal neurones in 9 abnormally reared (Table 1) and 14 normal control[1,4] cats was determined by the retrograde transport of horseradish peroxidase (HRP) injected at 10–12 sites within the contralateral visual cortex. One month

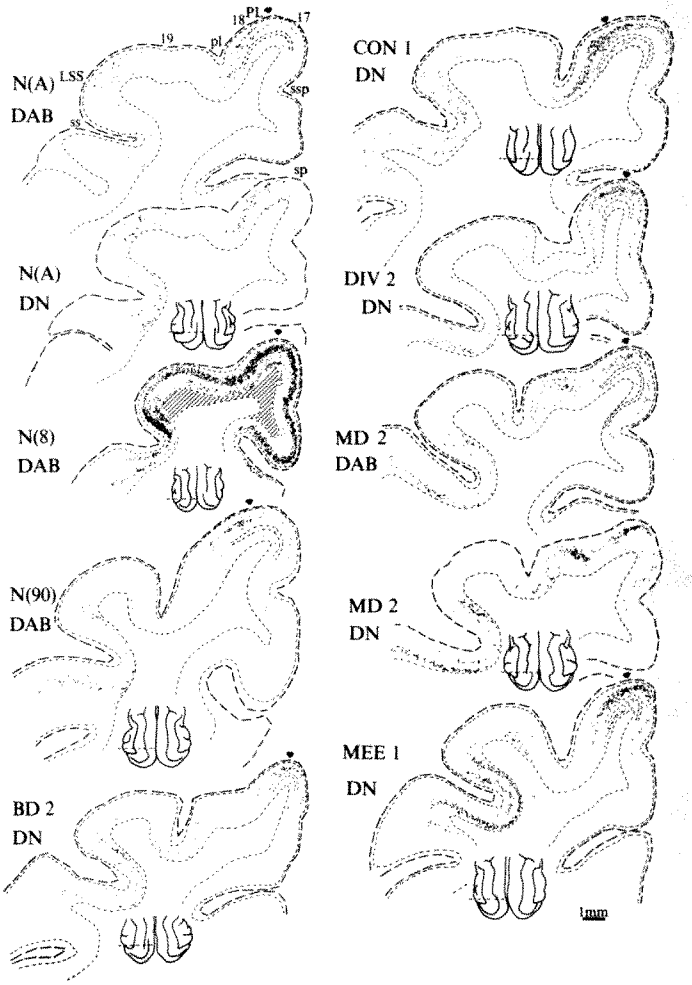


Fig. 1 Computer microscope[26] charts of the distribution of HRP-labelled callosal neurones in 80-μm thick coronal sections at comparable rostrocaudal levels of the visual cortex in different animals. Multiple, 0.5-μl injections of HRP (Boehringer grade I, 33% in sterile saline) were placed in the contralateral cortex. After a 2-day survival time, adjacent, 1-in-4 series of 80-μm sections of each brain were processed with diaminobenzidine (DAB)[1,4] and *O*-dianisidine (DN)[27]. Adjacent sections were reacted with the two substrates as shown for the normal adult, N(A), and for MD 2. In all animals, the injected postlateral and lateral gyri (areas 17, 18, 19) were invaded almost completely by dark HRP precipitate; HRP-filled neurones were found over most of the mediolateral extent of the ipsilateral lateral geniculate nucleus. Each HRP-labelled neurone is represented by a black dot. The level of each section is indicated on the inset drawn from a dorsal view photograph of the corresponding brain. In each section the thin, interrupted lines mark the lower boundaries of layers I, IV, V and VI (in N(8), lower boundaries of layers IV and V are not shown). In the DN sections from N(A) and MD 2 only the lower boundary of layer VI is marked. Cross hatching in N(8) indicates a region of displaced (migrating?) neuronal somata. The two kittens N(8) and N(90) come from a different study[5,6]. Their ages at the time of HRP injection were 8 and 90 days, respectively. Abbreviations: ss, pl, ssp and sp: suprasylvian, postlateral, suprasplenial and splenial sulci, respectively. PL: postlateral gyrus. LSS, 19, 18 and 17 indicate the lateral suprasylvian visual areas, and areas 19, 18 and 17, respectively. The arrow indicates the boundary between the latter two areas.

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before death, the strabismic animals could visually guide their paws with either eye; single unit recording from their areas 17 and 18 between the time of HRP injection and death showed that unlike normal cats, but as in monocularly squinted cats[7], most cortical neurones had monocular receptive fields.

In normal adult cats, the cortical volume containing callosal neurones (callosal efferent zone; CZ) consists of two radially separated laminae, located in layers III/IV and VI(subzones a and c, respectively). Subzone a is wider and richer in callosal neurones. Its medial boundary can extend to the crest of the lateral–postlateral gyrus in area 17 (Figs 1, 2, N(A)) and occasionally, at rostralmost levels, as far as the suprasplenial

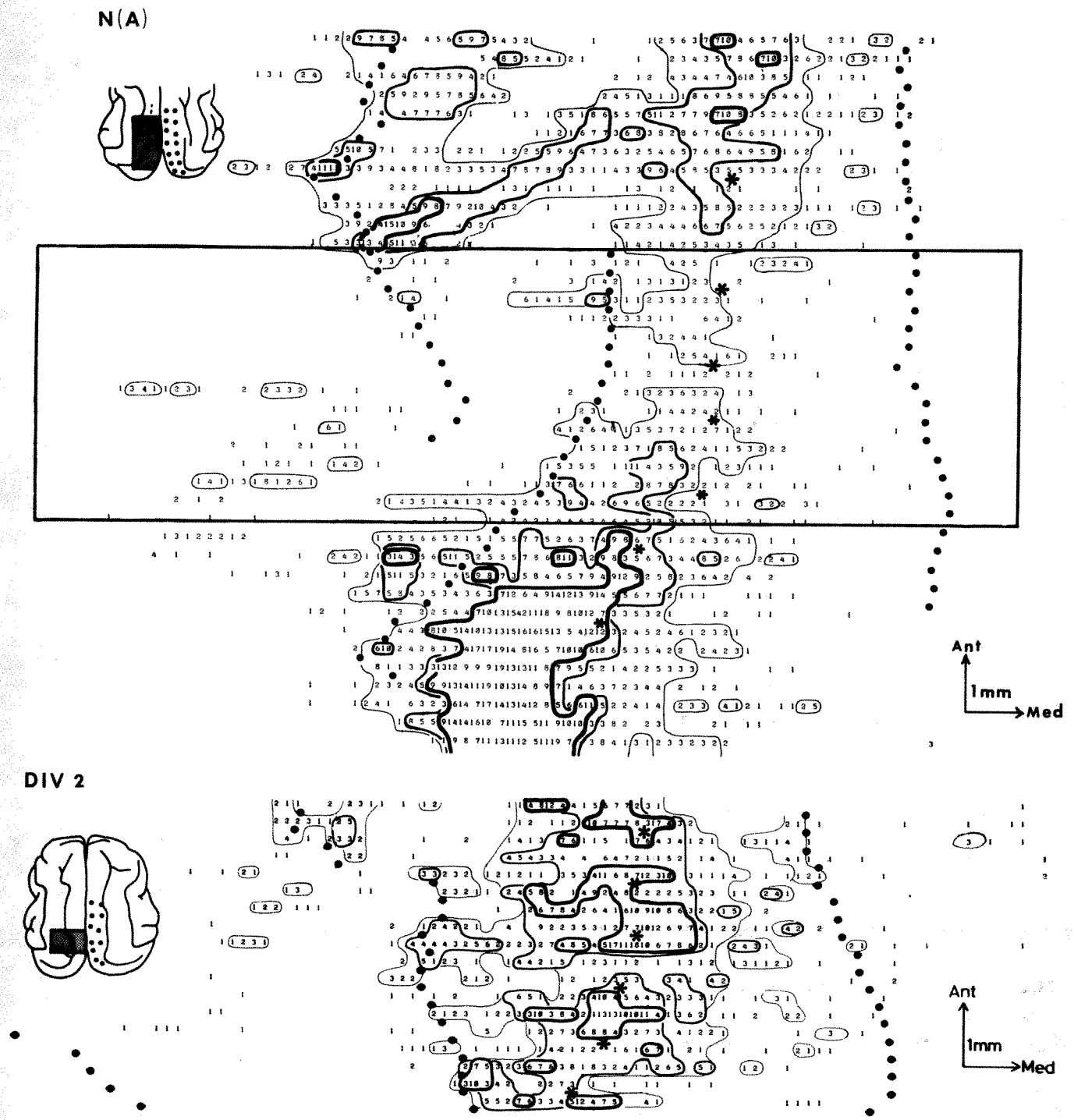


Fig. 2 Computer reconstructions of part of callosal subzone a in a normal adult cat (N(A)) and in a cat raised with divergent strabismus (DIV 2), processed with *O*-dianisidine. Flattened views of the postlateral and lateral gyri (stippled area in dorsal view insets). Dotted lines indicate, from left to right, the fundi of the lateral, postlateral and suprasplenial sulci. A rarely seen branch of the postlateral sulcus was found in DIV 2. The asterisks mark the boundary of areas 17 and 18. The part of the reconstruction of brain N(A) within the rectangle corresponds in rostrocaudal coordinates to the part reconstructed for DIV 2. The neurones in layers II–IV of each section were projected onto a line running parallel to the pial surface and 400 μm deep; the line was then divided into bins of 200 μm. Each horizontal row of numbers in the reconstruction represents the counts of labelled cells for each bin of one section. Contour lines of increasing thickness are drawn around regions where the summed count for any two adjacent bins is 1, 2 and 4 times, respectively, the average count for individual bins (all bins containing at least one labelled neurone were used to obtain this average). The sections were spaced 320 μm apart in N(A) and 280 μm apart in DIV 2. Because of this, the scales of the rostro-caudal axes in the two reconstructions are different. Sites of injection are marked by dots on the insets. For each animal, the homogeneity of the injection site was judged from that of retrograde labelling in the ipsilateral lateral geniculate body.

Table 1 Experiments on the effects of abnormal visual experience on the development of the CZ

Animal	Age at first operation and at death‡	Angle of squint (deg)
BD 1	7; 128	
BD 2	6; 141	
MD 1*	8; 173	
MD 2*	8; 146	
CON 1†	8; 250	26
CON 2†	9; 246	39
DIV 1†	9; 288	33
DIV 2	6; 349	45
MEE 1*	1; 124	

BD, Binocular lid suture; MD, monocular lid suture; CON, bilateral section of lateral rectus muscles (convergent strabismus); DIV, bilateral section of medial rectus muscles (divergent strabismus); MEE, monocular enucleation. Angle of squint was determined by ophthalmoscopic projection of retinal landmarks in paralysed animals (in normal animals about 3.50 (ref. 25)).

* Injected contralateral to open (remaining) eye.

† Electrophysiological experiment.

‡ Day of birth = day 0; eye opening normally around day 8.

sulcus; its lateral boundary is irregular and susceptible to individual variation. However, callosal neurones are generally found along the medial banks and sometimes down to the fundi of the lateral and postlateral sulci.

At the ages at which experimental rearing was started, callosal neurones are found throughout areas 17 and 18 (Fig. 1, N(8)) and (especially in subzone c) are more densely packed than in the adult. In normal animals the callosal neurone distribution is apparently mature at 3 months (Fig. 1, N(90)), 34 days before our youngest kitten was killed.

In the binocularly deprived cats (Fig. 1, BD 2) the mediolateral and radial distributions of callosal neurones seem to be very similar to those of normal adults. The CZ may be slightly narrower and callosal neurones a little less densely packed than in normal cats; additional quantitative data are necessary to decide this point.

In all other experimental animals (Fig. 1, right-hand column), subzone a extends further medially in area 17 than in binocularly deprived and normal animals. Callosal neurones are found along the medial bank of area 17 and reach the fundus of the suprasplenial sulcus along almost all its rostrocaudal extent. In CON 1 and DIV 2 a few neurones are also found in the part of area 17 between the suprasplenial and splenial sulci and in the dorsal bank of the latter (see Figs. 1, 2). Because of the normal irregularity and variability of the lateral border of the CZ, it is difficult to determine whether the CZ is also wider than normal in area 18. It is not narrower, so that the presence of callosal neurones in the medial part of area 17 cannot be ascribed to an overall medialward displacement of the CZ.

In the monocularly enucleated cat (Fig. 1, MEE 1) subzone c contains more callosal neurones than in normal or in the other experimental animals.

These results indicate that visual experience can affect which juvenile callosal neurones (or axons) are stabilised and/or which are eliminated, although it is possible that it induces formerly non-callosal neurones to produce callosal axons. The present limited effects suggest either that we did not find the most effective paradigm of visual deprivation or, more likely, that information of non-visual origin is also used in the development of the CZ. There seem to be two possible sources for such information: (1) the topographic (or retinotopic) organisation of structures projecting to or receiving from the cortex containing the callosal neurones; (2) an innate programme within the callosal neurones themselves.

The abnormal distribution of callosal neurones and terminals in Siamese cats[2,8] seems to support the first possibility. However, the development of callosal connections in binocularly deprived Siamese cats must be investigated to differentiate the effects of genetic abnormalities in the retino-geniculo-cortical topology from those of strabismus, which also affects this genotype. The mechanisms, time course and site of action of factors influencing the development of the CZ remain to be determined. The enlargement of the CZ may be related to the almost complete loss of binocular responsiveness of cortical neurones provoked by strabismus[7], monocular deprivation[9] or enucleation.

The reduction of the CZ is probably one aspect of a more general ontogenetic diminution of neurones and/or neuronal connections (for examples see refs 10–13). Our results and those obtained in Siamese cats[2,8], chicks[14] and monkeys[13,15] suggest that transitory neonatal connections can provide a substrate for the plasticity of neuronal connectivity. However, after transitory callosal neurones have been eliminated modifications of callosal terminals may still occur[16].

One functional consequence of the enlarged CZ is related to the fact that the tangential position of callosal neurones determines their receptive field location. Thus, the receptive fields of callosal neurones in the expanded CZ probably include an abnormally large portion of the visual field. However, they should still be concentrated near the vertical meridian[8,17–19], represented at the 17/18 border[20,21].

Callosal neurones are thought to integrate the cortical representation of the visual field and subserve binocular interactions contributing to stereopsis and possibly eye movements (for reviews see refs 22, 23). In the amphibian *Xenopus laevis*, binocular vision seems to be subserved by intertectal connections whose development is affected by visual experience[24], permitting compensation for normal or experimentally induced changes in ocular alignment. It is attractive to view the modifications we observe in the cat CZ as partial compensation for the disturbance of the binocular visual field, although the adaptiveness of the changes remains to be demonstrated.

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Note added in proof: Three more kittens deprived of vision by bilateral eyelid closure for 1–4 months after birth have now been examined. In all these animals MRP-labelled callosal neurones were fewer than in normal cats and showed radial and tangential distributions similar to that of binocularly deprived animals described above. Quantitative analysis of the reduction in number of callosal neurones following binocular deprivation is under way.

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Electroconvulsive shock treatment decreases β-adrenergic receptor sensitivity in rat brain

ELECTROCONVULSIVE SHOCK (ECS) is one of the most effective forms of therapy available for patients with depression[1]. Its mechanism of action, however, is not clear. Like other antidepressants, ECS probably augments the postsynaptic availability of neurotransmitters, for it increases the release and turnover of noradrenaline (NA)[2–4], decreases NA uptake[5], and raises the activity of tyrosine hydroxylase. Also, synthesis of cyclic AMP stimulated by NA (NA-sensitive adenylate cyclase) is reduced in brain tissues obtained after chronic treatment with ECS[6,7]. It has been reported that chronic administration of antidepressant drugs reduces β-adrenergic receptor density in the rat brain[8]. Because ECS is another form of treatment for depressive illness, we examined whether chronic administration of ECS has effects similar to those of other antidepressants on β-adrenergic receptor sensitivity in rat brain. We report here that we observed a significant decrease in β-adrenergic sensitivity following chronic ECS treatment. The relationship between such observed effects and clinical response requires further investigation.

Binding studies with radiolabelled ligands like ^{3}H-dihydroalprenolol (^{3}H-DHA) or ^{125}I-hydroxybenzylpindolol (β-adrenergic antagonists) have been used for identification and characterisation of β-adrenergic receptors in the brain[9–11] and other tissues[12]; thus, they provide a method for direct quantitation and study of the kinetic properties of these receptors.

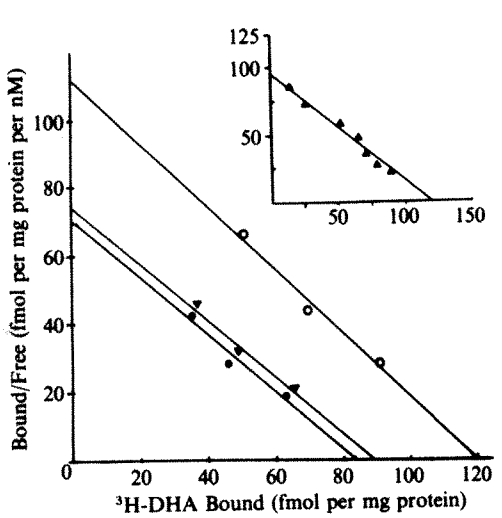


Fig. 1 Scatchard analysis of specific ^{3}H-DHA binding to the brain particulate fractions obtained from control rats (○), DI-treated rats (●) and ECS-treated rats (▲). The experimental conditions are described in Table 1 and each point is the mean obtained from six animals. Receptor density (B_{max}) and dissociation constant (K_D) were calculated from this figure and are shown in Table 1. Inset: Experimental conditions are described in Table 1. The specific ^{3}H-DHA binding to particulate fractions was determined as a function of increasing ^{3}H-DHA concentrations (0–4.5 nM) in a control rat. Scatchard analysis of the obtained data as shown in the inset gave a B_{max} of 115 fmol ^{3}H-DHA bound per mg protein and an apparent K_D of 1.12 nM. This experiment is one of several experiments carried out in identical conditions and giving similar results.

Table 1 Effect of chronic treatment with desipramine and electroconvulsive shock on receptor density and dissociation constant of β-adrenergic receptors in rat brain homogenates

Treatment	Receptor density (fmol per mg protein)	% of control	*P* values as compared with control	Dissociation constant (nM)
Control ($n=6$)	119±8.8			1.06±0.05
Desipramine ($n=6$)	84±3.5	71	<0.001	1.19±0.06
ECS ($n=6$)	87±3.5	73	<0.001	1.14±0.06

Male Sprague–Dawley rats (100–150 g) were subjected to ECS (75 mA for 1 s through transcorneal electrodes) once daily for 12 d. Another group was injected with despiramine (10 mg per kg, intraperitoneally) once daily for the same period. Control rats were injected with normal saline and were handled similarly to the ECS rats, but did not receive shocks. The rats were killed 1 h after the last treatment, and the cortices were removed, homogenised in 50 volumes (w/v) of Tris buffer (0.05 M, *p*H 7.4), and spun at 49,000*g* for 10 min. This process was repeated, and the pellet was finally suspended in Tris buffer. Binding of ^{3}H-DHA was carried out in triplicate by incubation of an aliquot of the suspension (0.3–0.5 mg protein) with ^{3}H-DHA at three concentrations (between 0.4 and 4 nM) in Tris buffer in a final volume of 1 ml, with or without 1 μM (−)propranolol, at 23 °C for 15 min. ^{3}H-DHA bound to the membrane was separated by filtration through a Whatman (GF/F) filter and washed three times with 4 ml buffer. The filter was placed in a BBS cocktail and counted in a Nuclear Chicago scintillation spectrometer. Specific binding is defined as the difference between total binding and the binding observed in the presence of 1 μM (−)propranolol. Receptor density, as shown in Fig. 1, was calculated by Scatchard analysis, and is the intercept on the *x* axis (B_{max}). K_D values were calculated by Scatchard analysis; *P* values were obtained by paired *t*-test (two tail).

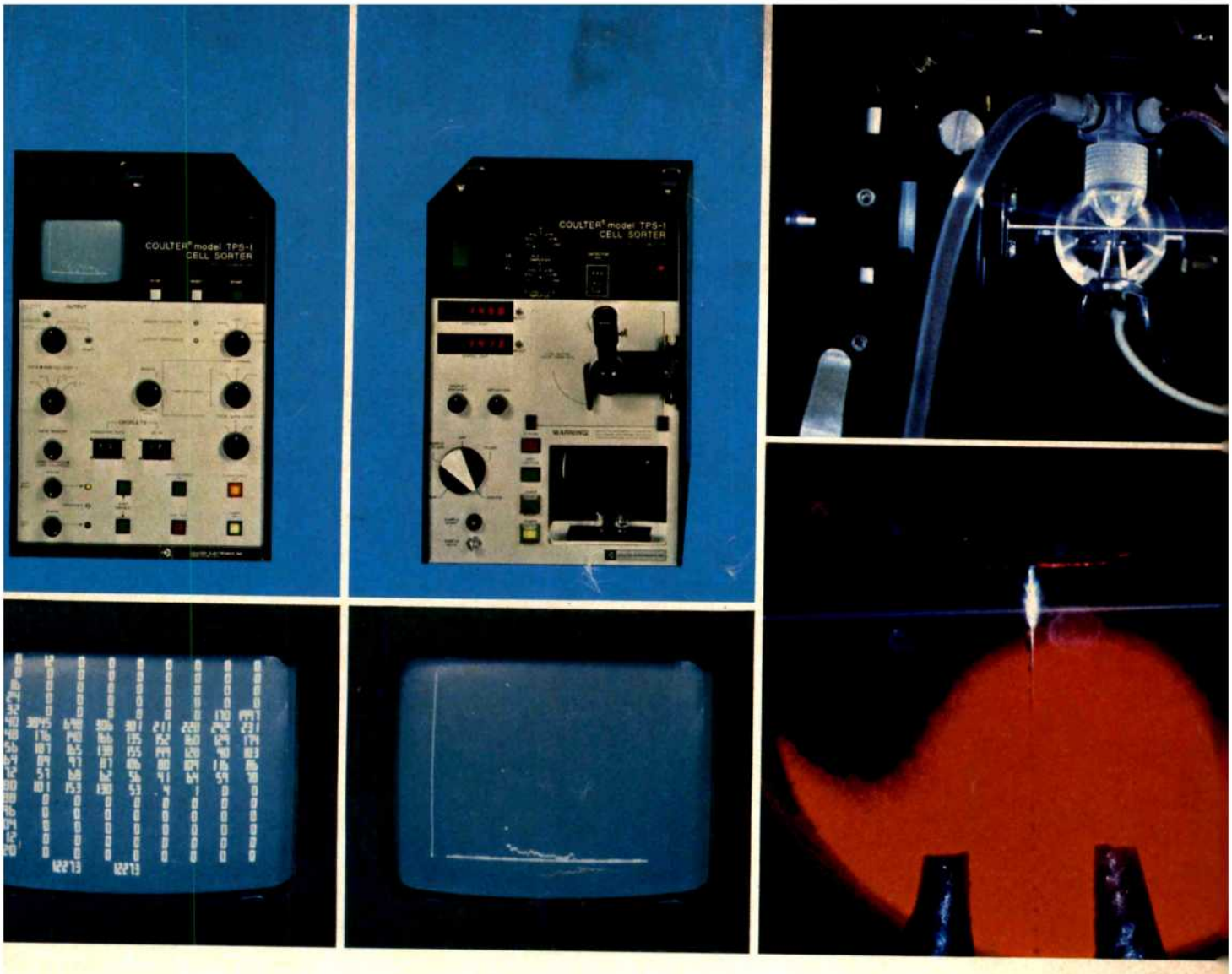
We therefore measured the binding ^{3}H-DHA to particulate fractions prepared from rat cerebral cortex. The method used was essentially that of Bylund and Snyder[9], and we obtained binding characteristics similar to those they reported. The binding was rapid, stereospecific to β-adrenergic agonists and antagonists and showed saturation kinetics similar to those reported. Scatchard analysis of the ^{3}H-DHA binding to rat cerebral cortex resulted in a straight line, indicating a single class of high-affinity receptors (Fig. 1, inset). The maximum number of binding sites (receptor density) observed (115 fmol per mg protein) and the observed apparent dissociation constant (1.12 nM) were also similar to those previously reported[8,9].

The effects of chronic treatment with ECS and desipramine (DI) on β-adrenergic receptor sensitivity (Table 1) were assessed by determining the maximum number of ^{3}H-DHA binding sites by Scatchard analyses (Fig. 1). Binding of ^{3}H-HDA to particulate fractions obtained from the cortex was carried out at three different concentrations of ^{3}H-DHA. The observed mean β-adrenergic receptor density for the control rats was 119±8.8 (fmol ^{3}H-DHA bound per mg protein). DI caused a 29% reduction in receptor density ($P<0.001$), in agreement with the results reported by Banerjee *et al.*[8]. Treatment with ECS resulted in a 27% decrease ($P<0.001$) in density. The mean apparent dissociation constants (K_D) of ^{3}H-DHA binding, determined by Scatchard analysis (Fig. 1), were similar for the treated and control rats (Table 1), indicating that the receptor density changes were not related to alterations in the affinity of receptors.

These results thus indicate that chronic ECS treatment produces a reduction in the sensitivity of β-adrenergic receptors in rat brain. This effect is similar to the effects of other antidepressants, for example, DI, presented here, and DI, doxepin and iprindole, reported by Banerjee *et al.*[8]. The results also suggest that a decrease in NA-stimulated synthesis of cyclic AMP in rats chronically treated with ECS, as reported previously[6,7], may be related to a decrease in β-adrenergic receptor sensitivity.

Treatment with ECS causes increased release and turnover of NA[4,5,13] and possibly of dopamine in various areas of the rat brain. Tricyclic antidepressants, on the other hand, inhibit reuptake of biogenic amines in synaptosomal preparations[14], and drugs like pargyline and phenelzine inhibit monoamine oxidase activity. These antidepressants have different modes of action

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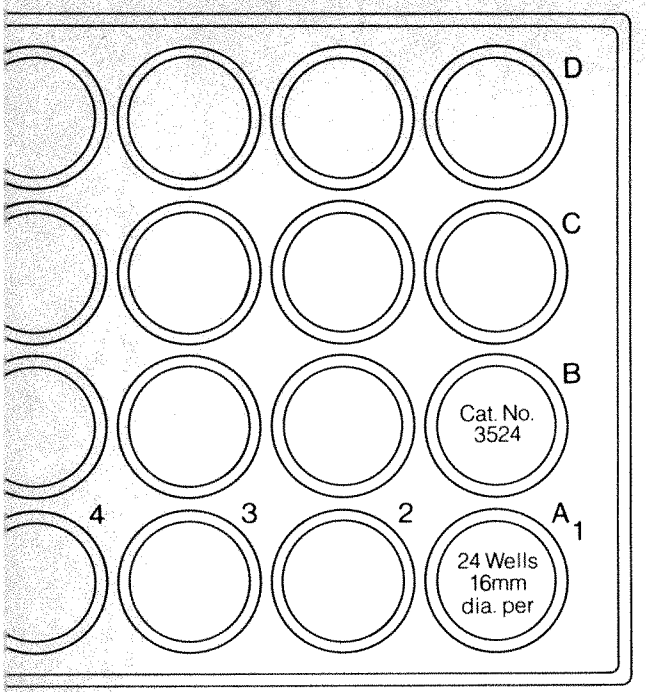
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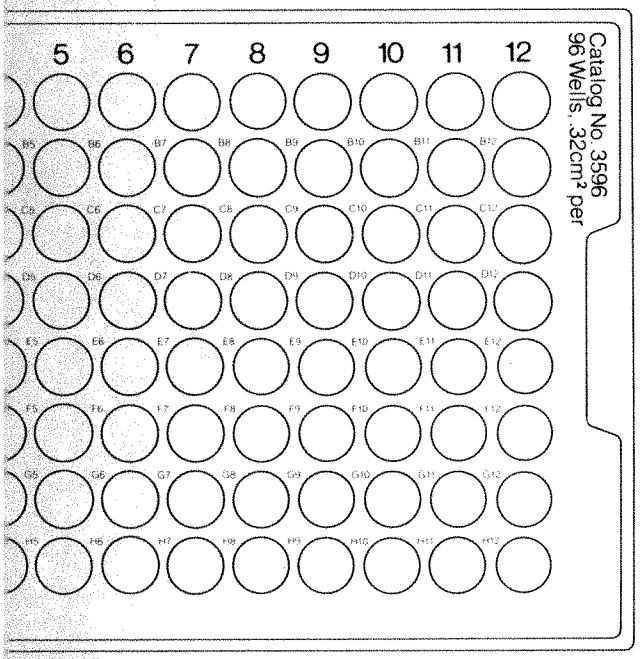
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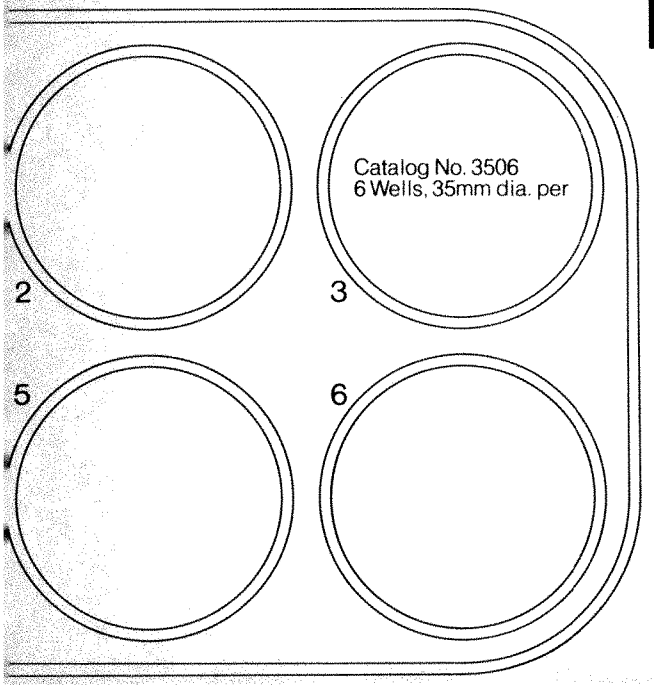
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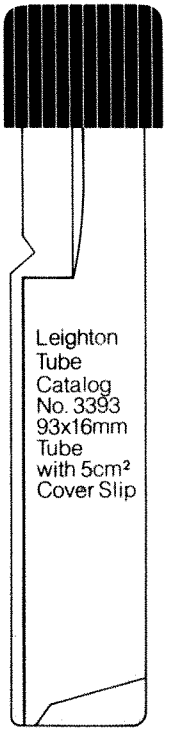
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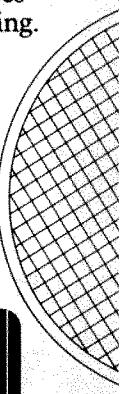
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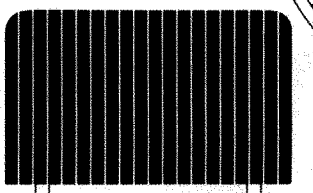


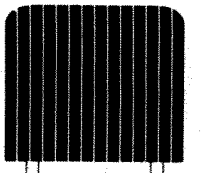




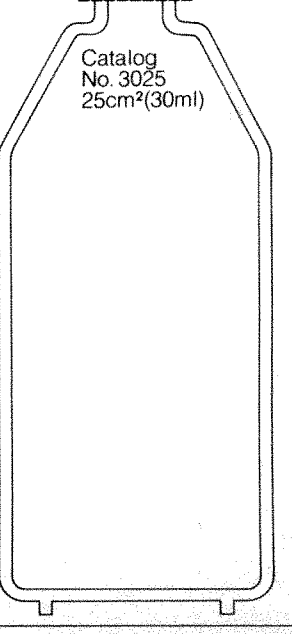






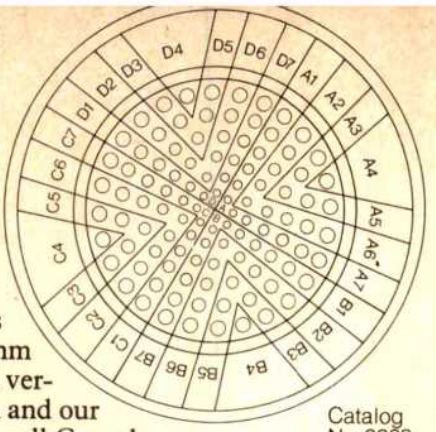




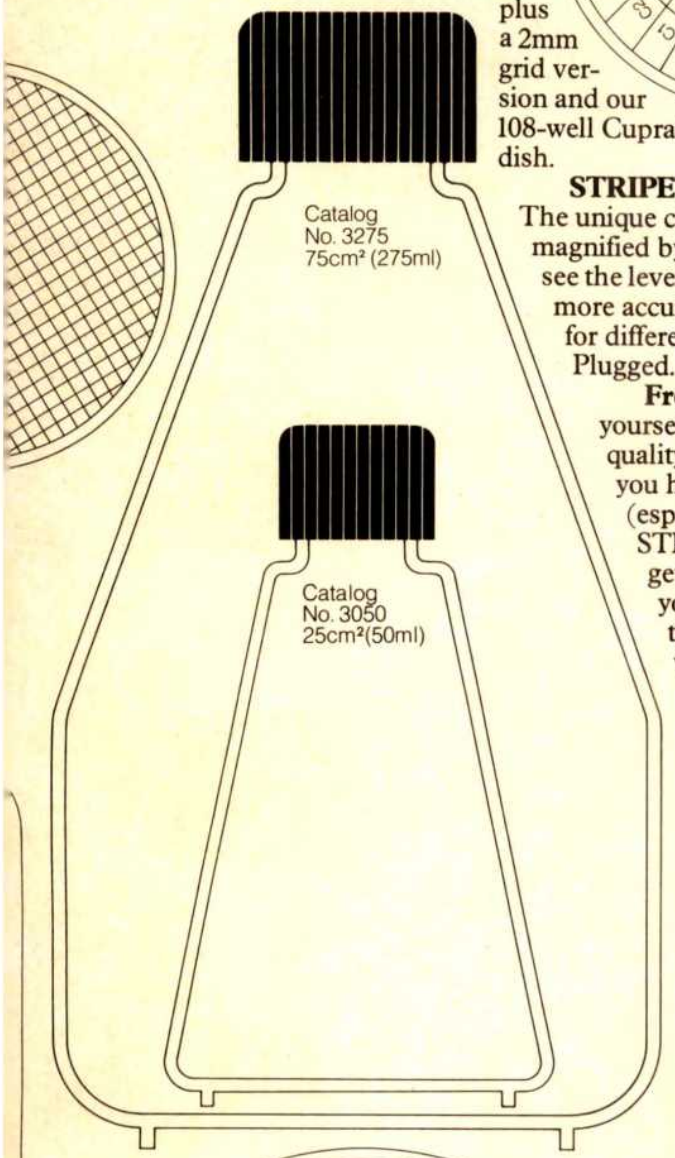


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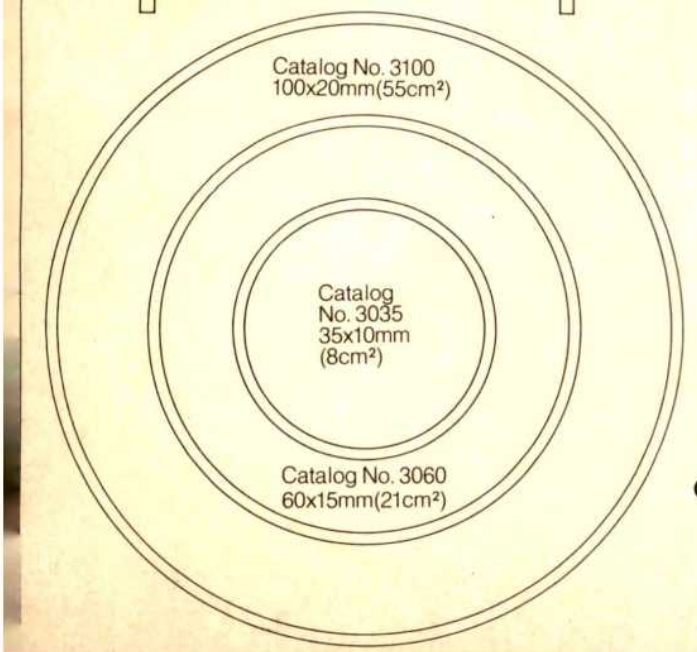


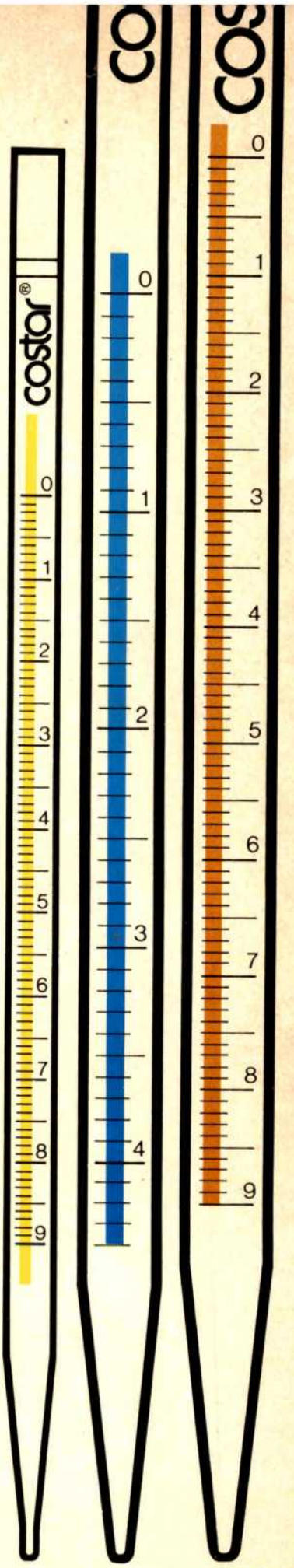
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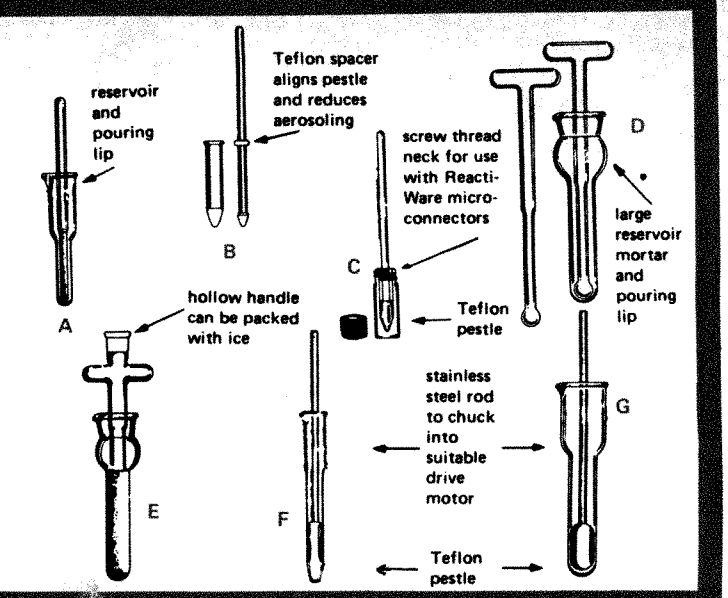
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E	TENBROECK, *all glass*	1	11 x 48	140	0.1 - 0.15
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on the biogenic amine system; however, all increase the biogenic amine concentration in the postsynaptic area, and reduce the sensitivity of β-adrenergic receptors[8] and the responsiveness of adenylate cyclase to NA, as reported by others[6,7,15,16] and presented here. Furthermore, Deguchi and Axelrod[17] and others[12] have suggested that continued exposure of adenylate-cyclase-coupled β-receptors to elevated levels of agonists lowers the sensitivity of the receptors; we might therefore assume that loss of β-adrenergic receptor sensitivity on chronic treatment with antidepressants is caused by overexposure of postsynaptic β-adrenergic receptors to elevated levels of NA. However, iprindole has no significant effect on re-uptake, release or turnover of catecholamines[18], and it is not certain whether it increases the levels of NA in the synaptic cleft; like other antidepressants, it reduces β-adrenergic receptor sensitivity[8] and decreases NA responsiveness of adenylate cyclase[16]. Therefore, as suggested by Banerjee *et al.*[8], other mechanisms may also be involved in the development of the reduction in β-adrenergic receptor sensitivity.

The clinical effects of antidepressants are observed only after 1–3 weeks of treatment. As the effects of antidepressants on β-adrenergic receptor sensitivity are observed after a similar treatment period, the two may be closely related. However, the time course of development of reduced β-adrenergic receptor sensitivity must be studied before such a relationship can be confirmed. It is also possible that the effects of antidepressants on β-adrenergic receptor sensitivity are secondary to their effects on the catecholamine systems.

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How does adrenaline accelerate the heart?

THE way in which adrenaline acts on the sinoatrial (SA) node to accelerate the heart rate has hitherto been obscure. However, in various other parts of the heart adrenaline increases the slow inward (Ca^{2+}/Na^{+}) current[1–4], and voltage-recording experiments have indicated that adrenaline also has this action in the sinus region[5–7]. In the voltage-clamp experiments reported here, we find that adrenaline does indeed increase the slow inward current in the SA node of the rabbit, but that it also augments the outward current which would tend to decelerate pacemaker depolarisation. We find that an additional current, i_t, is activated within the range of voltage where the pacemaker depolarisation occurs: this could be important both in normal pacemaking and in adrenaline-induced acceleration.

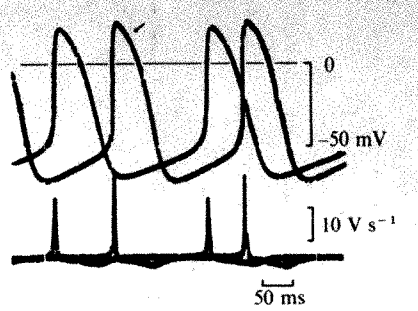


Fig. 1 Intracellular record of spontaneous activity in the rabbit SA node before and during (trace indicated by arrow) perfusion with adrenaline, 5×10^{-8} M. Lower trace shows the rate of change of voltage. In this and in the following figures traces have been recorded on tape during the experiment and overlapped on a storage oscilloscope on replay.

Figure 1 shows the effect of adrenaline on the intracellular voltage record and the rate of change of voltage (dV/dt) in a small, spontaneously firing preparation (about 0.3 mm × 0.3 mm) from the rabbit SA node. The overshoot of the action potential increases, as does dV/dt during the upstroke. As any fast sodium current present was blocked by the presence of tetrodotoxin (5×10^{-7} g ml^{-1}) these effects are probably caused by an increase in the slow inward current[8]. Figure 1 also indicates that the adrenaline produces a slightly more negative maximum diastolic potential, and an acceleration of the rate of repolarisation, both of which imply an increase of total membrane current in the outward, rather than the inward direction.

Voltage clamp of SA nodal tissue is much more difficult than that of other cardiac tissues; only recently has a method, pioneered by Noma and Irisawa[13], been developed for successfully clamping the mammalian sinus. In this, very small (0.3 mm × 0.3 mm) preparations which are still spontaneously active are dissected from the SA node and voltage clamped using two microelectrodes. Good voltage-clamp results can be obtained over a range of potentials, including the pacemaker range, although in response to large depolarisations and hyperpolarisations voltage control is often lost.

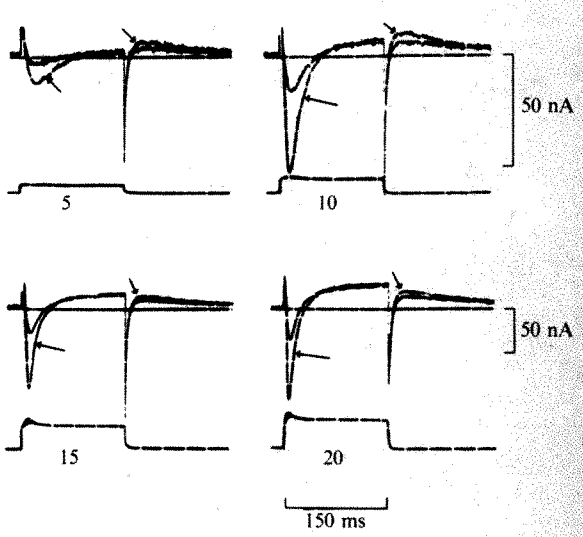


Fig. 2 Increase of $i_{Ca/Na}$ and i_K in adrenaline. Voltage-clamp depolarisations of 5, 10, 15 and 20 mV from a holding potential of −45 mV (lower traces in each panel) were given before and during the action of adrenaline, 5×10^{-7} M. All current traces recorded in adrenaline (indicated by arrows) have been shifted upwards by 2 nA. Solid lines represent zero current.

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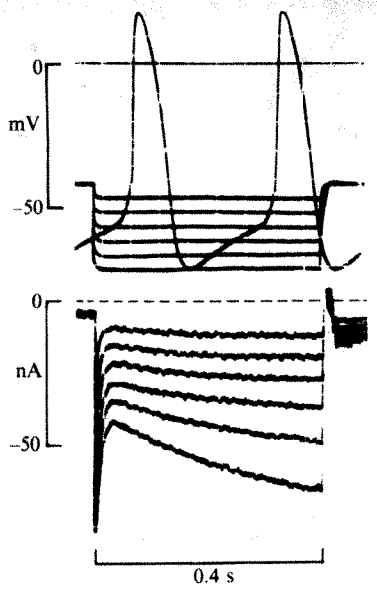


Fig. 3 *a*, Superimposition of pacemaker activity and voltage-clamp hyperpolarisations from a holding potential of −42 mV in the same preparation; *b*, currents recorded during the clamp hyperpolarisations. Note that the time-dependent changes in i_f are greater at more hyperpolarised levels.

With this method it can be shown that in the rabbit SA node adrenaline does indeed increase both slow inward current ($i_{Ca/Na}$) and outward potassium current (the current termed i_K by Difrancesco Noma and Trautwein[10]). Figure 2 shows the increase in peak $i_{Ca/Na}$ current caused by adrenaline during a 10-mV depolarising voltage-clamp pulse and the increase in the i_K-current tail recorded on return to the holding potential of −45 mV. These results correlate with previous findings that adrenaline increases both the slow inward and the plateau outward current in other cardiac tissues (for review see ref. 11).

As indicated in Fig. 1, the positive chronotropic effect of adrenaline is mediated by an increased rate of pacemaker depolarisation (PD). An increase of i_K will, however, tend to decelerate the PD. Although the larger $i_{Ca/Na}$ would by itself accelerate the PD in its later stages immediately preceding the upstroke, is it unlikely that combined with the augmented i_K it could account for the increased rate of change of voltage during the whole course of the PD seen in adrenaline.

What, then, is the mechanism of the increased pacemaker rate seen in adrenaline? We have found that an additional current, which has been previously noted in the rabbit SA node[9] and in the frog sinus venosus[12] and which we will call i_f, is important both in normal pacemaker activity and in adrenaline-induced acceleration. Hyperpolarising voltage-clamp pulses from a holding potential of −42 mV (Fig. 3) reveal that i_f is a slowly increasing inward (or decreasing outward) current change whose amplitude increases with the clamp pulse amplitude. In Fig. 3 the voltage protocol has been superimposed on a record of spontaneous activity in the same preparation to show the relevance of i_f to the potential range of the pacemaker depolarisation. Addition of adrenaline, 10^{-7} M, causes a substantial and reversible increase in the time-dependent change of i_f, as shown in Fig. 4. In the unclamped preparation such an increase would cause a faster PD, as observed during adrenaline action in Fig. 1.

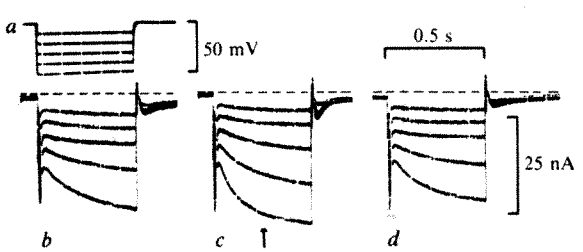


Fig. 4 Increase of i_f in adrenaline. i_f changes are activated by voltage-clamp hyperpolarisations from a holding potential of −36 mV. *a*, Voltage protocol used throughout the experiment; *b*, control; *c*, during adrenaline, 10^{-7} M; *d*, return.

Investigating the ionic nature of the i_f current system is difficult as it is not possible to hyperpolarise the small preparations used by more than 50 mV without risking membrane injury and consequent breakdown of the voltage clamp. We have therefore been unable to determine whether i_f has a reversal potential close to the equilibrium potential for K^+ ions, that is, at about −100 mV, which would show whether or not it could be equated with the i_{K_2} current of the Purkinje fibre. Nevertheless, the resemblance to i_{K_2} is striking: i_{K_2} is deactivated by hyperpolarisations into the same voltage range as i_f and the change in i_f produced by adrenaline strongly resembles that produced in i_{K_2} by a voltage shift of its kinetics. Regardless of whether it becomes possible to test this resemblance further experimentally, it is already clear that i_f has an important role both in normal rhythmic activity in the SA node and in mediating the response to adrenaline.

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Role of the dendritic release of dopamine in the reciprocal control of the two nigro-striatal dopaminergic pathways

USING cats implanted with push–pull cannulae, we have previously demonstrated a reciprocal regulation of the activity of the two nigro-striatal dopaminergic pathways. Asymmetric fluctuations in the spontaneous release of dopamine(DA) were simultaneously seen in the two caudate nuclei (CN)[1]. As revealed by the changes in DA release from nerve terminals, the unilateral nigral application of DA[1] or of drugs enhancing the dendritic release of DA, such as amphetamine or benztropine (2), reduced the activity of ipsilateral dopaminergic neurones and induced an opposite effect in the contralateral side. Conversely, the unilateral nigral blockade of dopaminergic transmission by local application of neuroleptics enhanced the release of DA in the ipsilateral CN and decreased the transmitter release in the contralateral structure[2]. Opposite changes in the release of DA were also seen in the two CN under unilateral delivery of sensory stimuli[3] or during unilateral electrical stimulation of the cerebellar dentate nucleus[4]. These latter effects were associated with asymmetric changes in the dendritic release of DA in the two substantia nigrae (SN) which were in an opposite direction to those seen in the two CN (an increased

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release of DA from nerve terminals corresponded to a decreased release of the transmitter from dendrites and vice versa). These experiments indicated that DA which is released from dendrites in one SN regulates the activity of the ipsilateral dopaminergic neurones; they also suggested that it contributes to the control of the contralateral dopaminergic neurones by influencing the dendritic release of DA in the contralateral SN. This hypothesis was confirmed in the present study by measuring the changes in DA release from nerve terminals and dendrites of the two pathways under pharmacological blockade or facilitation of dopaminergic transmission in one SN.

Adult cats of both sexes were anaesthetised with halothane and implanted with four push–pull cannulae (0.9 mm diameter), one in each SN and each CN, to measure continuously the release of ^{3}H-DA synthesised from ^{3}H-tyrosine, as previously described[5]. An artificial cerebrospinal fluid (CSF) containing ^{3}H-tyrosine (50 Ci mmole^{-1}, 50 μCi ml^{-1}) was introduced in each structure at a rate of 1 ml h^{-1} and ^{3}H-DA was estimated in serial 10-min superfusate fractions. ^{3}H-DA was separated from ^{3}H-tyrosine and ^{3}H-labelled metabolites by ion-exchange chromatography and alumina adsorption. α-Methyl-*p*-tyrosine (α-MT, 10^{-4}M), an inhibitor of DA synthesis; or (+)amphetamine (10^{-6} M) were applied to the left SN 22 h after the onset of superfusion of the four structures with ^{3}H-tyrosine to block or facilitate local dopaminergic transmission, respectively.

The unilateral nigral application of α-MT for 60 min markedly stimulated the release of ^{3}H-DA in the ipsilateral CN and induced an opposite effect in the contralateral structure. Moreover, in contrast to that observed in the ipsilateral side, this treatment stimulated the dendritic release of ^{3}H-DA in the contralateral SN (Fig. 1). The unilateral nigral application of (+)amphetamine for 10 min not only induced asymmetric changes in the release of ^{3}H-DA in both CN (as previously shown[2]), but also in both SN. These effects were in opposite directions to those induced by αMT (Fig. 2).

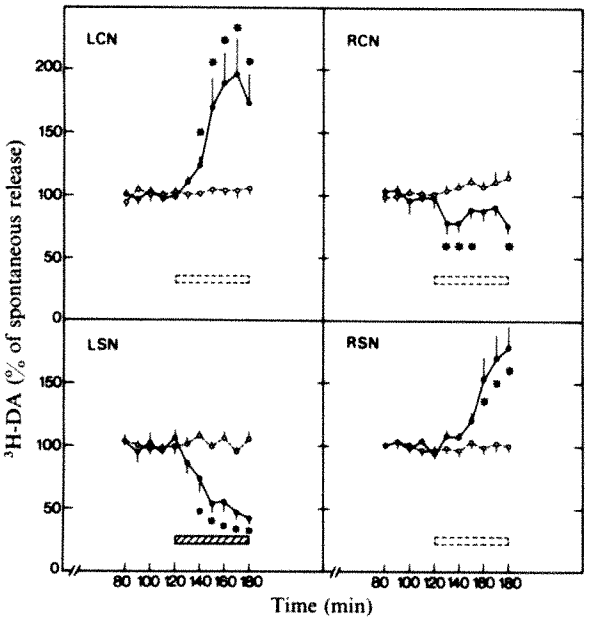


Fig. 1 Effects of α-methyl-*p*-tyrosine application into the left substantia nigra on the release of ^{3}H-dopamine from the two caudate nuclei and the two substantia nigrae. Four push–pull cannulae were simultaneously implanted in the left (LCN) and the right (RCN) caudate nuclei and in the left (LSN) and the right (RSN) substantia nigrae in anaesthetised cats. The four structures were perfused with an artificial CSF containing L[3,5-^{3}H]tyrosine (50 Ci mmol^{-1}, 50 μCi ml^{-1}, 1 ml h^{-1}. ^{3}H-Dopamine (^{3}H-DA) was estimated in 10-min successive superfusate fractions. The average quantities of ^{3}H-DA released in superfusate fractions of the SN (0.4 nCi) and the CN (0.6 nCi) were, respectively, 20 and 30 times the blank value. α-Methyl-*p*-tyrosine (α-MT, 10^{-4} M) was introduced for 60 min into the CSF superfusing the LSN (hatched bar). In each animal and for each cannula, ^{3}H-DA in each successive fraction was expressed as a percentage of an average spontaneous release calculated from the five fractions collected before the treatment. Data are the mean ± s.e.m. of results obtained with five animals (—•—). $*P < 0.05$ when compared with corresponding control values obtained in five untreated animals (---○---). ▨, α-MT application.

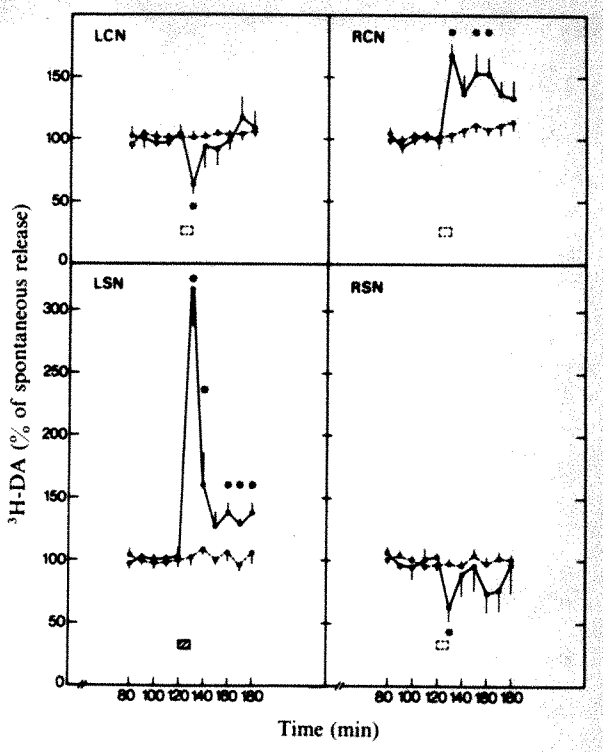


Fig. 2 Effects of (+)amphetamine application into the left substantia nigra on the release of ^{3}H-dopamine from the two caudate nuclei and the two substantia nigrae. Five animals were treated as described in Fig. 1 except that (+)amphetamine (10^{-6} M) was applied for 10 min into the left substantia nigra (LSN). Data are calculated and expressed as in Fig. 1. $*P < 0.05$ when compared with corresponding control values obtained in five untreated cats (---○---). ▨, (+)Amphetamine application.

To confirm that the contralateral effects induced by the unilateral application of α-MT or (+)amphetamine were mediated by the changes in DA release induced in the ipsilateral SN and were not secondary to those observed in the ipsilateral CN, these drugs were applied to the left CN and ^{3}H-DA was simultaneously measured in both CN and both SN. α-MT (10^{-4}M) and (+)amphetamine(10^{-6}M) which, respectively, reduced and enhanced the local release of ^{3}H-DA in the left CN, failed to change ^{3}H-DA release in the contralateral CN or SN (Figs. 3, 4). Also, marked changes in the dendritic release of ^{3}H-DA were seen in the ipsilateral SN (Figs. 3, 4). They were parallel to those detected in the CN, as the dendritic release of ^{3}H-DA was reduced by α-MT and enhanced by (+)amphetamine.

Several conclusions on the influences of DA can be drawn from the present study and are summarised in Fig. 5. First, the activation of ipsilateral dopaminergic neurones induced by the unilateral application of α-MT demonstrates that DA released from dendrites tonically inhibits the activity of the ipsilateral neurones. This may mainly result from a reduced availability of DA at dopaminergic receptor sites located either on dopaminergic cells or their dendrites[6,7] or on nigral afferent fibres[8,9] (Fig. 5*a*). Second, the effects of α-MT and (+)-amphetamine on the dendritic release of ^{3}H-DA imply that these drugs, respectively, stimulated or inhibited the firing rate of the ipsilateral dopaminergic neurones through their effects on

he dendritic release of DA. Thus, the changes in DA dendritic elease induced by local modifications of the dopaminergic ransmission in the ipsilateral CN further support the postuated[10] existence of a striato-nigral neuronal loop controlling the ctivity of the dopaminergic neurones (Fig. 5*b*). Third, the symmetric changes in DA release seen in both CN under the nilateral application of various stimuli[1-4] cannot be related to odifications of ^{3}H-DA release in one of the CN (Fig. 5*c*). ndeed, the changes in ^{3}H-DA release from nerve terminals in ne CN induced by the local application of α-MT or (+)-mphetamine did not affect ^{3}H-DA release in the contralateral N (Figs 3, 4). We therefore postulate that the asymmetric hanges in DA release observed in the two CN result from the hange in the dendritic release of DA occurring in one SN (Fig. *d* or *e*). This is well illustrated by the experiments shown in Figs and 2. DA released from dendrites in one SN must thus

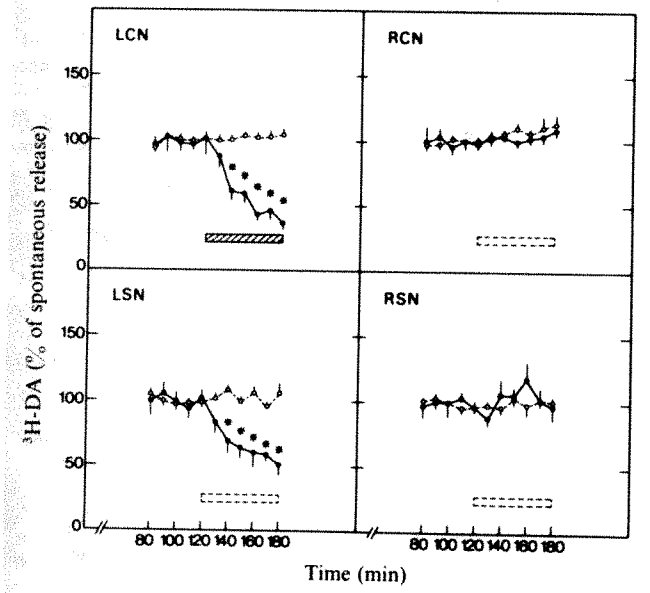


Fig. 3 Effects of α-methyl-*p*-tyrosine application into the left caudate nucleus on the release of ^{3}H-dopamine from the two caudate nuclei and the two substantia nigrae. Five animals were treated as described in Fig. 1 except that α-methyl-*p*-tyrosine (α-MT, 10^{-4} M) was applied for 60 min into the left caudate nucleus (LCN). Data are calculated and expressed as in Fig. 1. $*P<0.05$ when compared with corresponding control values obtained in five untreated cats (---○---). ▨, α-MT application.

egulate the activity of a nigral efferent pathway involved in the ontrol of the activity of the contralateral dopaminergic eurones. This could occur as DA acts not only on the opaminergic neurones in the pars compacta but also on other eurones in the pars reticulata[11]. DA could also indirectly affect he activity of the pars reticulata neurones by modifying the elease of transmitters from nigral afferent fibres[12]. As cells of he pars reticulata project to several structures in the brain[13-17], he pathway involved remains to be determined. However, we an still discuss whether the final input of this pathway occurs in ie contralateral SN (Fig. 5*e*) or contralateral CN (Fig. 5*d*). ourth, the effects of unilateral nigral αMT or (+)amphetamine idicate that the asymmetric changes in the dendritic release of)A in both SN are responsible for the opposite asymmetric anges in DA release in both CN. Indeed, the changes in the endritic release observed in the right SN during the application f these drugs in the left SN cannot be related to the opposite anges in ^{3}H-DA release from nerve terminals seen in the ntralateral CN (Fig. 5*d*). If this was the case, parallel changes ould occur in the CN and the SN as observed during the local plication of (+)amphetamine or α-MT in the CN (Figs 3, 4). s there is apparently no direct anatomical connection between

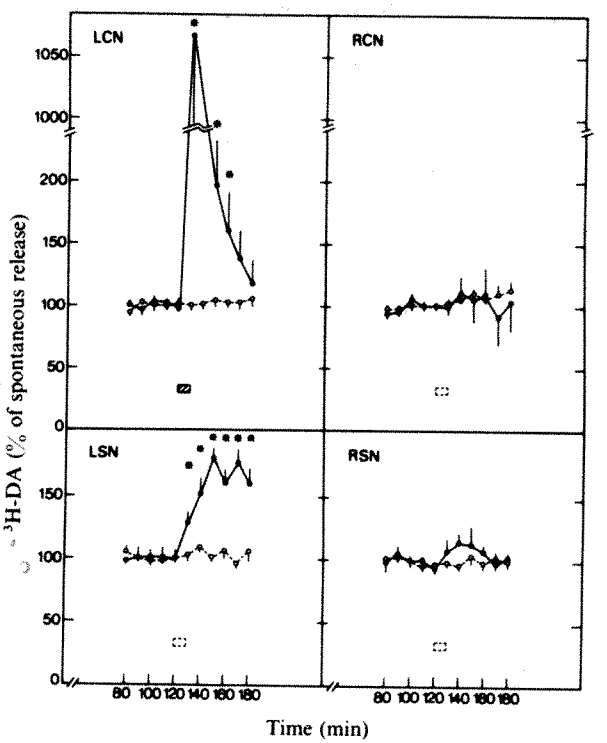


Fig. 4 Effects of (+)amphetamine application into the left caudate nucleus on the release of ^{3}H-dopamine from the two caudate nuclei and the two substantia nigrae. Seven animals were treated as described in Fig. 1 except that (+)amphetamine (10^{-6} M) was applied for 10 min into the left caudate nucleus (LCN). Data are calculated and expressed as in Fig. 1. $*P<0.05$ when compared with corresponding control values obtained in five untreated cats (---○---). ▨, (+)Amphetamine application.

the two SN, a polysynaptic pathway activated or inhibited by DA released from dendrites in one SN must be involved in the reciprocal control of the dendritic release of DA in the contralateral SN and thus in the regulation of the activity of the contralateral dopaminergic neurones (Fig. 5*e*).

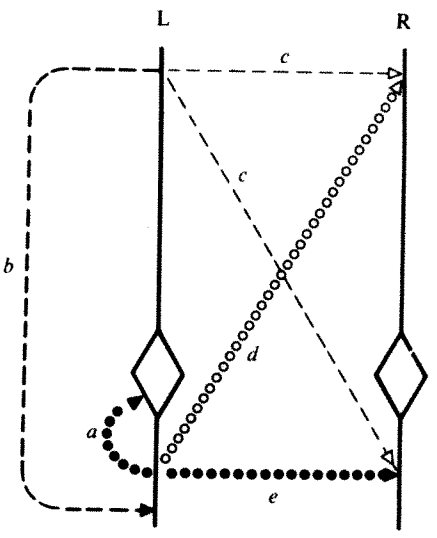


Fig. 5 Influences of dopamine released from dendrites and nerve terminals. Possible direct and indirect influences of dopamine (DA) released from nerve terminals (– – –, – – –) or dendrites (○, ●) of the left (L) dopaminergic neurones on both nigro-striatal dopaminergic pathways (L, R). Our results indicate the existence of the influences *a*, *b* and *e*, and exclude *c* and *d* (see text).

Finally, we should consider why no contralateral effects were observed during the local application of α-MT or (+)amphetamine in the left CN despite the changes in the dendritic release of ^{3}H-DA induced in the ipsilateral SN. Recent experiments suggest that the striato-nigral substance P neurones regulate the activity of the dopaminergic neurones by acting on DA dendritic release[18]. Moreover, in these experiments, there was no effect in the contralateral SN and CN[19], as was the case when dopaminergic drugs were applied in the left CN (Figs. 3, 4). Therefore, the striato-nigral substance P neurones may not only regulate the activity of the ipsilateral nigro-striatal dopaminergic neurones but could also influence the nigral cells involved in the reciprocal regulation of the two dopaminergic systems, and thus counteract the effect of DA on these cells. In fact, substance P nerve terminals are distributed in both the pars compacta and the pars reticulata[20–22] and substance P activates most cells in the SN[23,24]. Furthermore, in contrast to that observed with substance P and its antibody, the unilateral nigral application of agonists or antagonist of γ-aminobutyric acid affected the release of DA in the contralateral CN[25]. These effects were distinct from those induced by dopaminergic drugs, as in all cases the changes in ^{3}H-DA release seen in the two CN were symmetric.

Regarding their role in the coordination of sensory motor processes, it has previously been assumed that the nigro-striatal dopaminergic neurones act merely by modulating the activity of striatal cells. The demonstration of the dendritic release of DA, its important fluctuations in various physiological and pharmacological situations, and its role in regulating the activity, not only of the dopaminergic neurones but also of other nigral efferent pathways, indicate that the nigro-striatal dopaminergic neurones also control the delivery of messages originating from the SN. Such processes, which could be shared by other neurones, will undoubtedly influence our view of the transfer of information in the central nervous system.

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The role of mitogenic lectins in T-cell triggering

THE process of T-lymphocyte activation requires the participation of metabolically active non-θ-bearing accessory cells[1]. As first conclusively shown by Habu and Raff[2], this requirement is also true of lectin-dependent triggering. Thus, T-cell activation by mitogenic lectins depends on the presence of Ia-positive non-T cells[3,4]. The mere binding of lectins such as concanavalin A (Con A) to the surface membrane of T cells does not trigger the cells to go through the mitotic cycle. This can only be achieved by the activity of growth factors present in conditioned media (CM) from Con A-stimulated cell cultures[5]. Such growth factors, however, cannot activate normal, resting T cells, although they are competent to maintain activated T cells in exponential growth for indefinite periods of time[6]. Hence, to be mitogenic a lectin must not only induce the *in situ* production of T-cell growth factors, but must also render resting T cells sensitive to the mitogenic activity of these growth factors. In this report we demonstrate that the binding of Con A to purified T cells can in a relatively short time, modify their functional sensitivity to growth factors, even though this binding is not sufficient to stimulate them to proliferate.

In our initial experiments we have used CM obtained at 24 h from Con A-stimulated spleen cell cultures. CM were always tested for T-cell growth factor activity on cultures of purified T-cell blasts[4]. Normal spleen cells, when cultured in such CM where the lectin has been inactivated (in our experiments by supplementation with the specific inhibitor sugar α-methyl-D-mannoside (α-MM)) are not induced to grow[6] (Figs 1, 2 and Table 1). To demonstrate that direct interaction with mitogenic lectins renders T cells sensitive to growth factors, we incubated normal spleen cells in medium, either alone or with 5 μg ml^{-1} Con A, for various periods of time. The cells were then washed

Table 1 Direct interaction of Con A with T cells induces responsiveness to growth factors by expression of acceptor sites

Expts 1 and 2

Additions to cultures	Spleen cell responses (c.p.m. per culture × 10^{-3}) after: Anti-Ig column Expt 1	Anti-Ig column Expt 2	Anti-I-A treatment and anti-Ig column Expt 1	Anti-I-A treatment and anti-Ig column Expt 2
None	1.6±0.2	1.5±0.2	0.3±0.2	0.4±0.3
Con A	339.3±11.2	165.2±4.9	3.6±0.4	2.6±0.6
CM	ND	3.7±0.3	2.1±0.4	0.8±0.3
Con A+CM	ND	81.4±5.7	79.4±3.9	45.9±3.9

Expts 3 and 4

	T-cell blast response (c.p.m. per culture × 10^{-3}) to CM absorbed on:	Expt 3	Expt 4
None	—	1.8±0.4	0.6±0.4
Con A	—	1.3±0.2	1.3±0.4
CM	—	25.8±2.7	18.6±1.9
CM	Normal spleen cells	19.3±2.5	15.4±2.1
CM	4-h Con A spleen cells	12.9±1.6	7.1±0.9
CM	T-cell blasts	9.2±2.0	0.7±0.2

In expts 1 and 2 normal C3H/HeJ spleen cells either untreated or incubated for 30 min in the cold with monoclonal anti-I-A (clone H-116/32, see ref. 14) antibodies were passed over anti-Ig columns (13) and then stimulated in culture (2×10^5 ml^{-1}) with Con A (5 μg ml^{-1}) or a standard preparation of CM at a 50% (v/v) final concentration. ^{3}H-Thymidine uptake was measured on day 3 of culture. This type of experiment has been reproduced on six independent occasions. In expts 3 and 4 T-cell blasts kept in standard CM for over 2 weeks were cultured (5×10^4 ml^{-1}) in the presence of either Con A (5 μg ml^{-1}) or CM (50%, v/v, final concentration) which had been absorbed for 30 min in the cold with the indicated cells (2×10^8 ml^{-1} CM). Cells which had been exposed to Con A for 4 h were washed five times with 20 mg ml^{-1} α-MM before use in the absorption. T-cell blast responses were measured on day 2 after initiation of culture. Mixing experiments showing that the loss of activity in absorbed CM is not due to inhibitory activities have been carried out and partially presented elsewhere [6]. All results are shown as the mean values and s.d. from triplicate cultures. ND, not determined.

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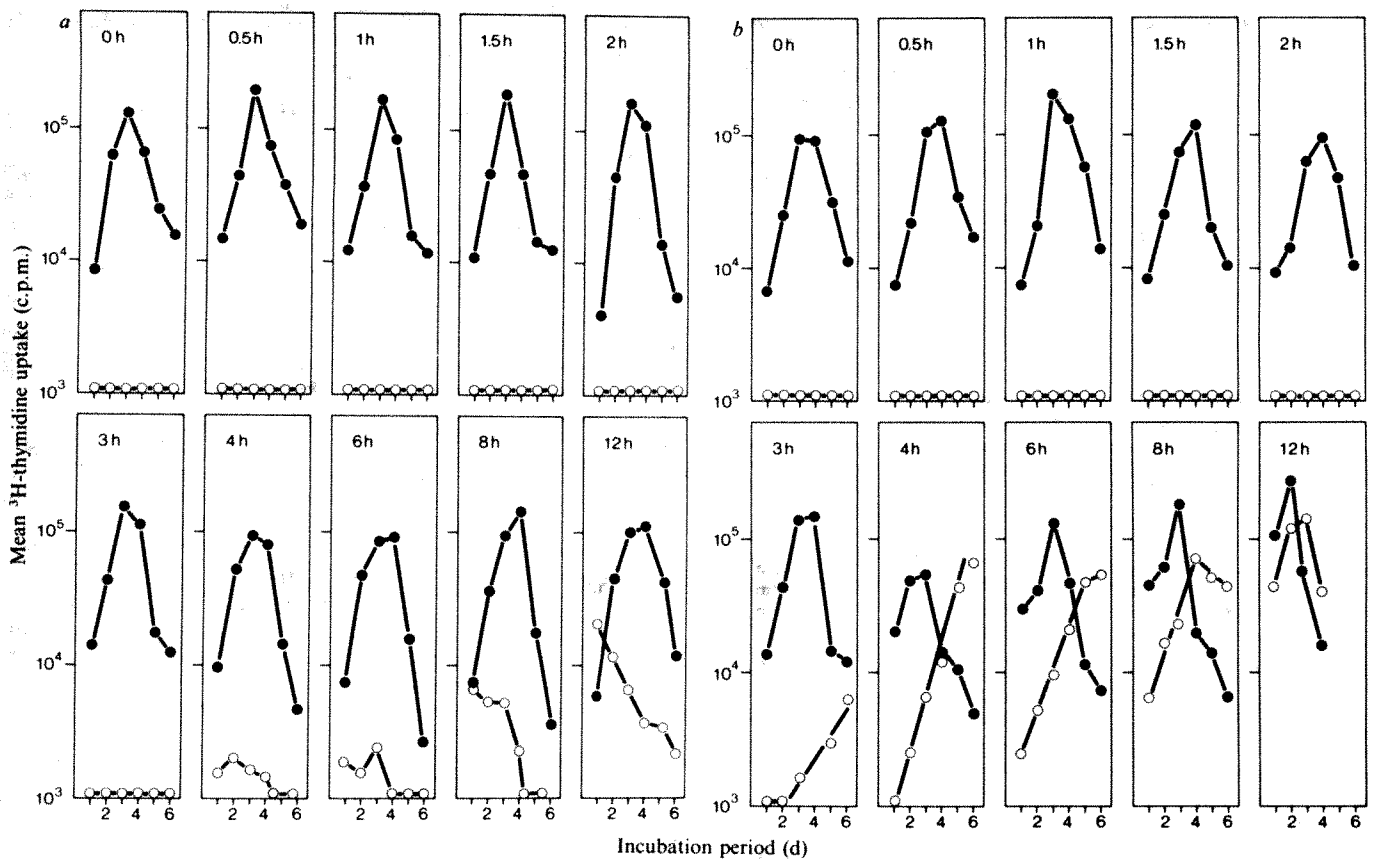


Fig. 1 Induction of responsiveness to growth factors by a short pulse of Con A. C3H/HeJ spleen cells (5×10^6 ml^{-1}) were incubated for the indicated periods of time, at 37 °C, in culture medium [RPMI-1640 containing 50 μg ml^{-1} neomycin, 10 mM HEPES, 5×10^{-5} M 2-mercaptoethanol and 10% fetal calf serum (GIBCO, batch L3644015)] in the presence or absence of 5 μg ml^{-1} Con A (Pharmacia). The cells were then washed five times in medium containing 20 mg ml^{-1} α-MM and recultured (2×10^5 ml^{-1}) either in medium or in growth-factor-containing medium (a 50% (v/v) concentration of CM obtained from 24-h cultures of Con A-stimulated spleen cells, supplemented with 20 mg ml^{-1} α-MM) or in medium containing 5 μg ml^{-1} Con A. The proliferative reponses in triplicate cultures were assayed every day as indicated after a 2-h pulse of ^{3}H-thymidine (Amersham TRK310; specific activity 2 Ci mmol^{-1}; 5 μCi ml^{-1}). *a*, Preincubation in Con A, cultures in Con A (●) or medium (○). *b*, Preincubation in Con A, cultures in CM (○); all cultures with cells preincubated in medium and set up in either medium or CM had values below 1,000 c.p.m. and are not shown. These cells were, however, functionally competent, as demonstrated by their responsiveness to Con A (●) in *b*. The results represent the mean values observed in triplicate cultures; the bars indicating s.d. are smaller than the symbols in the figure. Identical results were observed in three independent experiments.

free of lectin and their reactivity to growth factors was assessed in secondary cultures.

As shown in Fig. 1, incubation of spleen cells with optimal mitogenic doses of Con A for less than 8 h does not result in initiation of growth; this is a good indication that the removal of lectin after preincubation was effective. Exposure to Con A for 8 or 12 h results in a limited degree of cell activation to DNA synthesis, which, however, steadily decreases from day 1. In contrast, Con A pulses as short as 3 h result in the acquisition of responsiveness to growth factors, as demonstrated by the exponential growth observed in such cultures from days 1 to 6. Note that the response to Con A in the secondary cultures is the same whether the cells had been preincubated in the presence or absence of Con A. These experiments demonstrate that normal splenic T cells, which do not respond to either CM or a short pulse of Con A, initiate exponential growth when cultured in CM after the Con A pulse.

As pointed out above, T-cell proliferation depends on growth factors which are induced by mitogenic lectins[4]. We have analysed in detail the kinetics of growth factor production to separate the two triggering events: induction of sensitivity to growth factors (shown in Fig. 1), and production of these growth factors. Normal spleen cells were cultured with 5 μg ml^{-1} Con A, and the culture supernatants collected after various incubation periods and tested for their content in T-cell blast specific growth-factor activity, as described elsewhere[4]. As shown in Fig. 3, before 6 h no growth-promoting activity was detected in the supernatants, and this activity became maximal only between 12 and 24 h.

The difference in the kinetics of these two fundamental steps in T-cell triggering should be remembered when interpreting published observations on the 'commitment' of T lymphocytes to mitosis after mitogen pulses[7–12]. It is clear that in any experimental conditions the limiting factor is the available concentration of growth factors; therefore, such experiments provide information on the conditions for the generation of these molecules.

As we postulate two independent events in the pathway of lectin-dependent T-cell triggering, and as the whole activation process is dependent on Ia-positive accessory cells[2–4], we investigated whether or not both steps show similar cellular requirements. Normal spleen cells were passed over Ig–anti-Ig columns[13] with or without previous treatment with monoclonal mouse anti-I-A antibodies[14]. Effluent cells from the columns were then either simply cultured with Con A (to assess the effectiveness of accessory cell depletion) or tested for their ability to acquire responsiveness to growth factors after a short exposure to Con A. As we reported previously[4] and as shown in Table 1, although depletion of B cells has no influence on the T-cell response to Con A, concomitant depletion of I-A-bearing cells results in the abolition of the responses. Such responses are, however, largely restored when preformed growth factors are added to the cultures. As already pointed out, the factors are not mitogenic in the absence of Con A (expts. 1, 2). These results indicate that Con A responses are dependent on the presence of accessory cells, because these Ia-positive cells are necessary for the generation of growth factors.

The experiment presented in Fig. 2 shows that the first step in

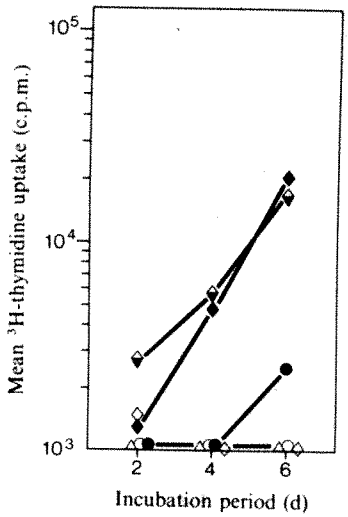


Fig. 2 Direct induction of responsiveness to growth factors in purified T cells by a short pulse of Con A. C_3H/HeJ spleen cells were treated with anti-I-A antibodies and passed through an anti-Ig column as described in Table 1, then cultured (2×10^5 ml^{-1}) in normal medium (○) or in medium containing 5 μg ml^{-1} Con A (●), a 50% final concentration of CM (◇) or both Con A and CM (◆). The same cells were also exposed to 5 μg ml^{-1} Con A for 4 h, washed five times in medium containing 20 mg ml^{-1} α-MM and cultured in medium (△) or in CM (◆).

triggering is independent of accessory cells. Purified T lymphocytes, depleted of Ia-positive cells, which do not respond to growth factors, acquire responsiveness to such growth factors during a 4 h treatment with Con A. After this short pulse, Con A is no longer required, but cell growth depends entirely on the presence of growth factors. We conclude that a direct interaction of the mitogenic lectin with the responding T cells is initially necessary for induction. Furthermore, this interaction is independent of the requirement for lectin and accessory cells in the generation of growth factors.

As we have shown before, responsiveness to growth factors is the result of the expression on the surface membrane of an acceptor site for those molecules[6]. We investigated whether or not cells pulsed with Con A for 4 h (which no longer required

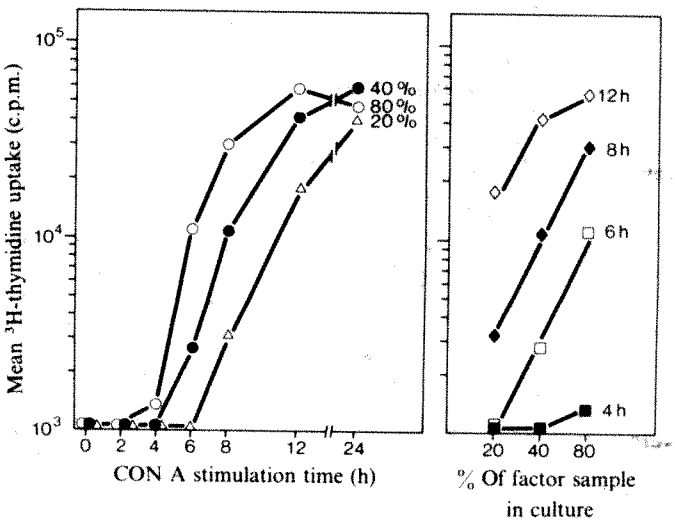


Fig. 3 Induction of T-cell growth factors in spleen cell cultures stimulated by Con A. C_3H/HeJ spleen cells (5×10^6 ml^{-1}) were cultured as described in Fig. 1 legend, in the presence of 5 μg ml^{-1} Con A. At the indicated times, CM from these cultures were collected, supplemented with 20 mg ml^{-1} α-MM and tested for growth-promoting activity in T-cell blasts which had been grown in standard CM for over 2 weeks. The test cultures contained 10^4 blasts in 0.2 ml and were supplemented by 80%(○), 40%(●) or 20%(△) concentrations of each supernatant. The results shown are the mean ^{3}H-thymidine uptake in triplicate cultures 3 days later. In the right hand panel, the same results are plotted to show the titration of the growth-factor activity in CM recovered after the various incubation periods. ◇, 12 h; ◆, 8 h; □, 6 h; ■, 4 h.

Con A for responding to growth factors) already bear such acceptor sites on the surface membrane. Acceptor sites were assayed by their ability to absorb out T-cell-specific growth-promoting activity from supernatants. Con A-pulsed cells were compared with normal spleen cells and with T-cell blasts. As shown in Table 1 (expts 3, 4), spleen cells exposed to Con A for 4 h have more acceptor sites for growth factors than normal spleen cells, but not as many as do full blast cells, which are, of course, much larger.

The present results demonstrate that direct interaction of a mitogenic lectin with purified T-cell populations does not trigger them into proliferation, but it induces, in a relatively short time, surface membrane expression of functional receptors for growth factors. In addition, the mitogenic lectin induces the production of growth factors in a process which depends on accessory, Ia-positive cells.

Assuming that T-cell triggering, in both allogenic and restricted responses, is not fundamentally different from the lectin-dependent responses studied here, we may speculate on the physiological significance of the two independent events which seem to be strictly necessary for induction. Thus, the bifunctional role of mitogenic lectins may be equivalent to the role of antigen and major histocompatibility complex products on accessory cells in the process of specific T-cell induction.

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Expression of V-region-like determinants on Ig-negative precursors in murine fetal liver and bone marrow

IDIOTYPES, as defined by Oudin[1], are antigenic markers associated with antibodies produced by a single individual. The demonstration of 'individual antigenic determinants' on human myeloma proteins[2] introduced the concept of idiotype as a clonal marker of Igs. However, as the operational definition of an idiotype is based on the specificity of anti-idiotype antisera, the extent of cross-reactivity of a particular idiotype, and therefore the definition of idiotype, depends on the criteria set for the preparation of reagents. In fact, a number of 'public' or 'cross-reactive' idiotypes are currently described which are produced by all individuals of a strain or species or even by different species[3]. Yet, idiotypes are exclusively considered as markers of

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V-gene products defining groups of related Ig molecules. The basic concept of Jerne's network theory[4], however, postulates that the universes of idiotypes and of antigens are identical. As a consequence, the finding of idiotype-cross-reactive determinants on non-Ig molecules should be the rule. We have observed[5] that a class of polyclonally distributed mitogen receptors on splenic B cells bear determinants cross-reactive with the public idiotype of the J558 myeloma protein of BALB/c origin. We have investigated the possibility that this finding might be related to the mechanism of generation and regulation of the antibody repertoire. Having no indications on the growth regulation of B-cell precursors, we started to investigate the antigenic relationship between Ig idiotypes and mitogen receptors on those cells. We report here the cross-reactivity between several germ-line idiotypes and non-Ig surface structures expressed on cells present in haematopoietic tissues. We hypothesise that growth receptors on precursor B cells have determinants cross-reactive with germ-line V-gene products, and that precursor cells are driven and selected by interactions between these receptors and molecules with anti-idiotype specificity also belonging to an incipient network transmitted in the germline.

Fetal livers were collected from BALB/c or (DBA/2× C57BL/6) F_1 embryos from day 13 to 15 of timed pregnancies. Fetal liver at that age does not contain detectable numbers of B cells as defined by surface Ig expression or reactivity to lipopolysaccharide (LPS)[6,7]. Adult bone marrow and spleen were obtained from BALB/c and C57BL/6 mice. We have used indirect immunofluorescence to detect the membrane expression of IgM and IgD, LPS-receptors[8] and determinants cross-reactive with a number of idiotypes of likely germline origin[9], namely the idiotypes on the following BALB/c myeloma proteins: J558 ($\alpha\lambda_1$, anti-α-1,3 dextran), W3129 ($\alpha\kappa$, anti-α-1,16 dextran) TEPC15 ($\alpha\kappa$, anti-phosphorylcholine), MOPC460 ($\alpha\kappa$, anti-dinitrophenyl) and MOPC104E ($\mu\lambda_1$, anti-α-1,3 dextran). Antisera to the first three proteins were raised in mice[5,10] while anti-MOPC104E was made in guinea pigs tolerant to mouse Ig. Three different anti-MOPC460 idiotypes were compared; conventional BALB/c, and rabbit antisera, and a monoclonal antibody[12]. Although all antisera reacted only with the immunising and with idiotypically cross-reactive proteins, they were additionally absorbed on Sepharose-coupled IgM, IgA, IgG, κ and λ myeloma proteins and pooled mouse Igs. Anti-J558 and anti-MOPC460 idiotypes were antibodies purified by affinity chromatography. As controls, an IgG1 fraction of a BALB/c anti-SRBC antiserum, the monoclonal antibody Sp-2 (IgG2b anti-SRBC)[13] and normal serum IgG from A/J mice were used. All reagents were labelled with trinitrophenyl[14] or arsonate[15] and used in indirect immunofluorescence[5,16].

Table 1 shows results of staining fetal liver, adult bone marrow and spleen cells with these reagents. All anti-idiotypes, but not normal IgG or anti-SRBC antibodies, stained a sizeable proportion of IgM-positive spleen lymphocytes (11–16% of all B cells). Nearly all cells stained by anti-idiotype were $\mu^+\delta^-$, while the majority of cells expressing LPS-receptors were $\mu^+\delta^+$ suggesting that the former are less differentiated than the latter. A relatively small proportion of fetal liver cells (0.6–1.2%) were also stained by the anti-idiotype reagents, while no cells expressed either Igs or LPS-receptors, or nonspecifically bound normal IgG. In bone marrow, anti-idiotypes stained ~4% of the total

Table 1 Presence of idiotype-cross-reacting structures on mouse fetal liver, adult bone marrow and adult spleen cells

Reagent	% Positive cells: Fetal liver (day 13–15)		Adult bone marrow: Total	Adult bone marrow: μ^+	Adult spleen	
Anti-Igs	<0.04					
Anti-μ	<0.04		3.4		44.3	
Anti-J558	0.73		4.0	1.0	6.9	
Anti-MOPC460*	0.82		3.6	1.2	6.6	
Anti-W3129	0.89	all μ^-	4.1	0.8	4.9	all μ^+
Anti-TEPC15	1.2		4.1	1.2	4.9	90% δ^-
Anti-MOPC104E	0.60		ND		5.5	
BALB/c IgG1 aSRBC	<0.05		ND		ND	
Sp-2 (IgG2b aSRBC)	<0.05		ND		<0.05	
Normal A/J IgG	<0.05		ND		<0.1	
Anti-LPS-R	<0.04		0.8	0.7	7.5	all μ^+ 80% δ^+

Anti-Igs and anti-μ antibodies were labelled with tetramethylrhodamine isothiocyanate. All anti-idiotypic antisera and anti-LPS-R antiserum[7] were labelled with trinitrophenylsulphonic acid and their binding to cells detected by exposure to fluorescein-conjugated anti-dinitrophenyl antibodies. Binding of guinea pig anti-MOPC104E was also detected with fluorescent rabbit anti-guinea pig Ig antibodies. The two methods of indirect immunofluorescence gave comparable results. Anti-δ antiserum was labelled with arsonate and detected with rhodamine-labelled rabbit anti-arsonate antibodies. Living cells in suspension in medium containing 10% fetal calf serum and 20 mM NaN_3 were exposed to one of the anti-idiotypic reagents for 30 min in ice, washed and exposed to the detecting reagent (see refs. 5, 12 for details). All cell samples strained with anti-idiotype or anti-LPS-R were counterstained with anti-μ. Spleen cell samples were also counterstained with anti-δ. The stained samples were observed under a fluorescence microscope (Zeiss Photomikroscop II or Leitz Orthoplan), equipped with vertical illuminators and combinations of filters and dichroic mirrors specific for fluorescein and rhodamine. Total cells in each field were counted in phase contrast. At least 2,500 cells in fetal liver samples, and 1,000 cells in bone marrow and spleen samples were counted in each experiment and for each reagent. The figures reported here are average values of six experiments with fetal liver cells, three experiments with bone marrow, and seven experiments with spleen cells from different strains, as we did not observe differences in frequency of positive cells among all the strains tested.

* The three different anti-MPOC460 idiotype antisera gave comparable results.
ND, Not determined.

Table 2 Simultaneous presence of more than one idiotype-cross-reacting structure on the membrane of fetal liver and spleen cells

Reagents		% Positive cells: Fetal liver	Spleen
Anti-MOPC460 +anti-W3129	Total MOPC460	0.44	6.5
	'Pure' MOPC460	0.18	3.4
	Doubles	0.26	3.1
	'Pure' W3129	0.27	3.0
	Total W3129	0.53	6.1
Anti-J558 +anti-W3129	Total J558	0.62	6.6
	'Pure' J558	0.25	1.7
	Doubles	0.37	4.9
	'Pure' W3129	0.17	1.4
	Total W3129	0.54	6.3
Anti-TEPC15 +anti-W3129	Total TEPC15	0.77	ND
	'Pure' TEPC15	0.62	
	Doubles	0.15	
	'Pure' W3129	0.40	
	Total W3129	0.55	
Anti-MOPC460 +anti-MOPC104E	Total MOPC460	0.39	ND
	'Pure' MOPC460	0 (<0.05)	
	Doubles	0.39	
	'Pure' MOPC104E	0.25	
	Total MOPC104E	0.64	
Anti-J558 +anti-MOPC104E	Total J558	0.60	6.8
	'Pure' J558	0.10	5.5
	Doubles	0.50	1.3
	'Pure' MOPC104E	0.25	4.9
	Total MOPC104E	0.76	6.2
Anti-TEPC15 +anti-MOPC104E	Total TEPC15	0.73	ND
	'Pure' TEPC15	0.07	
	Doubles	0.64	
	'Pure' MOPC104E	0.06	
	Total MOPC104E	0.70	

Anti-W3129 was labelled with arsonate and detected with rhodamine-labelled rabbit anti-arsonate antibodies. Anti-MOPC104E was used unlabelled and detected with rhodamine-labelled rabbit anti-guinea pig antibodies. Anti-J558, anti-TEPC15 and anti-MOPC460 were labelled with trinitrophenylsulphonic acid and detected with fluorescein-labelled anti-dinitrophenyl. The cell suspensions were exposed to a pair of anti-idiotypic antibodies for 30 min in the cold, washed and exposed to a mixture of the fluorescent detecting reagents. Positive cells out of the total cell population were counted as indicated in Table 1 legend. For the evaluation of 'double' versus 'pure' cells, each field was observed under specific illumination for fluorescein and for rhodamine. At least 100, in most cases 200, positive cells were scored in each combination.

cells, the majority of which were μ^-. None of the cells binding anti-idiotypes contained intracytoplasmic IgM, as demonstrated in experiments (not shown) where cells were stained on the membrane with anti-idiotypes, and in the cytoplasm, after fixation with anti-μ antibodies. It seems, therefore, that these cells are not those described in bone marrow and fetal liver by Raff *et al.*[17].

Confidence on the specificity of the anti-idiotype staining of Ig-negative fetal liver and bone marrow cells was provided by the lack of binding in the same staining conditions of other reagents such as mouse anti-sheep red blood cell (SRBC) or rabbit anti-LPS-receptor (LPS-R) antibodies. It is also clear that the molecules detected by anti-idiotypes on fetal liver are not idiotypes of passively acquired material IgG, as no cells were detectable in the same samples with anti-mouse Ig+L antibodies.

Further evidence for specificity of anti-idiotype binding to target cells was provided by double-staining experiments shown in Table 2. Note that, although we consistently observed the presence of two such idiotype-cross-reactive structures on the same cells both in spleen and fetal liver, a proportion of the positive cells was stained in all cases by only one reagent in the pair.

Table 3 shows results of experiments designed to characterise the membrane structures detected by some anti-idiotypes. The binding of anti-J558 and anti-MOPC104E idiotypes, but not that of anti-MOPC460 idiotype, was specifically inhibited by α-1,3-containing dextran. These results suggest that the staining of fetal liver cells, as well as that of spleen cells[5], by anti-J558 and anti-MOPC104E idiotypes is a specific reaction between anti-idiotypes and a membrane structure which is (or is associated with) a dextran binding site.

Having stressed the specificity of binding of purified anti-idiotype antibodies to Ig-negative cells in fetal liver and bone marrow, we must discuss the specificity of these anti-idiotype reagents. The antisera or purified antibodies used in these experiments are indeed anti-idiotypic when studied serologically for reactions with Ig molecules. On the other hand, the same reagents used to stain intracytoplasmic Igs of mature plasma cells in spleen, revealed frequencies of positive cells around 1–3%. These figures, although one order of magnitude lower than those observed by membrane staining of the same samples, were definitely higher than expected for 'individual specificities' and more compatible with V-region isotype specificities[9]. In fact, all the myeloma proteins used in these experiments carry 'public' idiotypes. It is likely therefore that the molecules we detect on the cell surface with these anti-idiotypes, do not cross-react with idiotypes in Oudin's *sensu stricto*, but rather with V-region determinants shared by related antibody molecules. Such V-region-like determinants are certainly not immunoglobulin in nature, or at least they are not associated with constant regions of known Ig isotypes (μ and L). In addition, the cross-reactivity observed here does not imply that we are dealing with products of Ig V-genes.

Functional studies in progress indicate that the cells described here are in fact B-cell precursors. Furthermore, the addition of anti-idiotypes to fetal liver and bone marrow cultures can be shown to influence B-cell development, suggesting that such idiotype-cross-reactive structures function as growth receptors. More than one specificity of idiotype-cross-reactive structure can be found on the same cells, both in fetal liver and spleen, and this parallels previous findings of multiple mitogen receptor specificities on the same B cell[18]. This is in contrast with the strict monospecificity of Ig molecules of a single cell, where all membrane Igs, both IgM and IgD, bear the same idiotype[19,20].

It is perhaps not fortuitous that such public idiotype-cross-reactive structures are present on fetal liver and adult bone marrow Ig-negative B-cell precursors. If the repertoire of antibodies is a closed network[4], and the germ-line specificities represent the main features of the system and are therefore anti-idiotypic, these 'idiotypes' on growth receptors of precursor cells would provide the internal conditions for inducing their proliferation, as well as a mechanism for selecting the desired idiotype specificities. These are minimal requirements for somatic generation and regulation of antibody diversity, and they assume that the repertoire selection is guided by idiotypes and not by combining site specificities. This proposal assumes that physiological mitogens which drive precursor B-cell development are anti-idiotypes and not the ligands to which the idiotype-bearing antibodies can bind. Only when the ligand can inhibit the reaction of anti-idiotypes with the triggering structures could we expect it to be mitogenic for precursors, as is the case for dextran[18,21]. Speculations on the selective pressures which result in antibacterial specificity of growth receptors on B cells, as well as of most myeloma proteins with antibody activity, will be developed elsewhere.

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Table 3 Inhibition of the staining of fetal liver cells by anti-idiotypic antisera

Reagent	Inhibitor	% Positive cells	% Inhibition
Anti-J558	None	0.37	—
	Dextran B1355	0.04	89.2
	Dextran B512	0.36	2.8
Anti-MOPC104E	None	0.50	—
	Dextran B1355	0.18	64.0
	Dextran B512	0.37	26.0
Anti-MOPC460	None	0.46	—
	Dextran B1355	0.39	15.3
	Dextran B512	0.41	10.9

The staining was performed in the presence of dextran B1355 (containing α-1, 3 and α-1, 6 linkages) or dextran B512 (containing primarily α-1, 6 linkages) at a concentration of 250 μg ml^{-1}.

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Tumour promoter TPA enhances transformation of human leukocytes by Epstein–Barr virus

TUMOUR-promoting phorbol esters have contributed to the concept of multiple-step carcinogenesis[1], as illustrated in the two-stage mouse skin carcinogenesis model[2]. Promoters are not significantly carcinogenic when tested alone, but decrease the threshold dose of carcinogens (initiators) and reduce the latency period for tumour appearance. 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) and several other diterpene esters represent potent tumour promoters[3–5]. Recently, it has been shown that these promoters efficiently induce persisting genomes of oncogenic herpesviruses, particularly of Epstein–Barr virus (EBV)[6–8], resulting in high percentages of viral antigen and particle-positive cells. Other observations showed that TPA enhances *in vitro* transformation of fibroblasts previously exposed to polycyclic aromatic hydrocarbons, UV light or adenoviruses[9–12]. These studies prompted us to analyse the effect of TPA not only on virus induction in EBV genome-harbouring cells, but also on EBV-induced transformation of human cord blood lymphocytes. Our data show that low concentrations of TPA significantly enhance transformation of lymphocytes by EBV.

Initial experiments were designed to investigate the effect of various concentrations of TPA on colony formation of human cord blood leukocytes after infection by transforming EBV derived from the B95-8 line[13]. As previously described[14–16], typical 'rod-shaped' EBV-transformed colonies appeared after about 2 weeks of incubation. Figure 1 shows that cultures treated with quite a wide range of TPA concentrations showed a marked increase in the number of colonies when compared with the untreated controls. The optimal concentration of TPA was 0.31 ng ml^{-1}. In these conditions six times more colonies were counted than in cultures without TPA; transformed colonies appeared earlier and were larger in cultures kept in the presence of TPA, although the morphology of TPA-treated and untreated cultures was similar.

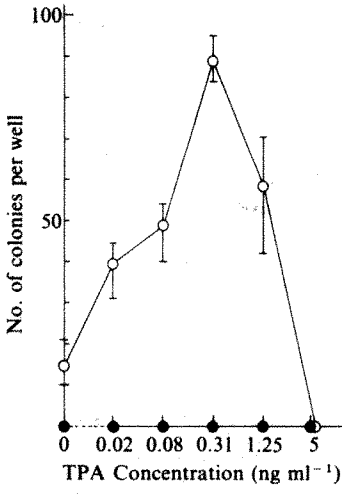


Fig. 1 Effect of TPA on colony formation of human cord blood lymphocytes after infection with EBV. Umbilical cord blood lymphocytes were prepared by a conventional Ficoll–Hypaque procedure[20], and infected with EBV derived from B95-8 cells. The virus concentration procedure has been described previously[14]. Colony formation by EBV-transformed cells in soft agar was determined as reported elsewhere[15]. The number of transformed cell colonies growing in agarose was quantitated under a low power microscope 2 weeks after infection and expressed as the mean value of four wells. The bars reveal the observed deviations. TPA was used in fourfold dilutions starting with 5 ng ml^{-1}. ●, Preparations without EBV; ○, preparations infected with EBV.

TPA has been reported to stimulate T lymphocytes specifically[17]. However, no transformed lymphoblastoid colonies were observed in cultures treated exclusively with TPA, although some differentiated cell colonies were detected. As the colonies in TPA-treated cells were also EB nuclear antigen-positive, these results show that they were indeed transformed by EBV. Similar experiments were carried out using peripheral leukocytes of an EBV-seroreactive healthy adult. The results also confirmed that at an optimal concentration of 0.31 ng ml^{-1} transformation efficiency was enhanced approximately fivefold (data not shown). Therefore, 0.31 ng ml^{-1} was used in further experiments by modifying the concentration of the virus used for transformation tests. As shown in Fig. 2, the maximal number of colonies was obtained at a virus dilution of 1:4 in all preparations tested. At this virus concentration fivefold higher counts of colonies were noted in TPA-treated cultures. The number of colonies decreased proportionally to the virus dilution, starting at a dilution of 1:4 in both types of culture. The virus–dose response was similar to that observed in previous studies[14]. At higher virus concentrations the decrease in the number of transformed colonies may be attributed to cell killing activity of the concentrated preparations[14].

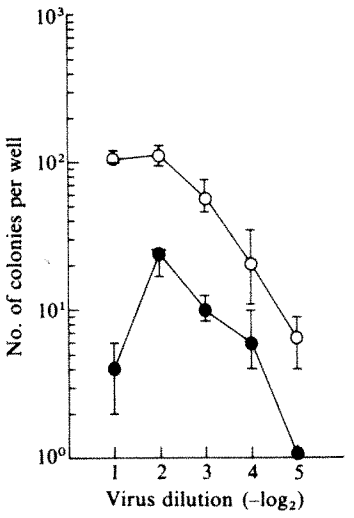


Fig. 2 Colony formation of human cord blood lymphocytes after infection with serial dilutions of EBV in the presence or absence of TPA. The procedures are the same as those outlined in Fig. 1. Cells are infected by serial dilutions of EBV and mixed with the agarose medium with (○) or without (●) 0.31 ng ml^{-1} TPA.

We then determined whether TPA also stimulates colony formation of established lymphoblastoid lines in agarose even without the addition of exogenous EBV and whether this effect is specific for EBV genome-harbouring cells. For this we used one EBV genome-negative line, BJAB, and four EBV-positive lines, P3HR-1, two Raji clones[18] and a newly established human cord leukocyte line, CBL-1 (about 2 months after establishment). The data show that except for CBL-1, TPA did not enhance colony formation of these cells. There was even a reduction in the number of colonies (15–40% less than in the control) which were also more dispersed in the presence of TPA. However, TPA caused an approximately threefold increase in colony formation in CBL-1 cells (Fig. 3).

We also investigated the effect of TPA on transformation by end-point dilution. Again, TPA increased the transformation efficiency in liquid culture systems. In the presence of 0.31 ng ml^{-1} TPA, two independent experiments yielded about fivefold higher TD_{50} per ml (7.9×10^{-5} and 2.5×10^{-5}) than in the untreated controls (1.6×10^{-5} and 5.0×10^{-4}).

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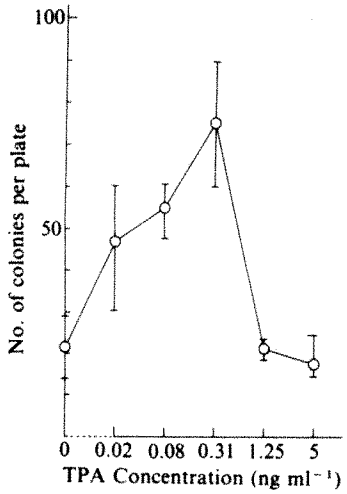


Fig. 3 Effect of TPA on cells of a newly established B95-8 EBV transformed cord blood lymphocyte line, CBL-1. The procedure for analysing the colony formation of cells from established lines was basically identical to that of the previous experiments[18]. 5,000 cells were seeded into each plate. The number of colonies was counted 7–9 days after inoculation. All values are the mean of three plates. Bars represent the deviations of these plates.

Our results indicate: (1) TPA at low molarity caused a three- to seven-fold enhancement of transformation of human leukocytes by EBV. (2) TPA does not increase the plating efficiency of three 'old' established cell lines in agarose regardless of their EBV genome state. (3) TPA enhances the plating efficiency of a newly established line in agarose. These results suggest that TPA acts on transformation steps rather than inducing nonspecific colony-stimulating factors that are known to promote cell growth in agarose[19].

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Oxidant damage mediates variant red cell resistance to malaria

IT is thought that the deleterious properties of thalassaemia (thal) and glucose-6-phosphate dehydrogenase deficiency ($G6PD^-$) are balanced in the population by a conferred protection against malaria, and that some characteristic of the variant red cell acts directly to inhibit the invasion or intracellular development of the malaria parasite. Indeed, such is the case for sickle cell disorders[1,2]. Recently, Pasvol *et al.*[3], having shown that fetal haemoglobin (HbF) (in both fetal and adult cells) inhibits malaria parasite development, have suggested that the slight persistance of HbF in children with thal trait is responsible for the selective advantage of the thal gene. They have reported no difference in parasite development in the red cells of adult carriers of thal. Evidence is presented here that both α- and β-thal trait red cells from adults are refractory to parasite development because of oxidant sensitivity, and, furthermore, that fetal red cells and $G6PD^-$ cells are refractory for the same reason.

Decreased protection against oxidant damage has frequently been suggested as a mechanism by which $G6PD^-$ cells could inhibit the optimal multiplication of the malaria parasite. Etkin and Eaton have demonstrated the production of H_2O_2 by a rodent malaria parasite[4], and the insufficient protective capacity of the $G6PD^-$ cell is well known[5]. The possibility that thalassaemic cells also inhibit parasite growth by their sensitivity to oxidant stress[6] has not previously been suggested. To study the role of oxidant sensitivity in resistance to malaria, *Plasmodium*

Table 1 Sensitivity to increased oxygen of *Plasmodium falciparum* in $G6PD^-$ and α- and β-thal red cells

RBC	Expt	Parasites per 100 red cells 30% O_2	17% O_2	Relative multiplication 30%/17%
Normal	1	3.2	9.3	0.34
	2	3.6	8.1	0.44
	3	2.1	5.0	0.42
	4	1.9	4.3	0.44
				0.41 ± 0.05
$G6PD^-$	1	0.4	4.4	0.09
	2	0.1	3.1	0.03
	3	1.2	4.6	0.26
	4	3.9	18.3	0.21
				0.15 ± 0.11
β-thal	1	0.3	1.8	0.17
	2	3.6	10.0	0.36
	3	0.6	4.1	0.15
	4	0.2	2.5	0.09
				0.19 ± 0.12
α-thal	1	1.3	8.2	0.16
	2	0.5	2.7	0.19
				0.18

Suspensions of variant and normal red cells (5% v/v) were inoculated with less than 1/30 cell volume of cells infected with *P. falciparum* (FMG/FCR-3). Cultures were maintained by the method of Trager and Jensen[7] as described previously[1]. After 2 d growth at 17% O_2, single cultures were split and incubated in either 17% or 30% O_2 for 2 additional days. Normal parasites per 100 red cells were counted on day 4 and the relative multiplication at 30% O_2 was calculated. Differences in the counts on day 4 between experiments result from different initial infections. Parasites with abnormal morphology at 30% O_2 were of a sufficient number to indicate that intracellular development was inhibited rather than invasion blocked. $G6PD^-$ red cells were obtained by screening[8] samples collected from male Negro blood donors. β-Thal red cells were from the parents of children attending a thalassaemia clinic (mostly mothers). α-thal red cells were verified by globin chain synthesis (H. Chang, New York Hospital). $G6PD^-$ experiments 3 and 4 were on the same sample. All others are from different individuals.

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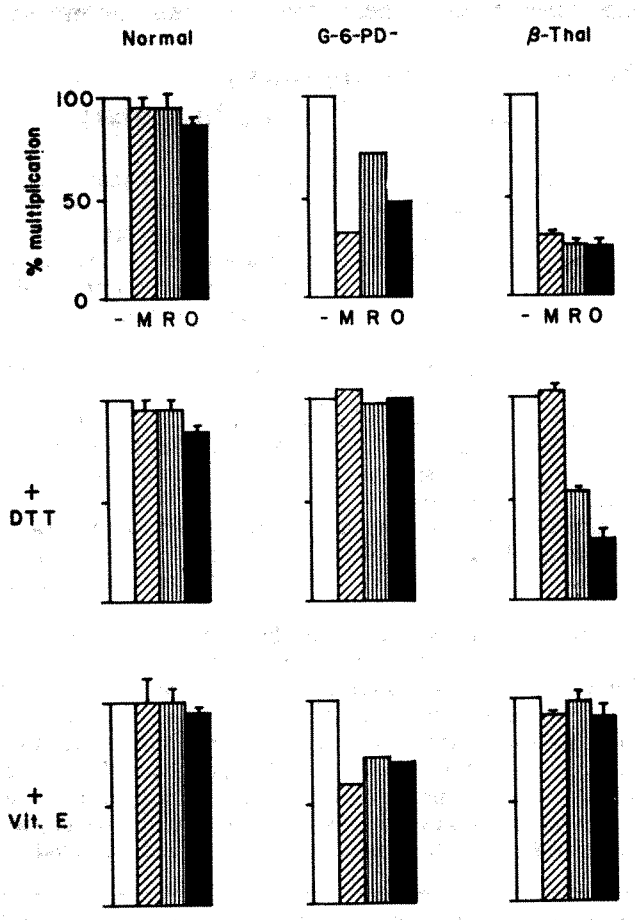


Fig. 1 Multiplication of *P. falciparum* in G6PD⁻ and β-thal red cells when oxidant damage is enhanced or inhibited. An experiment identical to that in Table 1 was carried out in the following conditions during the second 2 d: 17% O_2 (−), +2.5 μM menadione (M), +2.0 μM riboflavin (R), 25% 0_2 (O). DTT (50 μM) and vitamin E (0.2 mg ml^{-1}) were added as indicated. Height of the bars represents multiplication relative to that at 17% O_2. Error bars for normal and thal cells show one s.e.m. from three experiments (different donors). G6PD⁻ values are the means from two experiments. Neither DTT nor vitamin E had significant effects on growth in normal cells at 17% O_2.

falciparum, the human malaria parasite, was cultured in G6PD⁻, α- and β-thal trait, and HbF-containing erythrocytes in conditions which either enhanced or inhibited oxidant damage.

Cultures of G6PD⁻ and both α- and β-thal trait red cells in RPMI-1640 medium in an atmosphere of 17% O_2/3% CO_2 were inoculated with less than 1/30 volume of infected red cells. All variants supported normal multiplication of the parasite for 4 d in these conditions. The erythrocytic cycle of the parasite is 48 h and an eight- to tenfold multiplication was seen at this time. Thus, after 2 d, virtually all parasites had invaded variant cells. If cultures were then transferred to an atmosphere of 30% O_2/3% CO_2, an inhibition of multiplication occurred that was greater in G6PD⁻ and thal cells than in normal cells (Table 1) ($P<0.01$). The response of these host–parasite systems to oxidative stress was further characterised by parallel incubation in either 25% O_2, 2 μM riboflavin, or 2.5 μM menadione (Fig. 1). Riboflavin was more inhibitory to parasites in β-thal trait cells than to those in G6PD⁻ cells, while menadione and high O_2 were inhibitory to both. Thus, riboflavin and menadione have different effects on parasitised G6PD⁻ cells. Both of these agents can participate in single electron redox reactions to generate free radicals[9,10], but menadione alone can catalyse H_2O_2 production when reduced by flavin dehydrogenases[10]. Indeed, the ability of the malaria parasite to oxidise external NADPH[11] suggests that a parasite dehydrogenase may be exposed to the red cell cytoplasm and

Table 2 Multiplication in medium without gluthathione

RBC	Multiplication relative to complete medium (mean ± s.d.) RPMI-1640 −glutathione	High K^+ RPMI-1640 −glutathione
Normal	0.84 ± 0.03 ($n=4$)	0.70 ± 0.01 ($n=2$)
β-thal	0.48 ± 0.14 ($n=7$)	0.82 ± 0.02 ($n=2$)
G6PD⁻	0.32 ± 0.04 ($n=2$)	
Fetal (HbF)	0.33 ± 0.18 ($n=3$)	

Parasites were cultured for 2 d at 17% O_2 in complete RPMI-1640 and then for 2 d in either medium with or without glutathione. In two experiments, growth was also tested in medium without glutathione, but containing high potassium[22]. All experiments were on samples from different donors.

that menadione contributes to the peroxide load in the red cell by interacting with this enzyme. This interpretation is consistent with the sensitivity of G6PD⁻ cells to H_2O_2 referred to earlier. The sensitivity of thal cells to riboflavin may then be explained by a sensitivity of these cells to free radical mediated oxidation. Vitamin E (α-tocopherol), which prevents the lipid peroxidation catalysed by free radicals[12], was found to completely reverse the sensitivity of thal cells.

Parasitised G6PD⁻ cells were only partially protected by vitamin E, but dithiothreitol (DTT) was more effective. This thiol-reducing agent is expected to reduce gluthathione, providing substrate for gluthathione peroxidase, and may, like NADPH[13], protect red cell catalase. Both of these activities would result in lower H_2O_2 levels in the cell and decrease the risk to the G6PD⁻ cell from peroxide production and NADPH utilisation by the parasite. Note that DTT also protected the thal cell from menadione induced damage, presumably by counteracting the H_2O_2 produced by this agent. Mechanisms of oxidant stress and damage are notoriously difficult to identify and prove. The explanation discussed here and shown in Fig. 2 is not meant to be accurate or complete but is offered for discussion and testing.

The situation *in vivo* is not completely comparable to that *in vitro*. Oxygen concentrations of 30% and 25% are not normal physiological conditions. However, riboflavin is found in the circulation and soluble quinones are found in some foods. The observations that these substances potentiate the resistance of variant red cells to malaria infection implicate nutritional interactions in *in vivo* protection. Indeed, such ideas may explain the variable levels of resistance to malaria measured in field studies[15,16]. In addition, the culture medium RPMI-1640 contains reduced glutathione (GSH). When GSH was left out of the medium, approximating the *in vivo* situation, parasite growth was inhibited in variant cells at 17% O_2 (Table 2) ($P<0.01$). Growth in HbF-containing fetal cells was also inhibited. HbF

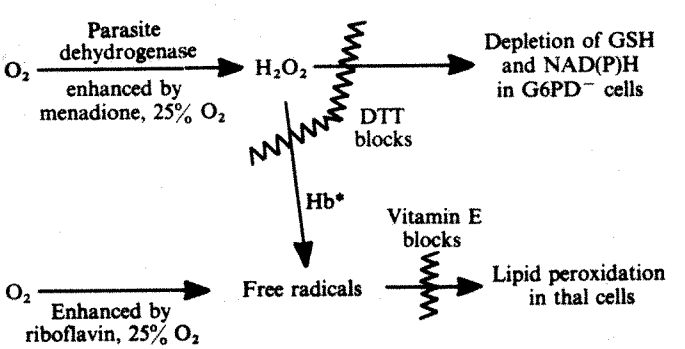


Fig. 2 Hypothetical model for the generation of oxidant damage during malaria infection of variant cells.* The interaction of H_2O_2 and HbF results in free radical production[14]. This may be enhanced in thalassaemia or HbF-containing cells.

was initially found to inhibit parasite growth in cultures in medium 199 (0.05 mg per litre GSH)[3], but this was not found here in complete RPMI-1640, which contains more glutathione (1 mg l^{-1}). Parasites in HbF cells were only inhibited in the absence of GSH. The sensitivity of fetal cells to lipid peroxidation[6] is consistent with this observation.

We have now seen that oxidant stress and oxidative damage act to limit malaria parasite multiplication in some red cell variants in conditions similar to those *in vivo*. In G6PD$^-$ cells, the production of peroxide by the parasite seems to overload the reducing capacity of the red cell. When catalase (50 μg ml^{-1}) was added to the medium to decrease H_2O_2 levels, parasites in G6PD$^-$ cells were protected, whereas those in thal cells were not (data not shown). Although some damage in the G6PD$^-$ host is prevented by vitamin E, there are other consequences of H_2O_2 exposure such as the depletion of GSH[17] and NADPH, metabolic requirements of the parasite[18,11]. *In vivo*, decreased GSH could lead to cross-linking of red cell membrane proteins[19] or Heinz body formation which may result in splenic sequestration and the selective removal from circulation of infected cells. This would explain the paucity of parasitised G6PD$^-$ cells seen in the blood of an infected heterozygous mozaic female patient[20].

As vitamin E was completely protective in thal red cells, free radical production was thought to lead to lipid peroxidation and membrane damage. To test this hypothesis, parasites in thal trait cells were cultured in a glutathione-free medium containing high (105 mM) K^+. One of the consequences of oxidative damage to the red cell membrane would have been increased cation permeability[21]. The high K^+ medium prevents red cell K^+ loss and maintains intracellular levels even when the membrane leaks. This protected the parasites from inhibition (Table 2). Thus, the primary lesion in the infected thal cell must be to the membrane. The parasite's requirement for a high potassium environment has previously been shown in the sickled HbAS cell which also leaks potassium and inhibits parasite metabolism[22].

Although HbF is clearly inhibitory in its own right, the adult thal trait is also resistant. Both the fetal red cell (data not shown) and the thal red cell are sensitive to riboflavin-induced oxidant damage. The basis for this sensitivity is being investigated. Their sensitivity is qualitatively different from that of G6PD$^-$ cells; the protection against malaria conferred by these traits may also differ. Nevertheless, the model of oxidant sensitivity presented here provides experimentally verified mechanisms for the resistance of these variants to malaria and for the subsequent enhanced survival of the carriers of these genetic traits.

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Nucleotide sequences of the attachment sites of bacteriophage Mu DNA

THE temperate bacteriophage Mu has the remarkable ability to insert its DNA in apparently random sites of the *Escherichia coli* chromosome (1–3). All Mu prophages have the same gene order[4], and the finding that the prophage and the phage maps are identical suggests that specific sites (attachment sites) at the ends of the Mu genome are used for the integration of Mu[5]. Phage particles do not contain the excised, free form of the Mu DNA; instead, the Mu genome is flanked by what seems to be heterogeneous bacterial DNA[6,7]. The variable DNA at both ends of Mu is thought to be generated when Mu DNA is transposed to many new chromosomal sites during replication and is subsequently packaged into phage particles together with adjacent host DNA. The nucleotide sequence analysis of the ends of Mu DNA reported here substantiates this hypothesis and identifies the attachment sites of Mu. Structural features of the attachment sites are presented.

The material for sequencing was derived in the following way. DNA fragments containing the Mu ends (Mu plus host DNA) were purified from mature phage DNA and inserted into plasmids. The cloning of the *Hin*dIII fragment, which contains the c-terminal or left end of Mu, has been described elsewhere[8]. Right-end fragments generated by a mixture of *Eco*RI and *Sal*I or *Eco*RI and *Bam*HI were inserted into plasmid pBR322 which had been cleaved with the appropriate restriction nucleases. The structures of these recombinant plasmids are outlined in Fig. 1; plasmids containing the left or right end of Mu are called pLM and pRM, respectively. The sequencing strategy (Fig. 2) involved end-labelling of small juncture fragments with [γ-^{32}P]ATP and polynucleotide kinase, separation of the two labelled ends by recleavage with restriction nucleases and deduction of the sequence by the chemical modification procedure of Maxam and Gilbert[9].

The sequence information we have obtained from three plasmids containing the left or right end of Mu demonstrates the validity of the concept that Mu DNA is covalently linked to variable host DNA sequences. For example, sequencing gels of two different left-end juncture fragments from pLM44 and pLM54, both read from the same 5′-labelled *Hpa*II site in Mu DNA into host DNA (Fig. 2), show sequences which match perfectly up to a certain nucleotide but which differ beyond this. In Fig. 3 we have aligned the juncture sequences of pLM and pRM plasmids. For comparison we have included both juncture sequences from a Mu prophage inserted in *lac*Z; details and implications of this latter experiment will be described elsewhere. All pLM plasmids contain a common sequence located next to a variable region different in each plasmid. The same is true for all pRM plasmids. We regard the common sequences as Mu and the variable sequences as host DNA, such that the end points of the Mu genome coincide with the first nucleotides of the common sequence. Although the host DNA sequences are largely different from one another, they show similarities in sequences directly adjacent to the Mu ends. At the left end in two out of four plasmids, the Mu sequences are preceded by

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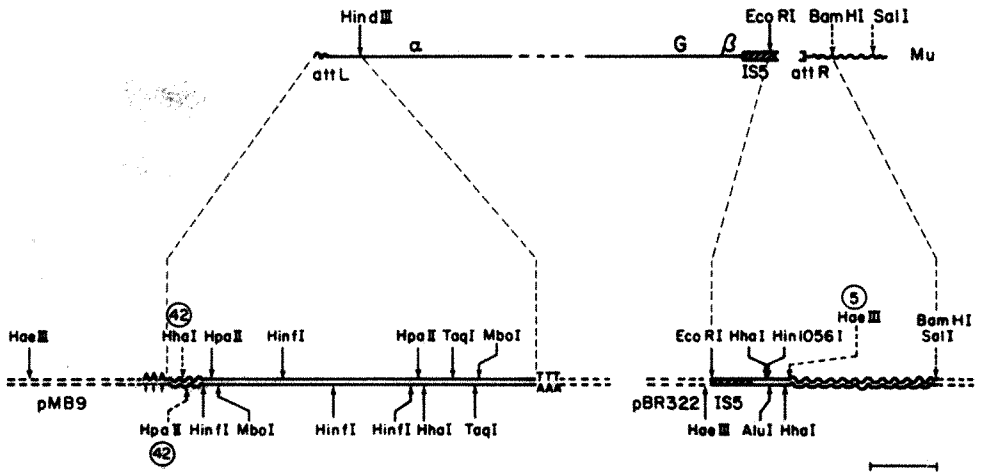


Fig. 1 Cloning of end fragments of the Mu genome and restriction enzyme cleavage map. attL: the leftmost 1,000-base pair long *Hin*dIII fragment of Mu DNA was isolated and inserted into the *Eco*RI cleavage site of pMB9 after tailing with terminal transferase[8]. A preliminary cleavage map of this fragment has been published previously[19], and we include here only cleavage sites that have been used for the identification of juncture fragments and the sequence analysis. pLM44, pLM54 and pLM42 were used here. attR: Mu 445-8 phage DNA[20] carried an additional *Eco*RI cleavage site (R.K. and D.K., unpublished) which is located in the region shown to be IS*5* (ref. 21). The rightmost *Eco*RI fragment of Mu 445-8 DNA was isolated, recleaved with either *Sal*I or *Bam*HI at statistically occurring sites in the heterogeneous host DNA and inserted into pBR322 (ref. 22), which had been cleaved with *Eco*RI + *Sal*I or *Eco*RI + *Bam*HI, respectively. The ligation conditions used were as described by Murray and Murray[23]. Three plasmids were analysed, pRM5, containing an *Eco*RI–*Sal*I insert 800 base pairs long, pRM8, containing an *Eco*RI-*Sal*I insert of 2,500 base pairs, and pRM1, containing an *Eco*RI-*Bam*HI insert of 400 base pairs. The heterogeneous *Eco*RI fragment overlapping attR of Mu 445–8 was also cleaved with various restriction enzymes to detect cleavage sites in the constant Mu part, which could then be used to determine the presence of Mu DNA in the pRM chimaeric plasmids. No cleavage sites were detected for *Hpa*II, *Hae*III and *Mbo*II, whereas *Alu*I, *Hha*I and *Hin*1056I cleaved at least once. Solid lines represent Mu DNA, wavy lines indicate host DNA, specific in each plasmid but different from one plasmid to another, and broken lines represent vector DNA. Cleavage sites are indicated by solid line arrows, broken line arrows labelled with numbers indicate that the cleavage site is only present in the plasmid indicated.

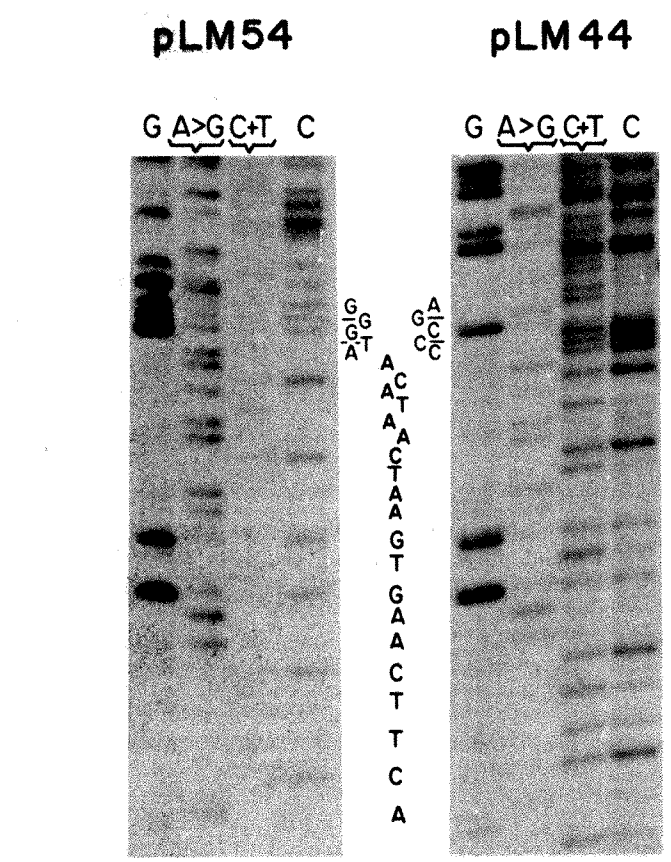
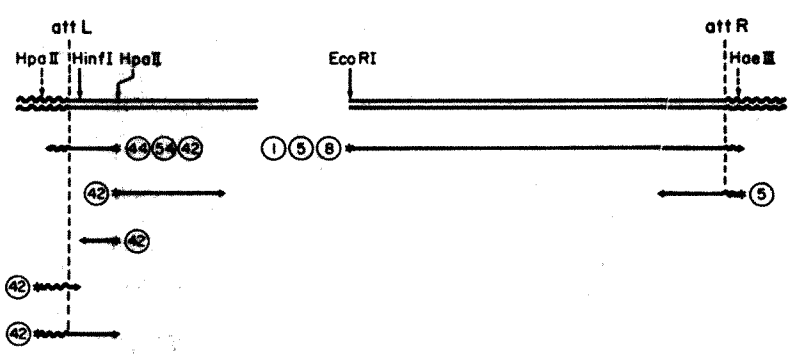


Fig. 2 Sequence analysis. Autoradiographs of sequencing gels showing the attL host DNA–Mu DNA junctures in pLM44 and pLM54. The autoradiographs show parts of the sequences (starting at nucleotide 19 of attL in Fig. 3) read from a labelled 5′ *Hpa*II end within Mu DNA through the juncture into the host DNA. Electrophoresis of the DNA fragments generated in the reactions described by Maxam and Gilbert[9] was on 20% polyacrylamide gels containing 7 M urea. For sequencing the attL juncture fragments of pLM44 and pLM54, the plasmids were digested with *Hpa*II, labelled with [γ-^{32}P]ATP and polynucleotide kinase[9], and recut with *Hae*III. The juncture fragments, now labelled only at the *Hpa*II site in the Mu part, were isolated from 2% agarose gels and sequenced. pLM42 contains a *Hpa*II as well as a *Hha*I site in the host DNA. The *Hha*I fragment overlapping the pLM42 attL junction was isolated after separation on 3.5% polyacrylamide gels; it was then recut with *Hpa*II and end-labelled. The two labelled internal *Hpa*II fragments were isolated from a 5% acrylamide gel, recleaved with *Hin*fI and the products separated on 8% polyacrylamide. All four labelled fragments were sequenced. In addition, the strands of the *Hpa*II juncture fragment of pLM42 were separated as described by Maxam and Gilbert[9] and sequenced. pRM plasmid DNAs were linearised with *Eco*RI, end-labelled and recleaved with *Hae*III to separate the two labelled ends. The juncture fragments were then purified on 2% agarose gels, re-isolated and sequenced. pRM5 contains an *Hae*III cleavage site in the bacterial DNA very close to the juncture. This site was labelled and the sequence was read over the juncture into Mu DNA. Solid lines indicate Mu, broken lines vector, and wavy lines host DNA, respectively. Asterisks mark the ^{32}P-labelled ends of a fragment. The arrow indicates the direction and length of the sequence that was read. We consider those sequences which were read from only one strand as preliminary. Numbers in circles refer to plasmid numbers and indicate that fragments from the corresponding plasmid were sequenced.



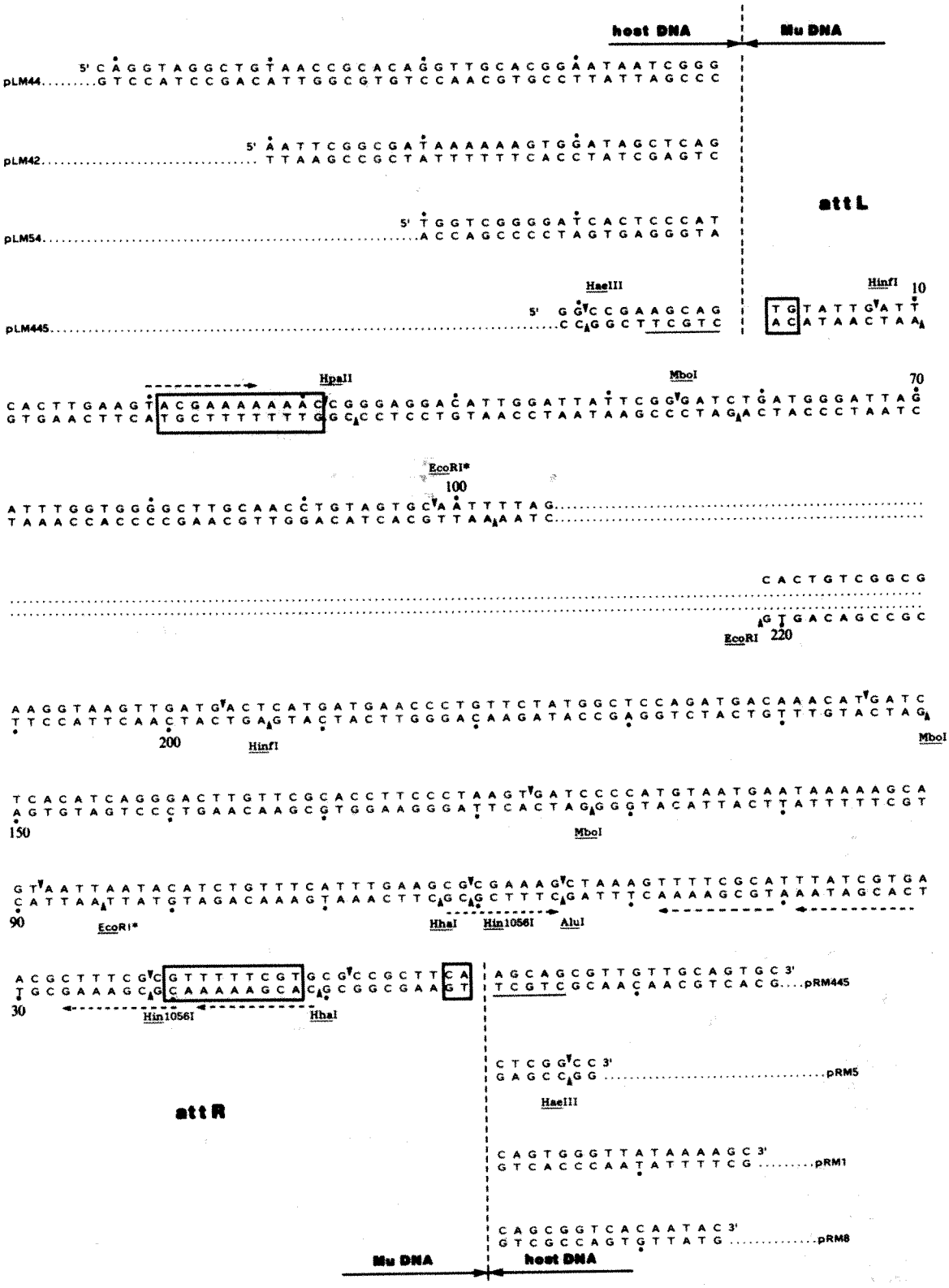


Fig. 3 Nucleotide sequence of the ends of bacteriophage Mu DNA. The central part of the sequence shows the common region of four pLM and four pRM plasmids. The unique sequences for the individual plasmids are shown to the left and right of the common sequence. The juncture sequences of pLM445 and pRM445 are included for comparison and will be described in detail elsewhere (manuscript in preparation); they show the juncture sequences of a Mu prophage inserted into the *lacZ* gene. Sequences found in inverted order at both ends are in solid line boxes. Broken line arrows indicate the sequence PyPuCGAAAA. The 5-base pair duplication of adjacent host sequences in pLM445 and pRM445 is underlined. The *Mbo*I and *Hin*fI cleavage sites in attR and all *Eco*RI* cleavage sites were inferred from the sequence; the presence of all other restriction enzyme cleavage sites have been demonstrated by mapping. Numbers indicate distances from the junctures in base pairs. Note that the attR sequence is derived from Mu 445–8 DNA; Chow *et al.*[20] have shown that in Mu 445–8 only ~150 base pairs next to the juncture are Mu DNA.

5'CAG and in one case by 5'CAT. At the right end in two of the four junctures the Mu sequence is followed by 5'CAG. We consider this to indicate some specificity of Mu insertion. In fact, it is possible that the specificity in host DNA sequences is more extensive and covers the first nucleotide(s) of the common sequence so that the definition of the exact end points of the Mu genome becomes somewhat arbitrary. Our positioning of these points, however, has been corroborated by the sequence analysis of two prophage junctures where the host DNA sequence is known (manuscript in preparation).

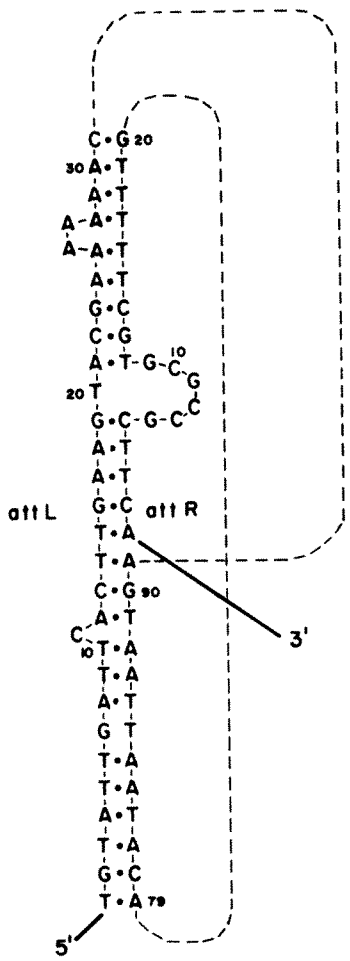


Fig. 4 Similar sequences at Mu attL and Mu attR. To illustrate the arrangement of similar sequences at the two Mu attachments sites, the DNA is presented in a single-strand stem-loop structure. Numbers indicate distances from the Mu–host DNA junctures. Nucleotides 1–20 of attR are paired with nucleotides 15–31 of attL and nucleotides 1–14 of attL are paired with nucleotides 79–91 of attR. Correct base pairing is indicated by dots.

The presence of defined juncture points between Mu and host DNA at both ends of the Mu genome suggests that specific Mu DNA sequences (hereafter referred to as Mu attL and Mu attR for left and right end attachment sites, respectively) at or near the junctures represent recognition sites for enzymes involved in Mu integration. In this respect, it should be noted that only one Mu-specific function, the protein coded for by gene *A*, is implicated in the integration process[10]. Assuming that both attachment sites are recognised by this protein we expect to find structural similarities between attL and attR. A first inspection shows that sequence similarities of the two attachment sites are confined to two base pairs at the very end and to a stretch of nine nucleotides (containing one mismatch) located between positions 21 and 31 in attL and between positions 12 and 20 in attR. Moreover, the sequence 5'PyPuCGAAAA which is part of the 9-base pair sequence common to both attachment sites is repeated with minor modifications five times within the first 62 nucleotides in attR but not in attL. This sequence might play a major part in the interaction of protein(s) with the attachment sites. Closer examination reveals that the first 31 nucleotides in attL are almost completely present in attR, but are arranged differently: the first 14 base pairs of attL are found 79 base pairs away from the juncture in attR, whereas nucleotides 15–31 in attL seem to correspond to the first 20 nucleotides of attR (Fig. 4). Whether this sequence arrangement has functional significance or is the result of evolutionary development remains to be determined.

In these structural aspects the Mu attachment sites differ markedly from IS sequences and other transposable elements, where the ends have been shown to contain more or less identical sequences arranged in inverted orientation[11–13]. The sequence differences encountered between Mu attL and Mu attR could reflect functional differences of the two attachment sites during integration and in the DNA replication and packaging processes. The insertion mechanisms of Mu and other transposable elements, however, may be closely related, as is indicated by the finding that Mu insertion, like the insertion of IS*1* (refs 12, 14), IS*2* (ref. 13) and Tn*3* (ref. 15) results in a duplication of host DNA at the host target site (Fig. 3)[16,17].

The sequences of the ends of Mu DNA shown here and those determined independently by Allet[18] differ substantially. The interpretation of Allet's sequencing data, particularly the location of the Mu ends and the finding of a 5-base pair inverted repeat sequence at the ends, is not consistent with our data. In addition, the sequences beyond the *Alu*I cleavage site at the attR end in Allet's sequence are completely different from the sequence we have determined for that region.

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Degradation of DL-leucine with longitudinally polarised electrons

MOLECULAR asymmetry represents a striking characteristic of living organisms and their products. The precise mechanism(s) involved in the origin of this asymmetry in nature is not known, although Vester[1] and Ulbricht[2] proposed that it may have originated through preferential destruction of one enantiomer of a racemic mixture by spin-polarised electrons arising from β-decay, or circularly polarised bremsstrahlung produced during their deceleration. However, tests of this hypothesis have yielded results which were either negative or, at best, of borderline significance[3]. Recently, however, Bonner *et al.*[4,5] reported the asymmetric degradation of the amino acid DL-leucine on irradiation with a beam of longitudinally polarised 120-keV electrons (degree of polarisation 10–27%). Electrons polarised

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Table 1 Effect of irradiation of samples of DL-leucine with longitudinally polarised electrons with spins antiparallel (AP) and parallel (P) to their momenta

a, Results of present study

Sample	Degree of electron polarisation (%)	Spin direction	Current density (nA cm^{-2})	Sample thickness (mg cm^{-2})	% Decomposition	Enantiomeric* composition (% D-isomer)
1	36	P	690	19	60	49.90±0.16(3)
2	42	AP	555	39	35	50.19±0.15(4)
3	42	AP	915	26	45	50.18±0.28(4)
4	42	P	645	30	45	50.29±0.25(12)
5	42	AP	1,110	31	55	50.20±0.11(8)
6	41	P	795	29	65	50.14±0.24(7)
7	42	P	930	29	75	50.26±0.25(3)
8	42	AP	360	17	65	50.15±0.17(4)
9	45	AP	360	27	40	49.97±0.12(5)
10	45	AP	300	36	40	50.14±0.12(4)
11	47	P	345	29	68	50.24±0.26(9)
12	46	P	390	20	77	49.99±0.30(7)
13	43	P	195	20	75	49.99±0.26(9)
14	43	AP	150	18	70	50.13±0.22(8)
15	43	P	96	23	72	50.14±0.22(4)
16	43	AP	114	23	60	49.92±0.15(8)
17	37	P	93	28	47	49.94±0.16(4)
18	41	AP	111	29	48	49.99±0.10(4)
19	43	P	84	20	65	49.92±0.20(8)
20	43	AP	87	20	78	49.84±0.19(6)
21	42	P	120	26	60	50.08±0.15(8)
22	44	AP	159	18	70	49.96±0.18(4)
23	41	P	27	8.7	65	49.92±0.25(6)
24	43	P	24	14	51	50.10±0.14(8)
25	43	AP	38	8.5	48	49.96±0.11(6)
26	41	P	27	10	60	50.06±0.10(5)
27	40	AP	36	9.2	56	50.08±0.17(5)
					Averages:	P(14 samples): 50.07±0.03†
						AP(13 samples): 50.05±0.03

b, Results of Bonner *et al.*

1	~20	AP	9.4	~20	52.9	49.29±0.20(10)
2	17–23	AP	15.0	~20	50.9	49.57±0.14(18)
3	27	AP	12.5	~6	54.9	49.70±0.16(15)
4	13–17	P	28.8	~20	73.9	50.40±0.12(22)
5	10–17	P	40.0	~20	75.6	50.37±0.14(28)
6	~20	P	14.4	~20	76.3	50.57±0.13(16)
					Averages:	P(3 samples): 50.45±0.06
						AP(3 samples): 49.52±0.12

% Decomposition is estimated from values measured by the enantiomeric marker technique[28] on preliminary samples and checked on representative samples (11, 12, 16, 18, 19, 21, 23–27).

* The error is the standard deviation from the mean for the number of analyses given in parentheses.

† The error is $\sigma/\sqrt{n}$ where σ is the standard deviation from the average %D for the n P or AP samples.

with their spins antiparallel (AP) to their momenta were reported to cause preferential destruction of the D-enantiomer. Moreover, the L-enantiomer of leucine was reported to be preferentially destroyed by electrons polarised with their spins parallel (P) to their momenta. These results were of special interest as electrons emitted in β-decay have AP spins and also it is the L-isomer of leucine which occurs in nature. The precise physical processes responsible for such an asymmetric degradation remain unclear. We report here that using an electron source[6] which provides a much higher degree of polarisation (typically 43%), we have reinvestigated the effect of irradiation of DL-leucine with longitudinally polarised electrons. In particular, the increased spin polarisation was expected to result in a more pronounced asymmetric degradation. However, no asymmetric decomposition was observed.

The polarised electron source used has been described elsewhere[6]. Briefly, spin-orientated helium (2^3S) metastable atoms are produced by optical pumping in a flowing helium afterglow. Electrons generated in chemi-ionisation reactions involving those metastable atoms and a reactant gas are polarised due to spin conservation[7]. The direction of the electron spin polarisation is easily reversed, without changing the beam trajectory, by reversing the sense of the circularly polarised optical pumping light. The electrons are extracted from the afterglow and formed into a collimated beam by a series of electrostatic lenses. The polarisation of the output beam is determined by measuring the Mott scattering asymmetry when the beam is scattered from a thin gold foil biased at 120 kV (for review see ref. 8). The electrons extracted from the afterglow are polarised transversely as this is convenient for Mott polarisation analysis. A Wein filter[9] located in the beam line is used to rotate the electron spins to obtain the beam of longitudinally polarised electrons required for sample bombardment.

Solid DL-leucine samples (0.6–2.5 mg) were mounted for irradiation on a thin sheet of aluminium foil backed by a layer of scintillation plastic and biased at 120 kV. Samples were held in place either by an extremely thin collodion film (1–16) or, as used by Bonner *et al.*[4,5], in a collodion matrix (17–27). Samples were irradiated with the beam polarised with electron spins parallel and antiparallel to their momentum vectors for target thickness ranging from 8.5 to 40 mg cm^{-2}. Combinations of electron beam current and irradiation times were varied to obtain sample decomposition (as measured by the enantiomeric marker technique[10]) of 35–80%. Although the electron source

used can produce microampere beam currents, we kept currents low to prevent heating of the sample. Despite the wide range of current densities (24–1,110 nA cm^{-2}) no significant variation was observed in the decomposition rate (0.016 mg nA^{-1} h^{-1}), which compared favourably with that of Bonner *et al.*[4,5] (0.015 mg nA^{-1} h^{-1}).

After irradiation, samples were dissolved in 3 M HCl in 2-propanol (2 ml) and maintained at 100° C for 3 h. After solvent evaporation, the resulting 2-propyl esters were treated overnight at room temperature with a 1:1 mixture of trifluoroacetic anhydride and methylene chloride (2 ml). The enantiomeric composition of the resulting *N*-trifluoroacetyl 2-propyl esters was determined by gas-liquid chromatography (GLC)[11] on a column (2.4 m × 2 mm) of SP-300 (5% *N*-lauroyl-L-valyl-*tert*-butylamide on Supelcoport; 100–120 mesh) using a Hewlett-Packard model 402 unit coupled to a Hewlett-Packard model 3370B digital electronic integrator. The precision and sensitivity of the method were confirmed using leucine of known enantiomeric composition processed in a manner identical to the irradiated samples. With 49.5% D-enantiomer our technique measured 49.56 ± 0.1% (5 analyses); with 50.5% D-enantiomer it measured 50.54 ± 0.08 (7 analyses).

Each irradiated sample of DL-leucine provided sufficient material for several determinations of enantiomeric composition. Before each determination either a sample irradiated with unpolarised electrons or a non-irradiated sample was analysed as a control to identify instrumental difficulties. To preclude bias GLC was carried out with the sense of polarisation of the bombarding electrons concealed.

As Table 1 shows, in spite of the wide range of experimental parameters investigated and attempts (samples 23–27) to duplicate as closely as possible the conditions reported by Bonner *et al.*[4], we observed no asymmetric decomposition of DL-leucine by longitudinally polarised electrons. Indeed we observed no single case of preferential decomposition of one of the enantiomers of DL-leucine on irradiation with polarised electrons.

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REPLY—We are indebted to Drs Walters and Hodge for making available to us an earlier version of the above manuscript in which only the first 22 experiments were reported. At our suggestion that these experiments were not comparable to ours due to the much higher current densities used, they undertook experiments 23–27, where the experimental parameters were apparently much closer to those we used. It is therefore particularly puzzling to us that these last five experiments also failed to show any asymmetric degradation.

In our linear accelerator irradiations we also occasionally (in about one-third of our experiments) observed no asymmetric degradation whatsoever. When an asymmetric effect was observed, however, it was consistently in one direction for AP electrons (three experiments; 43 GLC analyses) and in the opposite direction for P electron (three experiments; 66 GLC analyses), with statistical significance (*t* test) at the 99.9+% confidence level. Clearly, there are unknown operational parameters over which we, at least, had incomplete control in our experiments.

Since the initial publication of our positive results we have reported[1,2] the discovery of the racemisation of amino acids by ionising radiation. It seems likely that such radioracemisation must also compete with asymmetric degradation[2] in irradiation experiments such as ours and those of Hodge *et al.*, whether because of the electrons themselves or their bremsstrahlung. Could it be that unknown experimental parameters might tip the balance between a slight asymmetric bias in such irradiations, or (with overriding radioracemisation) none at all? Future experiments may shed light on this or other possible reasons for the unexplained discrepancies in our results and those of Hodge *et al.*

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Errata

In the letter 'Calcium conductance of acetylcholine-induced endplate channels' by P. D. Bregestovski, R. Miledi and I. Parker, *Nature* **279**, 638–639, the greek letter tau (τ) was omitted from six places in the text. These are: para 2 line 13; para 6 lines 1, 4, and 6 (twice); para 9 line 4. These errors are corrected in reprints of the article.

In the article 'Morphology and intracortical projections of functionally characterised neurones in the cat visual cortex' by C. D. Gilbert and T. N. Wiesel, *Nature* **280**, 120–125, in para 4 line 8, for diaminobutyric acid read 3,3'-diaminobenzidine.

Corrigendum

Since publication of the letter 'A Morarian age for the "younger Moines" of central and western Scotland' by M. A. J. Piasecki and O. van Breemen, *Nature* **278**, 734–736, the term 'Grampian group' has been changed to 'Grampian Division'. This follows discussions with an IGS working party and others. The term 'Division' is as used by Johnstone *et al. Mem. Am. Ass. Petrol. Geol.* **12**, 159–180 (1969).

matters arising

Mechanisms for radiation-induced shrinkage of voids

EVANS[1] has recently reported on the radiation-induced shrinkage of voids in molybdenum and its alloy TZM (Mo, 0.5% Ti, 0.1% Zr). Electron microscope measurements made on 3-mm disks irradiated in a fast reactor at 450±25 °C reveal that initially produced swelling of 0.57 and 0.34% at a total neutron dose of 3.5×10^{26} m^{-2} equivalent to 17.5 displacements per atom (d.p.a.) reduced on re-irradiation to 0.20 and 0.01% at a neutron dose of 8×10^{26} m^{-2} or 40 d.p.a. in molybdenum and TZM respectively. A related though opposite behaviour is found in the vacancy type loop structure, typically 5 nm in diameter, whose concentration increased to 6×10^{22} m^{-3} and 1.3×10^{23} m^{-3} at the highest dose level in molybdenum and TZM respectively.

Three mechanisms can give rise to negative swelling rates. First, solute atom segregation at the void surface considered by Marwick[2] which makes the voids biased against vacancies, a possibility discussed by Evans. Second, shrinkage due to thermal emission from voids having a radius less than a critical value[3-6], a mechanism which requires high temperatures and is unlikely to be important in the present context. The third mechanism[5,6], which is applicable at relatively low temperatures of 450 °C and in conditions where vacancy loops are produced, seems to be pertinent for the observations reported for molybdenum and TZM. The trapping of a fraction ε of the radiation-produced vacancies as vacancy loops or as impurities would require that a certain minimum dislocation density ρ_{crit} should develop so that the bias Z_i would allow a sufficient number of vacancies to go to neutral sinks like voids. An expression for ρ_{crit} involving the recombination parameter α, dose rate K and vacancy and interstitial diffusion constants D_V and D_I respectively can be obtained within the framework of the BEK model[7]. The voids will therefore shrink if the total dislocation density due to network and loop dislocation is less than[5,6]

$$\rho_{crit} = (\alpha K/D_V D_I)^{1/2}\varepsilon/(Z_i - 1)$$

The high vacancy migration energy of about[8] 2.0 eV for molybdenum will make D_V small and ρ_{crit} large at low temperatures. Though accurate values of the parameters are not known an estimate using $\alpha/D_I = 10^{20}$ m^{-2}, $K = 10^{-6}$ d.p.a. s^{-1} and $\varepsilon \sim (Z_i - 1) \simeq 0.05$ would give ρ_{crit} in the range 10^{15}–10^{16} m^{-2}. This compares very favourably with the observed vacancy loop densities of 9×10^{14} m^{-2} and 2×10^{15} m^{-2} for molybdenum and TZM respectively calculated from the loop size and concentration values reported. The mechanism therefore seems to be relevant for explaining the radiation-induced shrinkage of voids observed by Evans. The reason that the voids should initially grow at these temperatures and dislocation density values needs careful examination and explanation.

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EVANS REPLIES—Krishan and Nandedkar correctly point out that negative swelling rates can arise in a situation where a fraction of the radiation-produced vacancies are effectively removed from the system in the form of vacancy loops. Their expression showing the importance of dislocation density is for the mutual recombination dominant regime. For situations where recombination can be ignored but where thermal emission of vacancies from the loops is still negligible it is easy to show that the condition for void shrinkage is given by $\varepsilon > (Z_i - 1).(Z_i + y)^{-1}$, where Z_i is the bias and y is the ratio of void sink strength to dislocation sink strength. This expression may be more appropriate to the molybdenum case if a vacancy migration energy of 1.3 eV (ref. 1) is accepted.

To apply the mechanism suggested by Krishan and Nandedkar to my void shrinkage results[2] one must, as they point out, explain the growth of voids in the earlier stages of irradiation. In theory this is not too difficult: if one considers the expression above, an initial high dislocation density (and low y) could allow growth of voids. If the dislocation density remains constant the associated increase in y actually leads to a novel saturation condition for swelling but a subsequent drop in dislocation density would lead to the required void shrinkage. However, this picture is too inconsistent with experimental observations to be seriously considered for the molybdenum situation. One example from the void shrinkage results is the simultaneous large increase in dislocation density contrary to the requirements above, but more generally there is the problem of void growth in molybdenum at temperatures down to 330 °C (ref. 3) ($\sim 0.21 T_m$) even when the void sink strength is dominant ($y \sim 10$), and where the Krishan and Nandedkar assumption $\varepsilon(Z_i - 1)^{-1} \sim 1$ would imply negative swelling rates. The only easy way out of this dilemma is to accept that in molybdenum $\varepsilon(Z_i - 1)^{-1} \ll 0.1$, and in contrast to materials such as 316 stainless steel[4,5], assume that the vacancy loops play a negligible part in swelling processes below the $0.3T_m$ threshold. The differences between cascade collapse behaviour in molybdenum and f.c.c. metals during ion bombardment[6] apparently support this viewpoint.

The suggestions of Krishan and Nandedkar may have applications elsewhere, for example, in a thermal cycling situation ((A. J. E. Foreman, personal communication) but cannot easily explain the molybdenum and TZM results. I still feel that the explanation already provided[3,7]—where the segregation of a transmutation impurity (probably technetium) to void surfaces imposes an effective bias on the voids simply by having a low exchange probability for a vacancy—fits the experimental facts reasonably well. Note that this explanation does not depend on the reverse Kirkendall flux that Marwick[8] has suggested for concentrated alloys. However, it does closely fit the surface energy barrier model recently suggested by Fikus and Johnson[9].

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Possible Sun–weather correlation

AS I have recently published[1] a re-examination of some of Xanthakis' work[2], in which he claimed remarkably high correlations between solar activity and an index of zonal precipitation (for example, $r = +0.77$ in the zone 70°–80° N and $r = -0.71$ for 60°–70° N), I feel I should comment on the recent exchange between Xanthakis and Gerety[3,4].

My re-examination was prompted by the unusually high correlations claimed, the questionable discussion by Xanthakis of 'X-wise distributions'[5] and, more particularly, by the arbitrariness of the derivation of zonal mean $R - R_0$ values. Xanthakis defined $R - R_0$ for each year and station as the actual total precipitation, R, at that station in that year minus the lowest annual precipitation, R_0, reported for that same station over all the years considered. In the calculation of the zonal means Xanthakis averages together stations having very different mean $R - R_0$ values, with no attempt at normalisation (apart from assigning almost arbitrary R_0 values). Furthermore the stations come in or drop out in random fashion according to whether records are available. Thus, even if a perfect correlation between $R - R_0$ values and solar activity existed at each station (which is certainly not the case), the averaging process would be expected to inject discontinuities whenever a station record began or ended. In this respect Xanthakis' method should lead to poor correlations. This makes the claimed high correlations even more remarkable, and indeed puzzling.

The data for 70°–80° N were re-evaluated because that zone contains the least data, Xanthakis published a full tabulation of his data for that zone[2], and his result for that zone was an apparently highly significant correlation ($r = +0.77$) which did not change sign. Like Gerety *et al.*[6] I found that Xanthakis had used only part of the available data, and my analysis using additional data does not support the claimed highly significant correlation. I obtain a correlation of +0.38 which, considering that the data have been smoothed, is barely significant at the 95% confidence level. Xanthakis rightly points out that a different data sample will lead to a different result. However, the great reduction in the observed correlation using the larger sample certainly throws into question the significance of Xanthakis' result.

More importantly, unlike Gerety *et al.*, I chose only to use data as described by Xanthakis, who stated (or certainly implied) that he used all the available data published in *World Weather Records*[7] except that "only stations with more or less extensive series of precipitation data have been considered"[2]. An examination of his Table 2*a* shows that he used stations with as little as 6 years (Ingoy) or 8 years (Myggbukta) of data.

Examination of *World Weather Records* reveals a complete set of data for Isfjord Radio, Spitsbergen for 1912–24, which Xanthakis omitted even though this meant that for 1912–21 his 'zonal mean' was based on only one station. For the interval 1951–60 there are data for five additional stations—Isachsen (10 yr), Sachs Harbour (5 yr), Clyde (9 yr), Barter Island (10 yr), Chokurdakli (2 yr), and Vize Island (8 yr). All but Chokurdakli lie between the meridians 156° W through 0° to 80° E as stipulated by Xanthakis.

The issue is thus not whether different samples of stations were used, but rather why Xanthakis used the particular stations (which give such remarkable results) and did not use others which detract from the significance of the results. Furthermore, why was no mention made and no reason given for omission from the zonal mean of apparently good data (available from the same data source) from long-record stations such as Isfjord Radio, Isachsen and Barter Island, when shorter records were used from Ingoy and Myggbukta? Why does the choice of stations for inclusion in the zonal means consistently lead to higher correlations than those found using larger data samples?

In an attempt to answer these questions I have tested for the possibility that the correlation coefficients found by Xanthakis using his samples of stations and those found by using the larger available data samples are drawn from the same population by a random or chance selection of stations. According to Snedecor[8] this is done by applying a Z transform to the correlation coefficients and a Student t test to the difference between the Z values.

Table 1 summarises the results for three latitude belts which have been independently assessed by Pittock[1] and Gerety *et al.*[6], following the procedures outlined by Xanthakis[2]. Assuming N independent data pairs as indicated for each latitude belt, the probability P that each pair of samples of stations is drawn at random from the same population is shown. N has been reduced to allow for the computed autocorrelations in the data (including smoothing) according to the standard formula given by Quenouille[9]. Taken separately, the result for each of the three latitude zones suggests that Xanthakis' selection of stations (a selection which gives apparently significant correlations) was probably not by chance. Combining the results, it seems that the joint probability that Xanthakis' favourable selection was by chance is less than 1 in 30,000.

I am grateful to Dr E. J. Gerety for supplying the results of his computations.

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XANTHAKIS REPLIES—Pittock claims that the X-wise distribution of the reported correlations between $R - R_0$ and I_a (ref. 1) is questionable. The same dispute was put forward previously by Roberts[2]. However, in the light of recent analysis by Gerety *et al.*[3] it seems that in the zone 50°–60° N their correlation between $R - R_0$ and I_a has changed sign from negative (1885–1913) to positive (1914–60)[5]. This change of sign of the correlation, which is

Table 1 Correlation coefficients, r, between smoothed $\overline{R - R_0}$ and Xanthakis' solar activity index Ia, for three latitude zones over the years indicated, as obtained by Xanthakis[2] with his selection of stations, and those obtained by Pittock[1] (for 70°–80° N) and Gerety *et al.*[6] (for 60°–70° N and 50°–60° N) using larger available data samples

Zone	Years	r		N	P
		Xanthakis	Larger samples		
70°–80° N	1912–60	+0.77	+0.38	19	~0.07
60°–70° N	1882–1960	−0.71	−0.35	31	~0.05
50°–60° N	1914–65	+0.79	+0.20	21	~0.009

P is the probability that the data samples in each latitude zone are drawn at random from the same population, where N independent data pairs are assumed to exist. N has been reduced to allow for the computed autocorrelations in the data (including smoothing) according to the standard formula given by Quenouille[9].

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also observed at other latitude zones as well[4], is what we have originally called X-wise distribution[1].

The values of R_0, at individual stations, were not assigned "almost arbitrarily" but, as Pittock also mentioned, they refer to the lowest annual precipitation reported for the same station over all the years considered.

Pittock re-examined the results for the latitude belt 70°–80° N which, as he agrees, contain the least data. But this zone was only examined[1] to get a general idea of the behaviour of the quantity $R - R_0$ over the polar regions.

As regards the stations Barter Island (70°08′ N) and Clyde (70°27′ N), which are located at the boundary of the latitude belts 70°–80° N and 60°–70° N, these were grouped with the data of the 60°–70° N belt (see Table 3 of ref. 1) and not in the 70°–80° N, because their $R - R_0$ values are negatively correlated with I_a index, that is, as is the case for the corresponding data of the stations in the belt 60°–70° N. It is obvious that if in the very few stations (1–6) of the zone 70°–80° N, two more stations are added, negatively correlated with I_a, the correlation coefficient will be reduced significantly. Also, as King points out, "Analyses combining data from zones exhibiting opposite solar-cycle effects will inevitably lead to the erroneous conclusion that no solar-cycle effect exists, as will an analysis of rainfall data from regions situated between zones in which the sunspot effect is opposite"[6].

The omission of the stations Isachsen (10 yr), Sachs Harbour (5 yr) and Vize Island (8 yr) during the interval 1951–1960 was considered necessary for homogeneity purposes of the record to avoid an abrupt increase of the number of stations in that period.

With regard to the results of Table 1 in Pittock's analysis, I should note that no selection of stations was made for the zone 50°–60° N, for which all stations published in *World Weather Records* were included in the calculations.

Only in the belt 60°–70° N, 20 Siberian stations (long 65°–170° E) were studied separately, because of the different behaviour of their $R - R_0$ values with respect to the corresponding values of the remaining 20 stations in that zone, as can also be seen in Fig. 5 of ref. 1. This separation in my opinion was necessary.

Finally these research studies have been extended to include all the northern and southern latitude zones more comprehensively[4,7,8].

PITTOCK ADDS—Xanthakis' X-wise distribution is questionable because it involves a post-hoc decision for each data point as to whether a positive or negative correlation is appropriate. This adds an extra degree for each data point and enables any random scatter of data to be turned into an impressive apparent correlation. Results obtained by such a process have no statistical significance.

Xanthakis defined R_0 as the lowest annual precipitation reported at that station over all the years considered, but in fact he rounded off R_0 values to the nearest 100 mm below the lowest annual preciptation for most of the stations, but sometimes to the nearest 100 mm above (for example, Myggbukta and Vardo) and in one case to 300 mm at Jan Mayen where the lowest annual preciptiation was 197 mm in 1926. "Almost arbitrary" seems to be a fair description of this process.

Xanthakis now admits that the stations included in his zonal means were selected on a post-hoc basis according to the signs of their individual correlations of R–R_0 with I_a. This clearly invalidates Xanthakis's claimed levels of statistical significance. As indicated in my discussion of X-wise distributions, any set of random data can be classified into sub-groups on a post-hoc basis so as to give apparently significant correlations within sub-groups.

Xanthakis has still not explained why he omitted from his calculations for the zone 70–80° N the data for Isfjord Radio, Spitsbergen for 1912–24 even though he used data from the same station for 1925–60.

When someone comes up with an *a priori* basis on theoretical grounds for grouping stations or data by area, zone or era, and then shows statistically significant correlations within such previously defined sub-groups, we may get somewhere. Selection of data on an arbitrary post-hoc basis proves nothing.

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Use of the solar limb effect to test photon decay and cosmological redshift theories

CRAWFORD[1] suggested photon decay as the result of a gradual energy loss of photons due to their interaction with a curved space-time caused by tidal stresses on the finite spread of the photons. He attempts to explain with his theory the Hubble redshift, the solar limb effect, and the photon decay observed from the Pioneer-6 spacecraft when close to the Sun. The main support for his theory comes from the apparent excellent agreement of the prediction of his theory and the observed solar limb effect. The solar limb effect the systematic shift of the wavelengths solar Fraunhofer lines towards red wh going from the centre to the limb of t Sun after solar rotation effects have be removed. The solar limb effect has be used in many other more or less exo theories involving photon deca cosmological redshifts and so on[2–12]. It therefore of interest to summarise t properties of the limb effect and t compare them, in this instance, with t Crawford theory.

Any theory put forward to explain t limb effect should explain the followin properties. (1) After removal of t Einstein gravitational redshift co responding to 636 m s^{-1}, solar spectr lines show a wavelength shift of Δ to t blue at the disk centre. (2) The magnitud of Δ depends strongly on the strength c the line used[13,14] on the position in the lin at which the shift is measured[15] and on th excitation and ionisation potential of th line[16,17]. For weak lines Δ corresponds t ~400 m s^{-1}; for strong lines to ~0 m s (3) When going from centre to limb th wavelength stays nearly constant until on approaches the limb closely. At 0.2 sola radius from the limb the wavelength start shifting towards the red. The absolut shift reaches 0 m s^{-1} at ~0.02 solar radiu from the limb and shows a slight redshif (~0.1Δ) closer to the limb[11], which prob ably disappears at the limb itself. Thi redshift has been called 'super gravitational redshift'. (4) In sunspots th limb effect is absent ($\Delta = 0$)[18].

It was recently shown[19] that all these properties can be explained qualitatively in terms of so-called convective blue shifts resulting from the bright upward-moving convective elements in the solar atmosphere (granules) contributing more to the average radiation than the dark downward-moving elements. Quantitatively, the calculated convective shifts fall short by a factor 2–3 of the observed ones, but that discrepancy may be caused by an inadequate granulation model based on only partially resolved observations of the solar granulation.

Crawford's theory predicts a redshifting of lines when going from the centre of the Sun to the solar limb corresponding to 911 m s^{-1}, which is independent of line strength and position in the line. The same shift should be present in sunspots. Crawford tests his theory using data obtained for relatively weak lines by Plaskett[20], Adam[21] and Higgs[22] after correction of the observed wavelengths for a postulated average radial flow of matter into the Sun of 360 m s^{-1}. He obtains good agreement between the variation of wavelengths between centre and limb predicted by his theory and the 'corrected' data. Had he used the limb effect in strong lines or in sunspots the agreement would have vanished. Had he not postulated the 360 m s^{-1} inflow, a procedure which in our view has no justification, the agreement

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would also have disappeared. Crawford also predicts a redshift of the solar lines at the solar limb of 911 m s^{-1} in addition to the 636 m s^{-1} Einstein gravitational redshift (1.547 km s^{-1} in total). But observations at the solar limb give a value close (within ~50 m s^{-1}) to the gravitational redshift leaving an order of magnitude disagreement with the Crawford theory.

There is in our view no justification of Crawford's theory in terms of the observations of the solar limb effect. A major part, and possibly all, of the solar limb effect can be explained by simple physical processes in the solar atmosphere involving convective, wave and pressure shifts without taking recourse to any of the many exotic theories proposed. Observational and theoretical studies of the solar limb effect, from the point of view of solar atmospheric dynamics are obviously most important in setting limits to the magnitude of the effect predicted by theories of photon decay.

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The problem of thrown string

OUR attention has been drawn to the problem of the thrown string[1,2] by Bass and Bracken[3], who related the probability density of the distance between the ends of the string when in space to the volume of the ellipsoid in which the string must be contained. We[4] have already suggested a modification to this theory in an attempt to explain the experimental results of Synge[2]. However, we have recently reconsidered the problem.

We felt that in order to test the validity of the different possible models, an experimental distribution was required

Table 1 Frequency distributions for $\lambda = D/L$

Interval	Frequency 'springy' string	Frequency flexible string
0.0–0.1	344	379
0.1–0.2	641	763
0.2–0.3	739	884
0.3–0.4	662	654
0.4–0.5	485	428
0.5–0.6	481	284
0.6–0.7	391	199
0.7–0.8	427	131
0.8–0.9	450	119
0.9–1.0	400	49
Total	5,020	3,890

based on a large set of observations. The problem of generating the necessary data was solved by posing the problem of the thrown string to our first year engineering students as an exercise in data collection, descriptive statistics and fitting models to data. Each student had to obtain observations for at least a hundred throws of the string. The students were divided into two groups, one used a rather 'springy' red plastic, waxed, string, the other a flexible white cotton string of the same physical dimensions. All the strings were 54 cm long. The pooled data for each group is given separately in Table 1 and the corresponding histograms are shown in Fig. 1.

The density function proposed by Bass and Bracken is shown in Fig. 1. This shows that the model does not give a realistic description of the physical situation.

Observing the thrown string lying on the table it seemed that the steady motion of a point moving along the string from one end towards the other would be some sort of continuous two-dimensional random walk. The problem is then not unlike that studied by Broadbent and Kendall[5] concerning the wanderings of

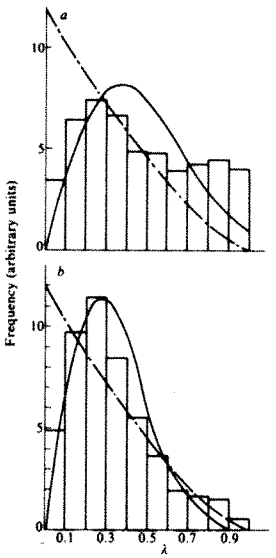


Fig. 1 Histogram and density functions for the 'springy' string (*a*); and for the flexible string (*b*). Bass and Bracken model (dashed line); Random walk model (solid line).

the larvae of the helminth *Trichostrongylus retortaeformis*. They assumed that the orthogonal X and Y components of position were independent random functions of time with zero mean and equal variance. X and Y were then assumed to be Wiener processes. This was not reasonable because it implies that the velocities in the x and y directions would be white noise. Perhaps it would have been more reasonable to have assumed that X and Y were the outputs of identical third order lagging systems excited by separate independent white noises. However, provided the systems were linear, this would not affect the result that after a given time X and Y would have independent normal distributions with zero mean and equal variance.

By analogy, our new hypothesis for the thrown string is that the distance D between the two ends will have probability density

$$f(D) = \frac{D}{\omega L^2} \exp\left(-\frac{D^2}{2\omega L^2}\right)$$

where L is the length of the string and ω is a constant dependent on the nature of the string and the experimental conditions. A similar result was reported by Kingman[6] using a slightly different model.

This density function is compared with the experimental results in Fig. 1 which shows that the proposed model reflects the general nature of the results reasonably well. The discrepancies between the theory and the experiment seem to be due to the lack of ability of the model to describe the upper tail of the distribution. We feel that the large frequencies at the upper tail are primarily a consequence of the impact of the string with the table resulting in a straightening out of the string; the effect is most noticeable in the 'springy' string.

We conclude first that the Bass and Bracken[3] and our previous[4] theories are false in that they are not borne out by experimental results. Second, a good approximation to the distribution is apparently given by the random walk model described above provided the string is not springy. The degree of flexibility of the string may be allowed for by suitable choice of the one parameter in this distribution. More experiments with different non-springy flexible strings are required to verify this second conclusion.

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ICE AGES

M

: Solving the Mystery

Professor John Imbrie and Katherine Palmer Imbrie

May 1979 £6.95 224pp 0 333 26767 2 illustrated

Knowledge that the world once endured an ice age has been widespread for more than a century. Indeed, the concept is now so familiar that nearly every winter storm prompts dramatic headlines: Is a new ice age upon us?

This book tackles that burning question and attempts to unfold, for the interested general reader and those with a more academic interest, the entire saga of the Ice Age 'mystery' – why ice ages occurred, what they were like, and when the next one is due.

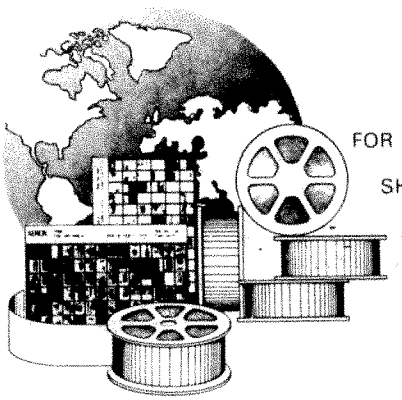
It is written by one of the principal scientists on the multi-million dollar CLIMAP project which set out to study changes in the climate of the earth over the past 700,000 years. Out of the group's analytical work came an exciting by-product: confirmation of the correctness of one of the several theories of what causes ice ages to occur. The theory that was confirmed, and is now explained along with many others in ICE AGES, is the Milankovitch, or astronomical theory which sees periodical glaciation as the result of three regular movements the earth makes as it travels around the sun: changing ellipticity of the earth's orbit, "wobble" of the earth's axis, and the changing tilt of the earth's axis.

"ICE AGES is fascinating in its scientific interest and also as a detective story. I recommend it to all." Dr. William F. Libby, Nobel Laureate.

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Transplantation Antigens — A Study in Serological Data Analysis

by **N.H. Selwood,** *National Tissue Typing Reference Laboratory, Bristol*
and **A.J. Hedges,** *Dept. of Bacteriology, University of Bristol.*

There can be little doubt that the urgent need which arose to resolve the antigen systems of the white blood cell has stimulated an intensity of activity and the generation of data on a scale previously unknown. This book attempts to simplify description of the many new and established methods which have been employed to extract information from basic serological data by dividing them into two main approaches, the statistical and the numerical. The text is directed generally at serologists and, although it inevitably contains some mathematics, these have been kept to a minimum and may be safely overlooked by those whose interest lies in the principles rather than the details. These principles do not relate only to the specialist field of HLA serology but are fundamental to all serological work. It has been the aim of the authors, wherever possible, to refer to basic concepts in order that the questions of applicability should be capable of the widest possible interpretation.

November 1978 — 156 pages
0471 99657 2 — $21.85/£9.50

Management of Energy/Environment Systems: Methods and Case Studies

edited by **W.K. Foell,** *Energy Research Center, University of Wisconsin-Madison.*

A book which concentrates on the solution of global energy problems through energy system management at regional and subnational levels. It arises from a joint study by authorities from the east and the west of three regional energy/environment systems with very different political, economic and social frameworks; these are the German Democratic Republic, the Rhône-Alpes region of France and Wisconsin in the USA. This is, therefore, a significant contribution to the management of the energy problems facing most industrialised societies. *(Wiley IIASA International Series on Applied Systems Analysis)*

July 1979 — 504 pages
0471 99721 8 — $39.50/£16.50

Biological Indicators of Water Quality

edited by **A. James** and **Lilian Evison,** *Division of Public Health Engineering, Civil Engineering Department, University of Newcastle-upon-Tyne.*

A book on the role of biological indicators in the assessment of water quality. It surveys the quality requirements for the various uses of water, such as supply, fishing, recreation etc. and discusses the various ways in which biological systems of classification have been developed. It covers the whole range from physiological tests to diversity indices and the spectrum of organisms from bacteria to fish. The practical value of the biological methods is stressed and their relation to chemical or physical alternatives. It covers a wide variety of experience from Europe, the United Kingdom and North America.
The book is based on papers given at a conference on the Biological Indicators of Water Quality held in Newcastle.

July 1979 — 624 pages
0471 27590 5 — $37.50/£15.00

Progress in Drug Metabolism Vol. 3

edited by **J.W. Bridges,** *Director, Institute of Environmental Health and Safety, University of Surrey,*
and **L.F. Chasseaud,** *Head of Metabolism and Pharmacokinetics, Huntingdon Research Centre.*

This is a review series intended to provide comprehensive, authoritative, readable, up-to-date and critical accounts by experts on the biological fate of a drug or xenobiotic in terms of absorption, distribution, transformation and excretion. The reviews are concerned with newer developments or progress in established areas, or they may retrieve and review the widely disseminated literature on a particular subject with the aim of keeping the reader more easily informed on the many and varied developments in drug metabolism studies.
Further volumes in this series are planned.

July 1979 — 382 pages
0471 99711 0 — $50.00/£19.25

Environmental Impact Assessment: Principles and Procedures SCOPE 5 2nd Ed.

by **R.E. Munn,** *Institute for Environmental Studies, University of Toronto.*

As the term is used in this book, environmental impact assessment is an activity designed to identify and predict the impact on the biogeophysical environment and on the health and well-being of humans of legislative proposals, policies, programme, projects, and operational procedures, and to interpret and communicate information about the impacts. Although the institutional procedures to be followed in the assessment process have been formalised in a number of countries, the scientific basis for these assessments is still rather uncertain. The published literature on the subject is scattered through many journals, and has not been evaluated critically in ways that are useful to managers and scientists. The first edition of this book appeared in early 1975. Since then, the number of jurisdictions requiring impact assessment has increased, as well as the literature on assessment methods. For these and other reasons, SCOPE convened a meeting in Toronto in October 1977 to prepare a second edition. A feature of the second edition is an Appendix which gives a selection of engineering references that may be of help when designing to meet environmental standards. *(SCOPE Series)*

July 1979 — 208 pages
0471 99745 5 — (paper only) $18.00/£7.50

Introduction to Marine Pollution Control

by **J. Williams,** *US Naval Academy.*

A holistic approach to marine pollution control drawing on research and developments in physics, chemistry, biology, law, and sociology. It emphasizes frequently encountered problems and situations, describes ocean characteristics that influence the spread of pollution, and offers insights into the nature of pollution, the origin of marine pollutants, and both chronic and acute effects. *(Ocean Engineering Series)*

July 1979 — 190 pages
0471 01904 6 — $29.00/£14.70



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reviews

A physicist who enjoys it

Rudolph Peierls

What Little I Remember. By Otto R. Frisch. Pp.219. (Cambridge University Press: Cambridge, 1979.) £9.50

MOST of nuclear physics has today become "big science", requiring large and expensive accelerators, with complex detection equipment and computers, used by large teams of research workers. Its senior practitioners have to spend much of their time and their energy on administration and organisation. This image of the new scientist does not fit Otto Robert Frisch at all. His very readable reminiscences are those of an individualist, at his best when working and thinking on this own, or with perhaps one close collaborator.

This characteristic comes out very clearly, together with the basic happiness of a man who enjoys what he is doing. Scientists tend to be driven by a variety of motives including, to varying degrees, ambition, a sense of duty to the community, curiosity about nature and its laws, and enjoyment in the design and execution of experiments. All these no doubt have some reality for Frisch, but the pleasure of doing what he does is clearly dominant. Ambition is the least of his concerns.

All this goes for his many other interests. Besides being an outstanding experimenter, with a flair for the simple and decisive experiment, he has many other interests and talents. This breadth probably owes much to his background. He grew up in Vienna, in a family belonging to the cultural tradition of early twentieth-century Vienna. His aunt, the famous nuclear physicist Lise Meitner, was his mentor in much besides physics. Next to physics his strongest line is music. He is a pianist of near-professional standard, and his hearing has absolute pitch. But here again he is less concerned with demonstrating his brilliance at the piano than in the pleasure of making music, and he can enjoy playing with others who may be much below his level in skill, as long as they share his love for music and his pleasure in making it.

He also likes drawing, and after every meeting the papers in front of him are covered with portraits or caricatures. Some of these are reproduced in the book. They are all recognisable likeness, and some bring out the personality of the subject excellently. His drawing is not of the standard of his music or his physics, but his enjoyment in the activity is again evident.

Presumably the drawings reproduced in the book are the most successful ones of many. The written characterisations of people, which, in his preface, he compares with the pencil sketches, also vary very much in depth. Niels Bohr and Lise Meitner come very much alive, as does George Placzek, with whom Frisch worked much in Copenhagen. But very little emerges of Chadwick, with whom he was closely associated in Liverpool and later in Los Alamos, beyond an account of their first encounter; and of some other colourful personalities there are only pale shadows.



George Placzek (1905-1955)

Sketch by O. R. Frisch

As a physicist, Frisch studied in the University of Vienna, where he received his PhD (he does not mention the subject of his thesis). After a year in a small firm making scientific instruments he was offered a position in the Physikalisch-Technische Reichsanstalt in Berlin, and after three years moved to Hamburg to work under Otto Stern, the authority on atomic beam research. That much migration was normal at a time when modern physicists formed a close-knit but widely-spread family.

But in 1933, when Hitler came to power, he had to be dismissed from his post as a "non-Aryan", and there followed the typical odyssey of a displaced academic of the time. A year at Birkbeck College in London under Blackett gave him the first acquaintance with life in England. This was the time when artificial radioactivity was discovered, and Frisch made his first venture into nuclear physics: he invented a clever device to observe very short-lived activities, and was able to discover new species of radioactive nuclei. He stayed in London only a year, and was then invited to Bohr's Institute in Copenhagen, where he remained until 1939.

This was a period of great excitement in nuclear physics. Fermi had started using the newly discovered neutron as a probe for studying nuclei, and neutron physics became one of the major subjects in many laboratories, including Copenhagen. Frisch became involved in this work, originally because he had a well-running counter suitable for detecting the activities produced by neutron bombardment. He describes the way the work was done, including the preparation of neutron sources by mixing radium and beryllium. A

particularly exciting moment was Niels Bohr's first pronouncement of the idea of the compound nucleus, as a comment after a seminar talk which highlighted the difficulties of the then conventional view in accounting for neutron resonances.

Then, in 1938, came the discovery by Hahn and Strassmann that radioactive barium was one of the products of the bombardment of uranium with neutrons. Frisch was visiting Lise Meitner in Sweden when she received the news of Hahn's result, and there is a fascinating account of the discussion which finally led them to realise that the uranium nucleus was undergoing "fission" (a term chosen by Frisch to describe the phenomenon). Before long he was back in Copenhagen and was doing an experiment which verified the suggested explanation.

By this time War was imminent, and Denmark was not a very safe place for a refugee from Hitler, so Frisch moved to Birmingham. He started some experiments relating to the fission problem which was still one of his main interest, but in a department not well equipped for nuclear physics, and with most of its members working on radar, progress was slow. He continued thinking about the implications and possibilities, including nuclear energy. Bohr had shown that a violent explosion was not possible with natural uranium, but Frisch started to question whether the possibility of separating the uranium isotopes in substantial quantities was really ruled out. He and Peierls concluded that a nuclear weapon did not seem to be impossible, and wrote a memorandum which led to an atomic weapons project being started in Britain. From then on most of his effort was devoted to this project. It was an odd situation that he and several others working on one of the most secret military projects were technically enemy aliens, and he tells of a number of ludicrous consequences of this. It soon emerged that he could work more usefully in Liverpool, where Chadwick's laboratory had a small cyclotron and was not preoccupied with other War research. He worked there, through the period of heavy air raids, until 1943, when it was decided to discontinue the British project during War time and send to the United States all those who could be useful to the American atom bomb work. So Frisch went to Los Alamos, and he gives a vivid description of life and work in this odd secret town. Very sensibly he was not attached to one of the large research groups there, but had a roving commission, helping with experimental troubles wherever appropriate.

One of his other accomplishments at Los Alamos was to learn driving, and he tells the story of his first accident, which brought him a broken rib and other minor injuries. He does not seem to remember another accident which became legendary at Los Alamos. Pulling aside to clear another car on a narrow lane without footpath, he knocked over an Army girl. The girl spent a few days in hospital and complained that Frisch had never visited her or shown interest. This was reported to him, and he promptly appeared in hospital, apologising and explaining that he had been concerned. "I enquired of the doctor about about your health and was glad to hear that there was nothing seriously wrong with you, mainly the effect of the fright".

After the War he became head of the Nuclear Physics Division of AERE, Harwell, the new atomic energy laboratory. According to his account of this period, he left the running of the division to his deputy, who enjoyed administration, and continued his individual research. Perhaps his diffident description exaggerates his lack of application to the work of running the division, but it is in character that he was not at his best in this kind of responsibility.

He had been in Harwell less than two years when he was offered the Jacksonian Professorship in Cambridge. The book is exceedingly brief about the 25 years he spent in Cambridge before retiring. He explains in the preface that his memory, like that of many others, is more complete about earlier events than about the more recent ones, and that it is embarrassing to write about people with whom one is still in touch. So the whole period, which includes his marriage and the growth of his two children, gets only a few pages. The story ends with a description of another ingenious device, a machine for measuring and evaluating bubble-chamber tracks, which he designed; he is now a partner in the firm manufacturing this commercially.

Three chapters of the book, Atoms, Nuclei and Research Resumed, contain expositions of parts of modern physics by way of background to the accounts of his own involvement with it. Being a physicist myself, I am not qualified to judge the appropriateness of these pieces. They are written with the clarity and simplicity to be expected from the author of *Meet the Atoms*, yet I wonder whether the reader who does not know the background can



Frisch with Lise Meitner

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The Structure of Matter

Robert M. Turnbull

A concise introduction to atomic, nuclear and particle physics for students in the early part of an undergraduate course. Deals with the principal topics in atomic and nuclear physics and goes on to describe the most recent findings in particle physics. Includes a problems section with answers.

256 pp 88 illustrations
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Avian Orientation and Navigation

Klaus Schmidt-Koenig

July 1979, x + 180pp., £12.50/$26.00 0.12.626550.X

During recent years there has been an explosion of ideas and research in the field of bird orientation and navigation, producing a vast amount of published material which makes a comprehensive account beyond the capacity of a single volume. The present book is more of a summary and review of the literature on all aspects of the subject over the last three decades or so, presenting the current state of knowledge and offering some speculations on future developments. Following a brief introduction to migratory achievements by birds, the main part of the book is devoted to the methodology and results of the experimental attempts to elucidate the mechanisms of orientation involved in bird navigation. In the final section, the hypotheses, theories and concepts that have been advanced to explain the phenomena of migration and navigation are discussed and evaluated on the basis of experimental evidence. The book is unique in its wide and objective coverage of the subject, and will be welcomed both as a reference source and as an introduction to the subject by researchers and students in zoology and animal behaviour, as well as by ornithologists and conservationists.

Climate and Weather in the Tropics

Herbert Riehl

July 1979, xiv + 612pp., £14.80/$31.50 0.12.588180.0

As tropical countries begin to play an important part in world affairs, the effect of tropical climates on agriculture, economic development and even global political relationships is increasingly felt. Therefore it is important that we understand the mechanisms of tropical weather systems and climates. New techniques with satellite and radar are available to help in gaining greater knowledge and international meteorological research has not been slow to exploit the advances made. The publication of these findings has made available much new information. With the current tendency to over-specialize within the broad meteorological spectrum has come a sudden burgeoning of related knowledge. With all this in mind the need arises for an up-to-date overview of tropical climate and weather. This book aims to present clearly and comprehensively the factual bases of the latest advances in tropical meteorology and their interpretation. Satellite and radar information is used extensively in the analysis of convective processes. Ferdinand Baer has contributed an extremely thorough chapter on numerical hurricane prediction; his discussion of the important advances made in the use of computers is applicable outside his particular field.

Society for General Microbiology No. 3

Microbial Polysaccharides and Polysaccharases

edited by R. C. W. Berkeley, G. W. Gooday and D. C. Ellwood

July/August 1979, xvi + 480pp., £16.00/$34.00 0.12.091450.6

Recognition by the Society for General Microbiology of the need to provide a compilation of what is known and what has been achieved, academically and industrially, in the science and applications of microbial polysaccharides was the factor that gave rise to the book. In a sense, it is a combined volume. It brings together papers from two symposia, that of the Society for General Microbiology entitled 'The Microbial Degradation of Polysaccharides' and that of one of its specialist groups, the Microbial Cell Surfaces and Membranes Group, on 'Microbial Extracellular Polysaccharides'. In this way it aims to consolidate discussion of topics from two related areas that have been separately treated in the past, microbial polysaccharides and polysaccharases. It aims, too, to assess current research in these fields and to suggest directions that further research might take in the short and medium term future. Many of the papers presented here are set solidly in a commercial context, emphasising the need to interpret theoretical research in an industrial light. The mixture of contributors is such as to reinforce this approach, comprising chemists, physicists and microbiologists from both academic and industrial backgrounds.

Applications of Graph Theory

edited by R. J. Wilson and L. W. Beineke

July/August 1979, xii + c.424pp., £18.50/$39.00 0.12.757840.4

One of the consequences of the recent rapid expansion of graph theory is that it has become increasingly difficult to determine what is currently known about any particular topic in the field. When it comes to the application of graph theory to the Sciences or Social Sciences this difficulty is compounded by the problem of understanding the language and literature of the area in question. In view of this the editors have collected together a series of expository surveys written by a distinguished group of authors, in the hope that such a collection might prove useful both to professional graph theorists interested in the applications of their subject and to those who may want to learn about the uses of graph theory. Unlike other books on this subject, this volume is written for graph theorists by people actively working in the various fields of its applications such as chemistry, geography, engineering and social sciences.

Integration of Analogue Electronic Circuits

J. Davidse

July/August 1979, x + 276pp., £18.60/$39.50 0.12.204450.9

In present day electronics monolithic technology constitutes a prominent vehicle for circuit implementation. In the context of digital circuitry its proliferation has been widespread. With analogue circuitry the pace of that proliferation has been somewhat slower: understandably so, however, when one considers the range of functions, the spectrum of applications and the variety of specifications characteristic of analogue electronics. The evolution of electronic techniques in this area has reached a point now where knowledge of the principles of monolithic analogue circuit design is indispensable for designers of analogue electronic circuits. That knowledge must also be at least desirable for designers of electronic systems and subsystems in which analogue circuits have applications. With this in mind, the Delft University of Technology has for some years been training students in analogue integrated circuitry. The purpose of the course, and of the book which resulted from it, was to present a comprehensive survey of this area of electronics and its development over the last decade or so.

Secretory Tissues in Plants

A. Fahn

July 1979, x + 302pp., £20.00/$42.00 0.12.247650.6

Secreted substances play an important role in the life of many plants, particularly in the interactions between plants and animals. They have also proved to be of great value to man throughout the ages. The tissues which produce these substances are themselves useful in research as they provide convenient systems for a variety of studies such as biosynthetic processes and short distance transport. Knowledge of their morphology, anatomy and ultrastructure is essential in order to understand how they function and the electron microscope has done much in recent years to further the investigations of the mechanisms of secretion and the relationships between structure and function. This book presents an overall picture of the structure and development of these tissues as determined by both electron and light microscopy studies. It has many illustrations and references. This comprehensive coverage of the subject will interest not only plant anatomists and physiologists but cell biologists, biochemists, endocrinologists and those studying the coevolution of plants and animals.

Advances in Insect Physiology Volume 14

edited by J. E. Treherne, M. J. Berridge and V. B. Wigglesworth

July/August 1979, viii + 442pp., £27.00/$57.00 0.12.024214.1

This highly acclaimed series aims to give a comprehensive survey of selected topics in insect physiology and to relate them to other areas of biological science. The reviews serve as a guide to recently acquired knowledge and also as a stimulus for further advances in the subject. **Contents: John Machin:** Atmospheric water absorption in arthropods. **Franz Englemann:** Insect vitellogen: identification, biosynthesis and role in vitellogenesis. **Arthur M. Jungreis:** Physiology of moulting in insects. **Tom Piek and K. Djie Njio:** Morphology and electrochemistry of insect muscle fibre membrane. **John Palka:** Theories of pattern formation in insect neural development. **Brian W. Staddon:** The scent glands of Heteropters. Subject index. Cumulative list of authors. Cumulative list of chapter titles.

Biomedical Research Applications of Scanning Electron Microscopy Volume 1

edited by G. M. Hodges and R. C. Hallowes

July 1979, x + 364pp., £29.50/$62.00 0.12.351001.5

Although scanning electron microscopy is becoming a routine method in biomedicine, until now there has been no publication dedicated to a discussion of its applications. The purpose of this open-ended series is, then, to review and evaluate these applications integrating current knowledge of structure, composition and function into a comprehensive framework. Each chapter includes a discussion of suitable preparative procedures and co-operative applications of other analytical techniques where appropriate. The scope of the series is such that it should serve as a comprehensive and stimulating guide to people entering the field of scanning electron microscopy as applied to biomedicine and prove an essential reference source for research workers including cell biologists, embryologists, developmental biologists, anatomists, pathologists and immunologists.

Biology of the Reptilia

edited by C. Gans, R. G. Northcutt and P. Ulinski

This widely acclaimed series provides an up-to-date and comprehensive review of the class Reptilia. Volumes 9 and 10 begin a new section in the series, providing the first treatment of the neurobiology of reptiles, a subject that logically ties together reptilian morphology with their physiology and behaviour.

Volume 9 Neurology A

September/October 1979, xvi + 462pp., £30.00/$63.00 0.12.274609.0

Contents: D. Starck: Cranio-cerebral relations in Recent reptiles. **James A. Hopson:** Paleoneurology. **Roland Platel:** Brain weight-body weight relationships. **David G. Senn:** Embryonic development of the central nervous system. **W. B. Quay:** The parietal eye-pineal complex. **Monika von During and Malcolm R. Miller:** Sensory nerve endings of the skin and deeper structures. Author index. Subject index.

Volume 10 Neurology B

October/November 1979, xiv + 376pp., £22.00/$45.00 (approx) 0.12.274610.4

Contents: P. J. Berger and G. Burnstock: Autonomic nervous system. **A. Kusuma, H. J. ten Donkelaar, and R. Nieuwenhuys:** Intrinsic organization of the spinal cord. **William L. R. Cruce:** Spinal cord in lizards. **H. J. ten Donkelaar and R. Nieuwenhuys:** The brain stem. **Martin Ernst Schwab:** Variation in the rhombencephalon. **Andre Parent:** Monaminergic systems of the brain. **M. G. Belekhova:** Neurophysiology of the forebrain. Author index. Subject index.

Food Science and Technology: A Series of Monographs

Brewing Science Volume 1

edited by J. R. A. Pollock

July/August 1979, xiv + 580pp., £30.00/$63.50 0.12.561001.7

It is intended that this volume and the subsequent volumes now in preparation should provide a comprehensive account of the present position of brewing science and its development.

Contents include: M. Moll: Analysis and Composition of Barley and Malt. **A. Mcleod:** The Physiology of Malting. **M. Verzele:** The Chemistry of Hops. **M. Moll:** Water in Malting and Brewing.

The Systematics Association Special Volume No. 12

The Origin of Major Invertebrate Groups

edited by M. R. House

July 1979, x + 516pp., £32.50/$68.50 0.12.357450.1

Two papers give a background setting to the early radiation of the Invertebrata; two more present a review of protozoan and metazoan radiation. Other contributors provide an analysis of the early evolution in invertebrate groups which include the Coelenterata, Bryozoa, Brachiopoda, Echinodermata, Graptolithina and possible early Chordata. In some cases several authors contribute to different aspects of radiation, for example that of Arthropoda. For the Mollusca there are four contributions from zoologists and paleontologists. A final chapter combines points taken from a recording of the meetings with written contributions to reflect the informed and lively exchanges that took place.

Organometallic Chemistry: A series of Monographs

Transition Metal Complexes of Cyclic Polyolefins

G. Deganello

August 1979, viii + 480pp., £34.50/$72.50 0.12.207950.7

The book is divided into five chapters dealing with metal complexes of cycloheptatriene, cyclotatetraene, cyclooctatriene, bicyclo [6.n.0] trienes and other polyolefins but each chapter also describes metal complexes of polyolefins related to these. Each metal or metal group is treated in order of increasing atomic number. The main topics discussed in detail are preparation, spectroscopic characterisation, structure, reactivity, and catalytic activity. Metal complexes of other organic moieties originated by rearrangement of the above polyolefins are also included. Particular emphasis is placed on NMR investigations and an appendix is added on the mechanistic aspects of the fluxional behaviour manifested in solution by several dimetal hexacarbonyl polyolefin complexes, studied by ^{13}C NMR spectroscopy.

Hormones in Blood 3rd Edition

edited by C. H. Gray and V. H. T. James

The three volumes provide a unique and definitive reference work which will be invaluable not only to the general scientific and medical reader needing to up-date his knowledge, but also to the specialist interested in the biochemical, physiological and clinical aspects of endocrinology.

Volume 1

July 1979, xx + 704pp. £37.50/$79.00 0.12.296201.X

Contents: D. R. Bangham: Foreword: standards for hormones. **A. Arimura and A. V. Schally:** Hypothalamic releasing and inhibiting hormones and factors. **U. A. Parman:** Insulin. **P. J. Lefebvre and A. S. Luyckx:** Glucagon. **P. H. Sonksen and T. E. T. West:** Growth hormone. **K. Hall and L. Fryklund:** Somatomedins. **S. Franks:** Prolactin. **T. Chard:** Human placental lactogen. **K. D. Bagshawe, F. Searle and M. Wass:** Human chorionic gonadotrophin. **W. R. Butt:** Gonadotrophins. **P. Mary Cotes and D. E. Gueret Wardle:** Erythropoietin. **P. G. Condliffe and B. D. Weintraub:** Pituitary thyroid-stimulating hormone and other thyroid-stimulating substances. **J. Robbins and J. E. Rall:** The iodine-containing hormones.

Volume 2

July/August 1979, c. 600pp., £27.50/$58.50 0.12.296202.8

Contents: D. R. Bangham: Foreword: standards for hormones. **G. N. Hendy, S. E. Papapoulos, I. G. Lewin and J. L. H. O'Riordan:** Parathyroid hormone. **S. E. Papapoulos, I. G. Lewin, T. L. Clemens, G. N. Hendy and J. L. H. O'Riordan:** Vitamin D. **L. J. Deftos:** Calcitonin. **B. A. Callingham and M. A. Barrand:** The catecholamines: adrenaline, noradrenaline and dopamine. **G. A. Smythe:** Serotonin. **J. A. Salmon and R. J. Flower:** Prostaglandins and related compounds. **S. R. Bloom:** Gastro-intestinal hormones I: pancreatic polypeptide, motilin, gastric inhibitory peptide, neurotensin, enteroglucagon and others. **G. J. Dockray:** Gastro-intestinal hormones II: gastrin, cholecystokinin and secretin. **C. R. W. Edwards:** Oxytocin. **C. R. W. Edwards:** Vasopressin.

Volume 3

August/September 1979, c. 700pp., £35.00/$78.00 (approx) 0.12.296203.6

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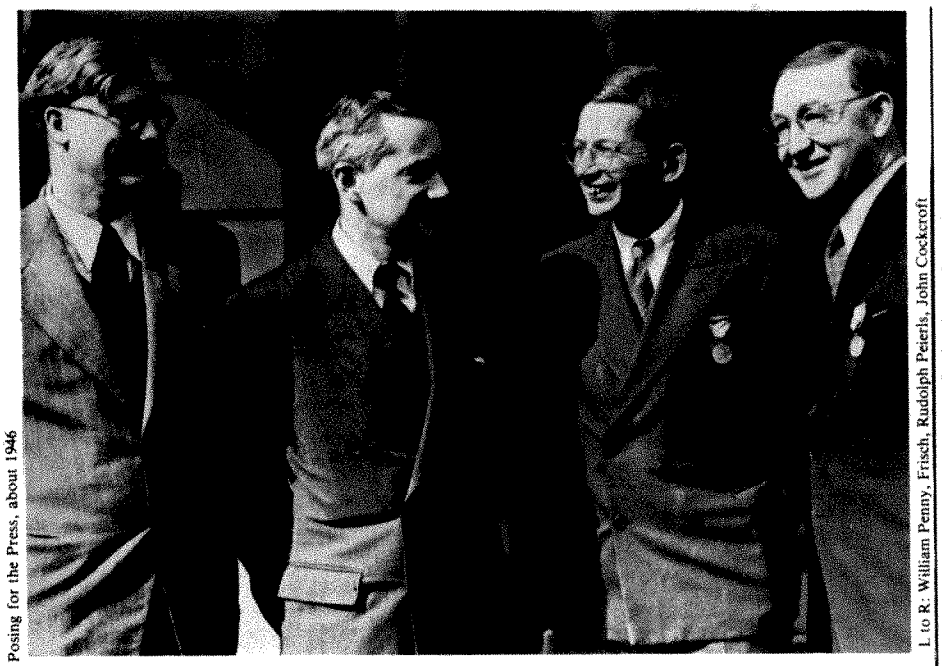
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Posing for the Press, about 1946

L to R: William Penny, Frisch, Rudolph Peierls, John Cockcroft

really assimilate enough information to help him with the biographical chapters which are the real meat of the book, and which seem quite intelligible on their own.

This is a happy book, from which the author's personality and his enjoyment of physics, of music, of life, emerges clearly. It is also a portrait of the pre-War world of physics, of the days of small numbers and small apparatus, of times when a physicist could think of an ingenious experiment today and set it up tomorrow. □

Sir Rudolf Peierls was Professor of Theoretical Physics at the University of Oxford until his retirement in 1974, and was previously Professor of Mathematical Physics at the University of Birmingham from 1937-63.

Gelada baboons

Ecological and Sociological Studies of Gelada Baboons. Edited by M. Kawai. Pp. 344. (S. Karger: Basel, Munchen, Paris and London, 1978). DM148; $74.

In a variety of ways, gelada baboons are one of the most unprimate-like primates: they are almost exclusively terrestrial; they feed extensively on grass; and they aggregate in large herds of unstable membership. Consequently, they are a species which might be expected to shed extensive light on relationships between ecological variables and behaviour and on the functional significance of behavioural differences between primates and ungulates.

The volume recently edited by Kawai provides a broad description of the ranging behaviour, social organisation and feeding ecology of the species in the Gich area of the Simien National Park in Ethiopia, but does little to provide satisfactory explanations of the geladas' many surprising characteristics. Four observers habituated several herds and learnt to recognise all individuals in one of them. As a result, they were able to examine both detailed social relationships among individual members of the harem-groups which aggregate to form herds, and relationships between neighbouring herds.

Twelve chapters, by combinations of the four authors, cover herd dynamics, inter-harem relationships, social behaviour within harems, the development of social behaviour and group structure, reproductive behaviour, communication, ranging behaviour and feeding ecology. Particularly in areas where it concentrates on answering questions rather than broad, quantitative description, the book reveals fascinating insights into the behaviour of the species: its description of herd stability, of grooming relationships within harems and of food intake in relation to abundance, provide novel coverage of important topics.

Sadly, the reader's interest is titillated more often than it is satisfied. Why do females periodically discipline their harem males by chasing them up cliffs? Why do harem masters tolerate the presence of extra males in their harems which, even if they are temporarily inhibited from breeding, are likely to contest their hegemony in future? And why do male geladas not control their females in as chauvinistic a fashion as male hamadryas baboons — which share a similar environment and social system? Attention to specific questions of this kind would have provided the book with a stronger connecting theme and reduced the extent to which it overlaps the previous volume on geladas in the same series.

T. H. Clutton-Brock

T. H. Clutton-Brock is at King's College Research Centre, Cambridge, UK.

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This book examines the relationship between food, energy and society. The authors analyse and compare the ways in which different societies, both 'developed' and 'undeveloped', use different kinds of energy in producing their food. They also investigate the effects of substituting other resources (such as labour or land) for some of this energy, and assess the nutritional quality of a variety of diets.

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Trace elements in metabolism

Trace Elements and Iron in Human Metabolism. By A.S. Prasad. Pp. 380. (Plenum: New York, 1979). Available in UK, Middle East and Africa from Wiley (Chichester, UK) at £17.50; $35.

IF a monograph is defined as a systematic and exhaustive examination of a particular subject, then this book, contrary to the statement in the Foreward, cannot be so described. It contains information about not only the well-established (chromium, copper, fluorine, iodine, manganese and selenium) and newer (nickel, vanadium, silicon and tin) trace elements, but also magnesium, iron and certain toxic metals. This last section is highly selective in its choice of metals (cadmium, lead and mercury) and seems quite out of place in the present volume; the brief chapters are superficial and contain statements that are either incorrect, or likely to mislead. Thus, for example: "hypertensive subjects have abnormally high concentrations of renal cadmium" (p354); "A similar protective effect of excess zinc against lead toxicity was noted in young horses" (p365); and, "The physiocochemical classification of mercury does not correlate well with its toxic properties" (p371).

Even the review of the field of essential metals is an enormous task for a single author, and although Dr Prasad has produced an informative summary, inevitably many of the recent developments have been omitted. The chapter on chromium (confined essentially to the glucose-tolerance factor), selenium (devoted largely to glutathione peroxidase) and the newer trace elements, for example, contain only 2,2 and 1 reference(s), respectively, to work published later than 1975, whilst in the chapter on iron (a detailed, 60-page, review) 94% of the 428 references are to publications before 1971. Also the references to published work are perhaps inevitably for such a large subject, rather selective. Nevertheless, the book provides a clear account, not by any means exhaustive, of certain aspects of the action of trace elements aiming to link molecular biology, biological and animal experimentation with human nutrition and clinical experience. This is a highly commendable aim and the book can be recommended to provide such a broad view which, if depth is required, will require supplementation with further reading. The individual chapters on copper, iron and zinc, in particular, are extremely thorough and informative.

The very readable text is, in some cases, rather repetitious largely due to the subdivisions in each chapter, such that the experimental work in animals is often separated from observations in man. Furthermore, the considerations of the functions and mechanism of action of the trace elements are somehow diluted by sections on toxicity and therapeutic measures. The book is well produced, but for such a wide general coverage of the subject, a more detailed index might have been an advantage.

With the provisos mentioned above, this book can be recommended for those who wish to quickly obtain a general view of the biology of trace elements with particular reference to man. Contrary to the title, the volume is not restricted to human metabolism, but at least in certain sections also deals extensively with studies in experimental and domestic animals. Thus, it covers much but not all, of the ground already surveyed by Underwood in the fourth edition (1977) of his book *Trace Elements in Human and Animal Nutrition* (Academic; New York, San Francisco and London).

N. Aldridge
M. Webb

N. Aldridge and M. Webb are at the MRC Toxicology Unit, Carshalton, UK.

- *The Sense of Order: A Study in the Psychology of Decorative Art* (Phaidon: Oxford, 1979), by E.H. Gombrich (for review, see *Nature*, **279**, 653; 1979), has been published in the United States by Cornell University Press (Ithaca, New York) at $38.50.

- *The Menace of Atomic Energy* (W.W. Norton: New York, 1977), by Ralph Nader and John Abotts (for review, see *Nature*, **274**, 95; 1978) has now been published in the UK by Melbourne House (London) at £7.95.

- *Rutherford and Physics at the Turn of the Century*, edited by M. Bunge and W.R. Shea (for review, see *Nature*, **279**, 741; 1979), was published by Neale Watson Academic (New York) and Dawson (Folkestone, UK), and not as stated.

Electromagnetic wave propagation

Methods in Electromagnetic Wave Propagation. By D.S. Jones. Pp. 887. (Clarendon/Oxford University Press: Oxford, 1979.) £22.50.

THE field of electromagnetism has, as the author claims, undergone considerable development in recent years. Motivated by such fields as satellite communications, radar and geophysical prospecting the search for better methods of solution to important problems in the areas of electromagnetic wave propagation and scattering has intensified. Existing classical methods of applied analysis have been extended, complemented or indeed supplanted by new numero/theoretic methods based upon modern applicable mathematics. Professor Jones, himself a leading researcher in the field, is to be congratulated on presenting these methods in a systematic manner and critically reviewing their application.

The book is very large, containing over 800 pages in 9 chapters. The first chapter is introductory and contains the mathematical features used in the remainder of the book. The problems of propagation in waveguides are discussed in chapters 2, 3 and 4. Solutions are found by finite differences (chapter 2) and by variational methods (chapter 4). As these problems can often be reduced to finding eigenvalues of operators, this is described in some detail in chapter 3. The implementation of variational methods to provide numerical bounds on approximate solutions is the main concern of chapter 5.

The advent of the digital computer has transformed the antenna design field and classically 'difficult' problems are now amenable to numerical analysis. Both wire and solid antennas are treated in chapter 6 where integral equations of various types are studied from analytical and numerical points of view. Guidance on methods for dealing with transient phenomena is given in chapter 7.

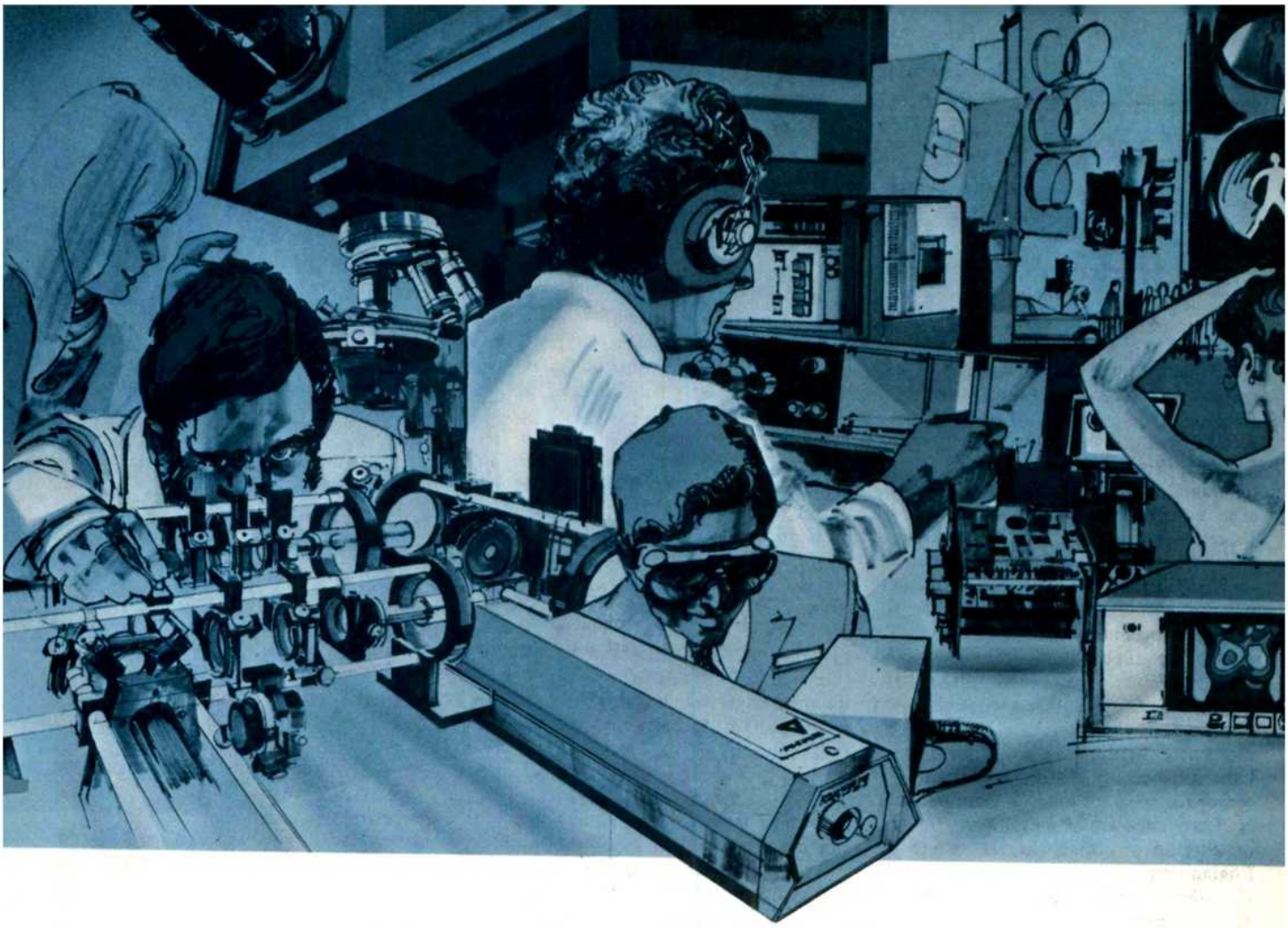
The most exciting material in the book for the reviewer is in chapter 8 where the geometric theory of diffraction (GTD) is developed from first principles. Very few authoritative accounts of this subject are available and the author has devoted one quarter of his book to it. The use of GTD in the design of reflector antennas has become increasingly important in recent years. Finally the problems of inverse scattering, that is, describing the source knowing its scattered field, is discussed in chapter 9. Nearly 400 references are listed, although few appear for 1977 and none afterwards.

It is perhaps churlish to mention omissions in a book of this length but no space has been found for recent work on general reflector synthesis involving Monge-Ampère equations or the use of complex coordinates and stereographic projection in geometrical optics.

The quality of the material and the clarity of its exposition makes this an important reference book for research workers and lecturers (there are numerous exercises) in the electromagnetic field.

B.S. Westcott

B.S. Westcott is Senior Lecturer in Applied Mathematics in the Faculty of Mathematical Studies, University of Southampton, UK.



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Nature Vol. 280 19 July 1979

new products for tissue culture

These notes, prepared from material provided by the manufacturers, are intended to give an outline of the range of products on the market. For more details use the Reader Enquiry Card bound inside the cover.

A feature on chromatography will appear in the 23 August issue.

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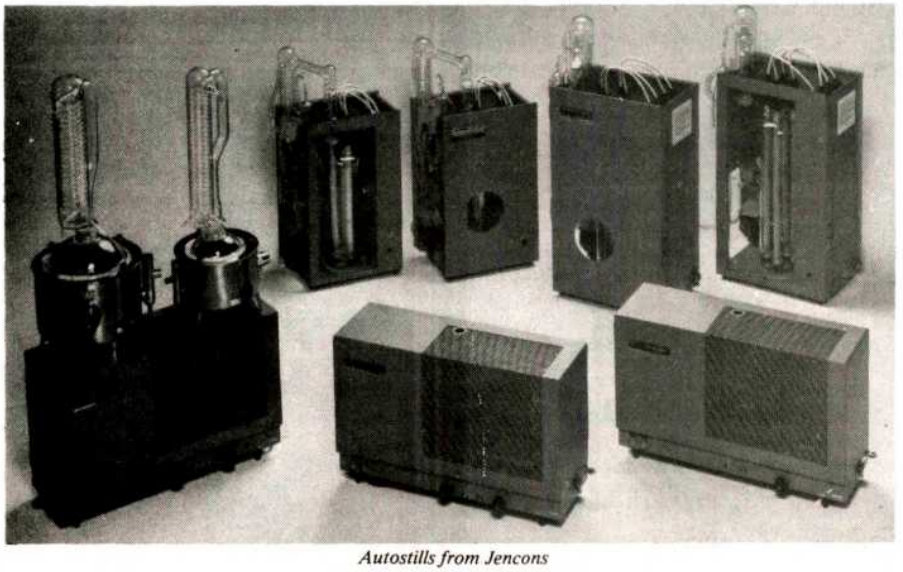
Boro Labs Ltd of Aldermaston have for several years been closely involved in the field of tissue culture as a result of their sole distributorship for the popular Salamander CO_2 incubator. The Salamander incubator is of all stainless steel construction and features a water jacket enclosing five sides of the chamber which ensures superb temperature control and stability. Dual circuit fail-safe thermostats ensure additional protection for valuable specimens. Automatic humidity control is available as an optional exta and the unit can also be supplied with automatic CO_2 changeover unit, and custom built access ports. The CO_2 monitor/controller is a purpose built katherometer unit utilising a thermistor sensor for speed of response and sensitivity. Boro Labs are also major distributors of Sterilin products in the south of England and are able to offer prompt service of this manufacturers wide range of disposable items.

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All growth media produced by Flow Laboratories are quality controlled using cells produced in house to ensure stringent growth test criteria. Different cell types are, of course, used for different media types. For example, RPMI 1640 medium is used for the growth of lymphocytes and is tested using EB3 cells, a lymphocyte cell line, along with human embryonic cells. These tests are in addition to pre-production checks on chemical quality and water purity, and the testing of the finished product for composition and physical properties.

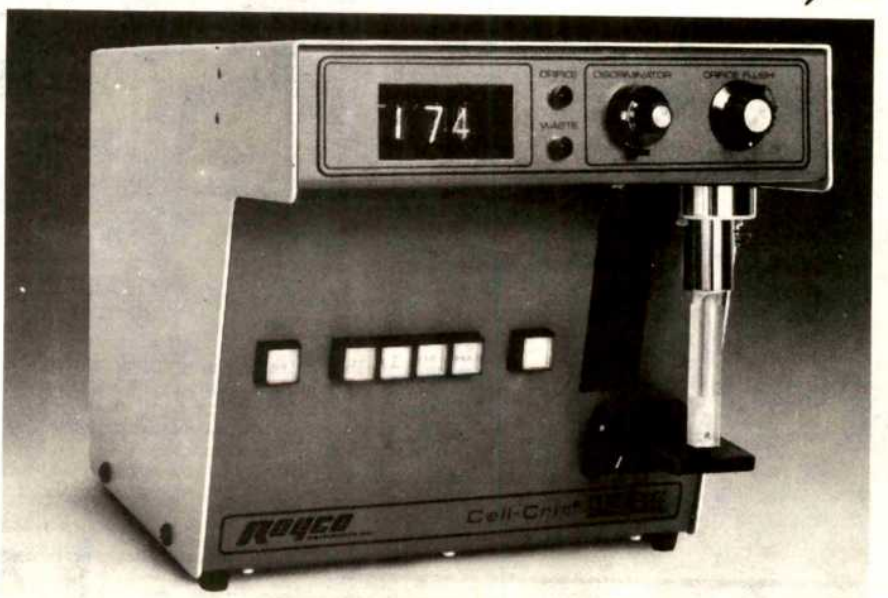
Sera for tissue culture are submitted to growth tests similar to those used for media, and also to special tests for mycoplasma. Should mycoplasma be found in serum or any other biological material, that material is discarded. All bovine sera are routinely tested for the presence of BVD and other viruses causing cytopathic and haemadsorbing effects and donor sera are tested for the presence of bacteriophage.

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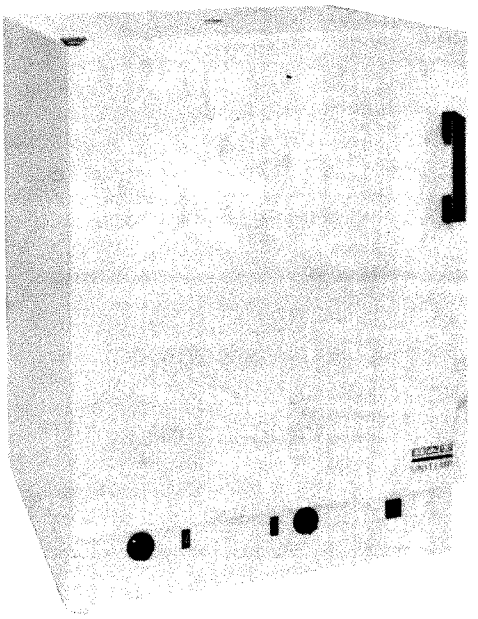
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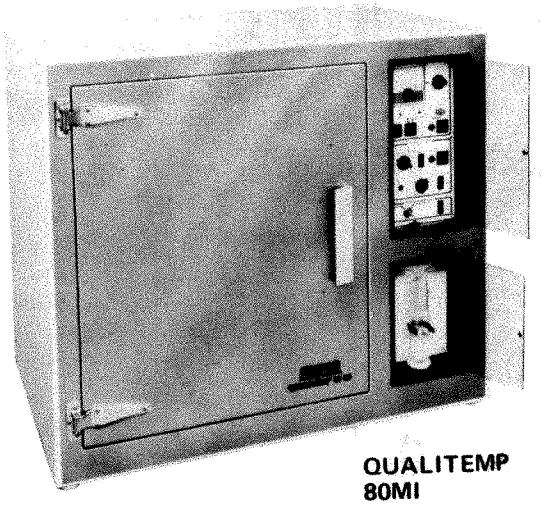
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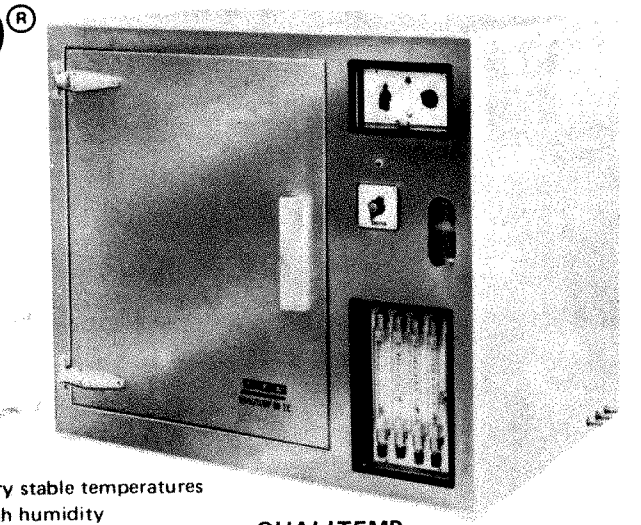
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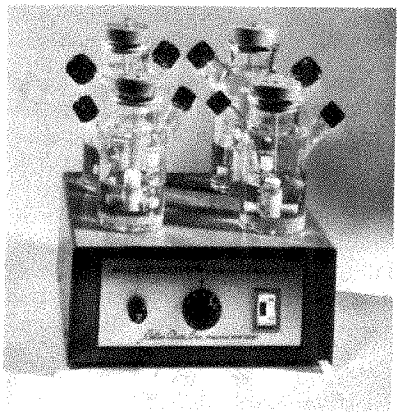
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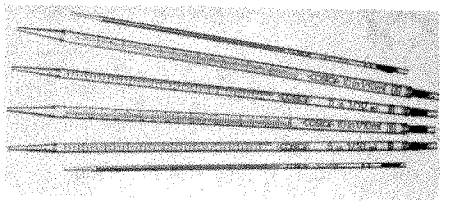
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A.R. HORWELL

With the ever-increasing interest in tissue culture, Arnold R. Horwell Ltd of London are now able to supply products from some of the leading manufacturers of the specialised equipment used in this field. Two ranges of disposable tissue culture equipment are available from Horwells, Sterilin and Costar. Costar have recently added a new triangular culture flask to their range; the design of the flask makes it easier to insert pipettes for feeding or fluid changes. Costar Stripettes are disposable sterile pipettes made from polystyrene which have a unique colour band running the length of the pipette; this band is magnified by the liquid contained in the pipette making it easier to observe the meniscus level. Existing Costar products include a range of traditionally-shaped culture flasks, multi-well cluster plates, as well as culture dishes. The Bellco Glass range of glassware for tissue culture includes two types of hanging-bar spinner flasks — one type with central rubber stoppers, and the other with a central plastic screw cap; sizes are from 25ml to 36 litres. A complimentary range of non-heating magnetic stirrers for the above flasks is available. These stirrers are designed for continuous operation, without the possibility of transferring heat to the culture mechanism; units can be supplied with one, two, four or nine positions for flasks. For continuous culture techniques, Bellco produce the Autoharvester. This is based on their cell production roller apparatus, and has facilities for adding and collecting media at pre-set intervals over as long a period as required.

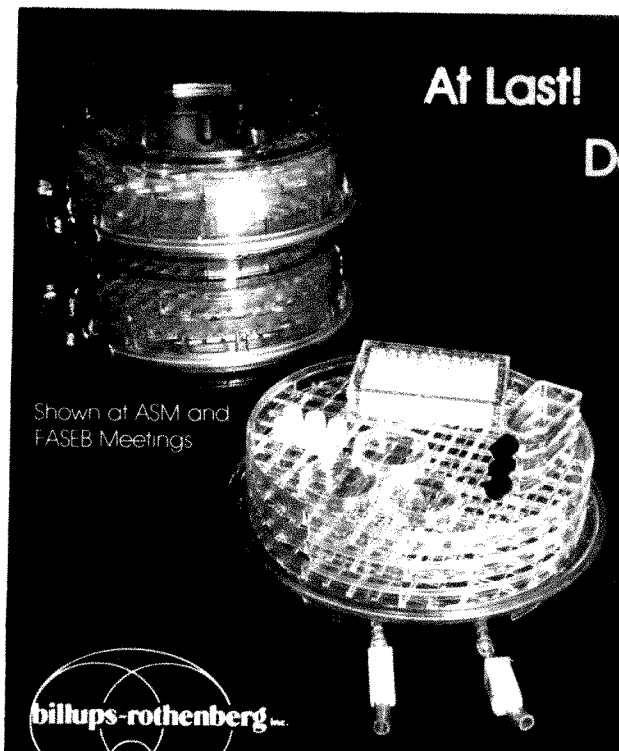


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With this Modular Incubator Chamber[1] you no longer have to worry about the inherent variability (due to CO_2, temperature and humidity fluctuations) of present day continuous flow and anaerobic incubators.

Simply place your experiment into the sealed chamber, purge the system with the required gas mixture and set in a controlled temperature environment.

Because each chamber is an isolated system you now have the flexibility to design experiments you previously thought were impractical or too expensive.

Chamber and tray components are made from rugged, non-toxic, uv-resistant polycarbonate.

Multi-purpose trays conveniently hold petri dishes, test tubes, T.C. flasks, microtest plates, etc.

Chambers can be stacked and interlocked for agitation or storage.

And now for the best part. The price of the chamber with 3 trays is only $95.[2] To order or for further information phone or write Billups-Rothenberg, Inc., Dept. N-1, P.O. Box 977, Del Mar, CA 92014 USA (714) 755-3309.

billups-rothenberg inc.

Dealer inquiries invited

[1]U.S. Pat No. 3886047 [2]U.S. Dollars F.O.B. Del Mar, CA.

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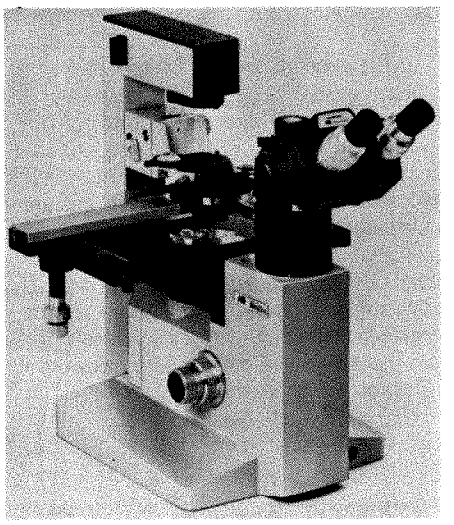
FROST INSTRUMENTS

Frost Instruments of Wokingham, UK, supply a varied range of equipment for tissue culture which includes a range of incubators to suit all requirements. A popular model is the Grant CO_2 incubator, which features a stainless steel interior with horizontal air flow system, and extremely sensitive control for both temperature and humidity. The inner chamber is of stainless steel and has positions for 11 shelves. The unit is guaranteed for 3 years.

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AMERICAN OPTICAL CORPORATION

The Scientific Instrument Division of American Optical Corporation have introduced the Biostar, a new inverted biological microscope which is readily adaptable to diverse applications and varied specimen preparation techniques. Each component of the instrument is designed for optimum performance in the inverted mode. The proven infinity corrected optical system is introduced to inverted microscopy for the first time. This system provides exceptional flexibility and better optical correction for lateral colour, astigmatism and curvature of field. The solid state illumination system of the Biostar is unique to this type of instrument, providing exceptional illumination



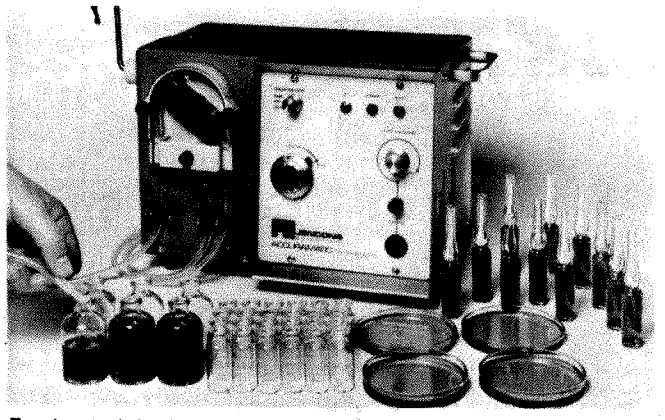
The American Optical Biostar inverted microscope

by means of a quick-change 20-W tungsten halogen lamp, a built-in field diaphragm, and a built-in dual view/multi-view adapter. To avoid light loss between illuminator and condenser, a highly corrected focusing system is used. An oblique illumination system is available for flask microscopy techniques. A wide selection of planachromatic and achromatic objectives (brightfield and phase/dark contrast) is available. The large 8″ × 10″ stage is solidly attached to the wide, sturdy stand.

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COLLABORATIVE RESEARCH

Collaborative Research have added endothelial cell growth supplement (CR-ECGS) to their expanding range of *in vitro* growth factors. This powerful adjunct for cell culture makes it feasible, for the first time, to grow and maintain human vascular endothelial cells at low cell densities in monolayer culture. When added at the recommended concentration to serum-containing medium, CR-ECGS promotes a six- to tenfold proliferation of human vascular endothelial cells over a 10-day period. CR-ECGS also supports the repeated passaging of established cultures. Collaborative Research human fibronectin (CR-HFN) is isolated from human fibrinogen. Human fibronectin adsorbs to the surfaces of any glass or plastic culture vessel or microcarrier and provides a suitable surface for the attachment and growth of anchorage-dependent mammalian cells. It is effective for plating fastidious cells, including many epithelial, endothelial, neural and myoblastoid cells which may otherwise not attach. It dramatically increases cloning efficiency and helps minimise the variability in plating efficiency associated with different serum lots. Investigators wishing to define the growth factor and nutrient requirement of cell lines, for example, will find CR-HFN a useful aid. The range of Collaborative Research tissue culture products now includes epidermal growth



ACCURAMATIC DUO-SPEED AMPOULE FILLER/MEDIA DISPENSER

PRICE £520 + VAT

Filling range: 3mm bore tube. Low speed 0.5ml to 20 ml
High speed 2ml to 75ml
8.5mm bore tube. High speed 25ml to 550 ml
Filling Time: Up to 14 seconds depending on volume required.
Electrical requirements: 210-250 V. 50Hz. 1 amp.

Duo-Speed Accuramatic is supplied complete with 3 mm bore and 8.5 mm standard bore tubing assemblies and a footswitch. Intermediate delivery tubing sizes to give deliveries other than those listed are available to suit your particular requirements.

For bacteriologists, microbiologists, virologists, pharmacists, pathologists, public health laboratories — anyone who has a requirement for an automatic dispenser.

Dispenses at the touch of a button 0.5ml to 550ml with accuracy of approximately ±1% midrange, or automatically with a variable interval between doses. Footswitch allows normal single-dose operation or acts as a safety overrider when automatic function is selected.

Peristaltic pump output is directed to delivery tube. Suspensions and sterile solutions can be filled. In-line filtration is easily set up. Stepless volume adjustment can be made while Accuramatic is running. The delivery nozzle can be remote from the machine such as in a controlled environment cabinet. Separately sterilised tubing assembly can be installed in pump and solenoid clamping heads in less than a minute. Delivery time between doses in automatic function can be varied from 0-5 seconds, and in manual function filling is as quick as the dexterity of the operator will allow. The Accuramatic incorporates a carrying handle and is easily portable.

Using the low speed of the Duo-Speed Accuramatic ensures accurate, steady and constant filling of small containers without splash or overspill and also prevents bubbles and frothing which can cause inaccuracies. The high speed setting is suitable for routine dispensing.

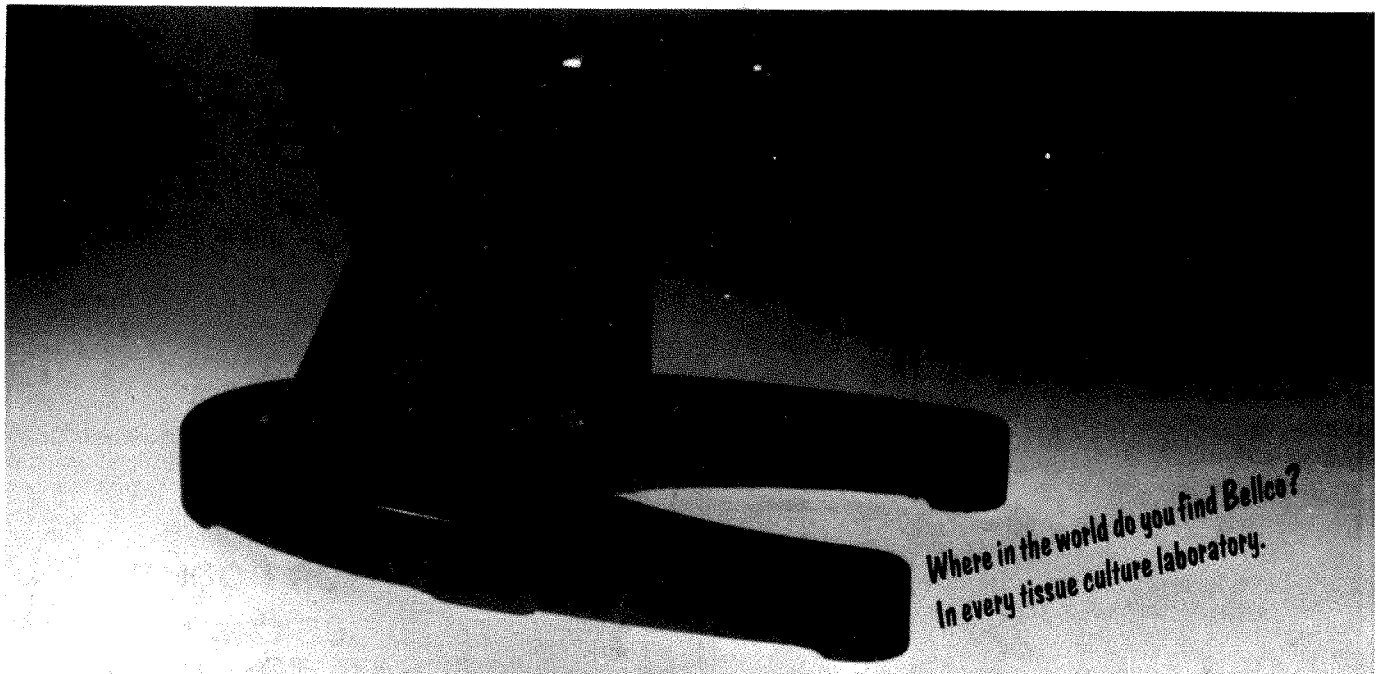
Accuramatic advantages

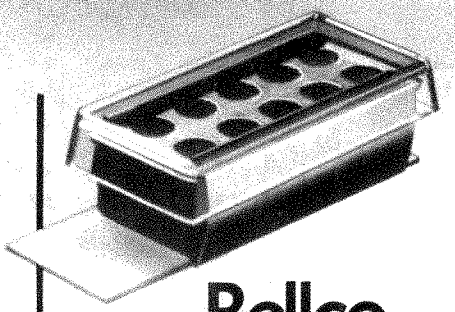
No valves, pistons or moving parts in contact with liquid, only silicone rubber tubing and glass fittings, so easily sterilised and changed. Printed circuitry is easily replaced, simple to service, front panel lifts away. Demonstration on request in most areas. The turn of a wheel — sets the delivery rate to your selected volume.

Jencons (Scientific) Ltd, Hemel Hempstead, Hertfordshire, England

BEST FOR DELIVERING THE GOODS

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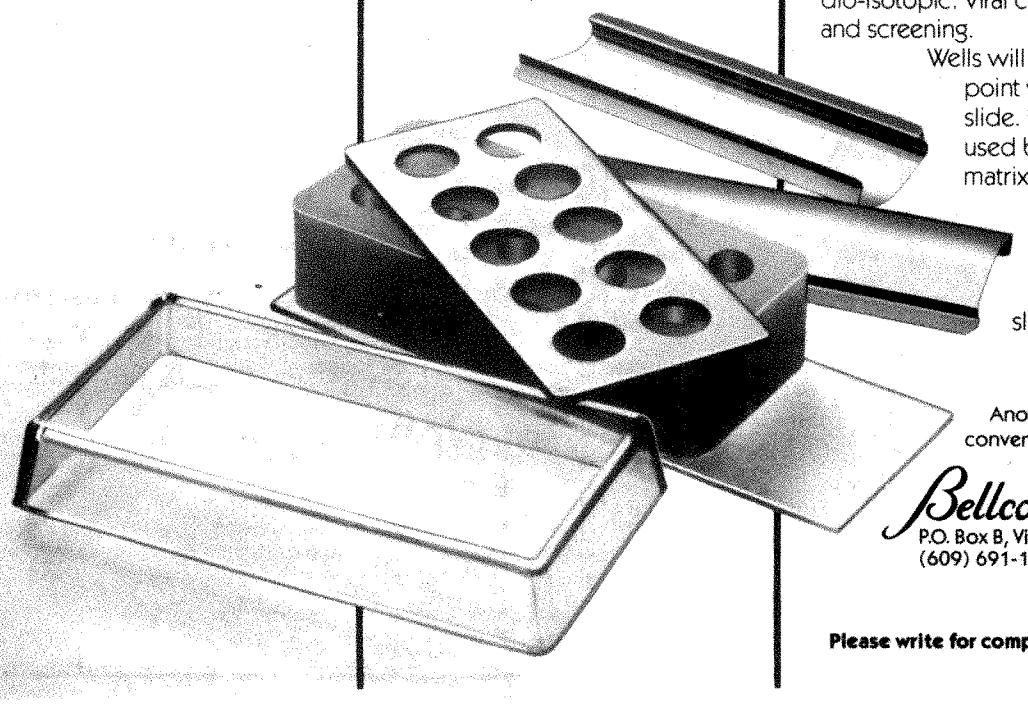
Bellco Micro-Slide Culture Chamber*

*U.S. PATENT 3,883,398

For convenient tissue-culture, immunology and virology . . . the Bellco Micro-Slide Culture Chamber. Non-toxic, autoclavable, with silicone-rubber matrix. Ten micro-culture wells. Each has 0.4-ml volume to provide ten 0.317 cm^2 monolayer growth areas. Use with standard 25 x 50 mm microscope slide. Stainless-steel side clips and top-pressure plate for quick, convenient assembly. Can be autoclaved intact with transparent cover.

On a micro-culture level, assay any substance that affects mammalian cells. Autoradiography. Cell differentiation. Cytotoxic antibody titrations. Immunofluorescence. Lymphocytotoxicity assays, optical or radio-isotopic. Viral cytopathogenic effect and screening.

Wells will not leak at contact point with microscope slide. Polyester film can be used between slide and matrix. Matrix will not disturb monolayers during disassembly. Results can be retained on microscope slides.



Another dependable, convenient product from

Bellco Glass, Inc.
P.O. Box B, Vineland, NJ 08360 USA
(609) 691-1075 TWX 510-687-8910

Please write for complete general catalogue

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OUR FIBRONECTIN MAKES YOUR CELLS GROW ATTACHED TO CULTURE FLASKS . . .
and other growth surfaces.

If you have had disappointing results trying to grow cells in culture, we may have the remedy . . . our Human Fibronectin (CR-HFN). This product has repeatedly proven effective in getting fastidious cells to attach to surfaces—and grow.

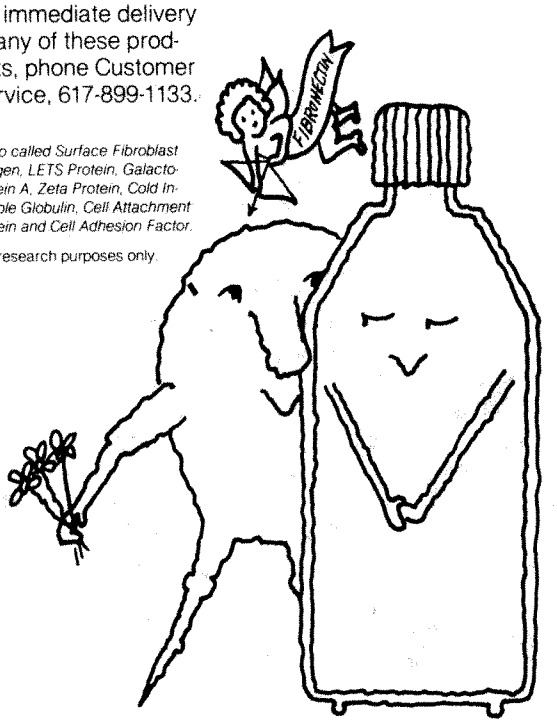
CR-HFN, — a glycoprotein cell surface antigen*— adsorbs to surfaces to provide a hospitable environment for anchorage-dependent cells. Cells which were previously impossible to culture, now grow. Plating efficiency is dramatically increased. And the variability of plating with different serum batches is reduced or eliminated.

At Collaborative Research, we're committed to the development of products which make tissue culture a dependable science. CR-HFN is the latest addition to our growing line of tissue culture products—including Epidermal Growth Factor, Fibroblast Growth Factor, Nerve Growth Factor, Multiplication Stimulating Activity, Endothelial Cell Growth Supplement, and Human Thrombin.

To find out more about these products and what they can mean for your research, write or call today. Or for immediate delivery of any of these products, phone Customer Service, 617-899-1133.

*Also called Surface Fibroblast Antigen, LETS Protein, Galactoprotein A, Zeta Protein, Cold Insoluble Globulin, Cell Attachment Protein and Cell Adhesion Factor.

For research purposes only.



Research Products Division
Collaborative Research, Inc.
1365 Main Street, Waltham, Mass. 02154
Tel (617) 899-1133 TWX 710-324-7609

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Telex: 270146F

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INCUBATORS

125 — 300 litres
+ AUTOMATIC CO_2
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INCUBATOR ROOMS

UP TO 60 CUBIC METRES
ACCURATE TO ± 0.2°C

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STOP PRESS

THE SECRET'S OUT

The
SALAMANDER
Tissue Culture Incubator

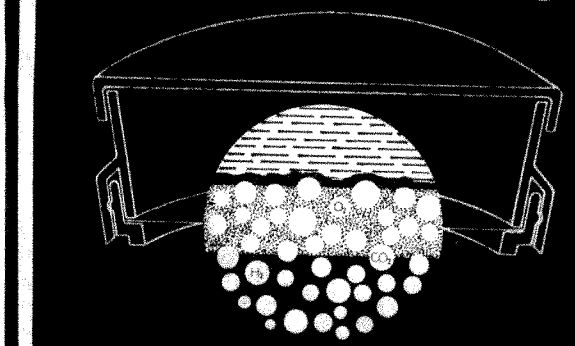
Now updated and available from the original designers.
For further details contact.

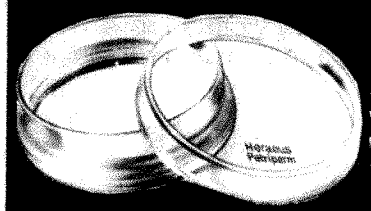
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A new approach in tissue culture





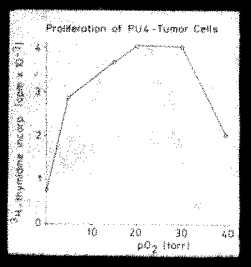
PETRIPERM

a novel culture dish with gas permeable membrane as cellular support.

Additional advantages of the PETRIPERM membrane are:

- UV permeable 200 nm
- Suitable for high power light and fluorescence microscopy
- The membrane can be cut for cell cloning, electron microscopy, staining and documentation
- Chemically resistant to acids, bases and organic solvents

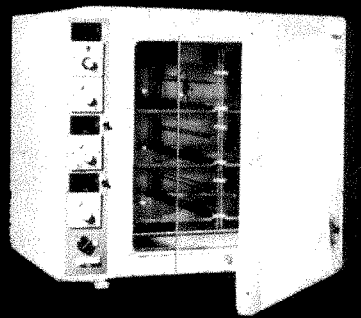
PETRIPERM is available either with a hydrophilic surface for optimal attachment, or with a hydrophobic surface for easy detachment of adherent cells.



In the intact animal, the pH and the partial pressure of both oxygen and CO_2 of the cell environment are under total biochemical and physiological control. This state is not even approximated by available techniques of monolayer cell culture in conventional tissue culture ware. Continuously changing gradients of these parameters in the micro-environment of cultured cells will obscure the biological significance of culture dependent phenomena. PETRIPERM is the prerequisite for the precise control of pO_2, pCO_2 and pH in the cellular environment as gases reach the cultured cells directly through the highly gas permeable [illegible] µm thick membrane.

Heraeus— Co_2-Incubator B 5060 EK/CO_2

We build the incubator with appropriate pCO_2, pH and pO_2 control units



W. C. HERAEUS GMBH
Produktbereich
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D-6450 HANAU

Heraeus

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Another *Exclusive* from Collaborative Research

Platelet Derived Growth Factor†

(CR™-PDGF)

Partially Purified from Human Platelets

Mitogenic for
Smooth Muscle Cells
Glial Cells
Fibroblasts
and more

Supplied Lyophilized in 100µg quantities,
Catalogue #40003
To order call Customer Service **(617) 899-1133**

Other Growth Factor Products from Collaborative:
Fibronectin (CR-HFN)
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Epidermal Growth Factor (CR-EGF)
Fibroblast Growth Factor (CR-FGF)
Multiplication Stimulating Activity (CR-MSA)
Endothelial Cell Growth Supplement (CR-ECGS)
plus antiserum and iodinated Growth Factors

Research Products Division
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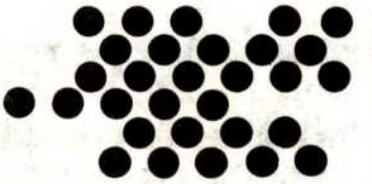
†Antoniades, H.N., Scher, C.D., and Stiles, C.D. (1979) Proc. Natl. Acad. Sci. U.S.A., 76, 1809-1813.

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Flow Laboratories —everything you need for tissue culture.

Liquid/Powder Media and Sera for cell culture

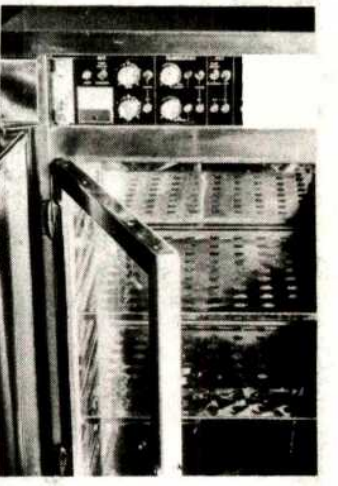




Leading world supplier of human and animal cell lines.

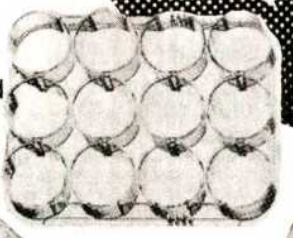


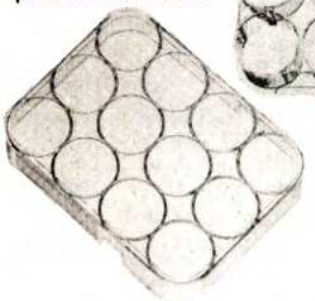
Laminar Flow Cabinets Recirculating, Horizontal and Vertical Flow.

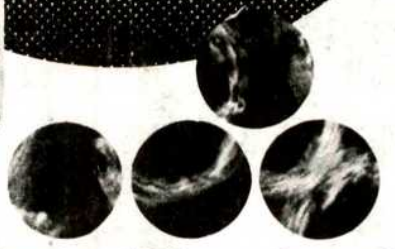


Automatic CO_2 Incubators.

Full range of tissue-culture treated plastic labware.







Superbead® Microcarriers provide large surface area for cell growth using minimum space.

Suppliers of quality tissue culture products to over 100 countries.

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McLean, Virginia, USA
Tel. (703) 893-5925
Telex 234 903010 Flowlab MCLN

Companies also in West Germany, France, Canada, Sweden, Australia, Singapore

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factor (CR-EGF), fibroblast growth factor (CR-FGF), nerve growth factor (CR-NGF), multiplication stimulating activity (CR-MSA) and human thrombin (CR-HT). Technical literature on these products is available from the manufacturers. In the UK, Collaborative Research products are available from Uniscience Ltd.

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HERAEUS

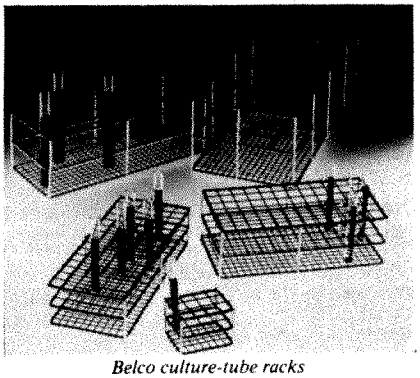
A new approach to tissue culture is announced by Heraeus, the Petriperm culture dish. This dish is fitted with a gas-permeable membrane which acts as a cellular support. The membrane can be cut for cell cloning, electron microscopy or staining. They are suitable for high-power light and fluorescence microscopy, transparent to UV light at 200 nm and resistant to acids, bases and organic solvents. Petriperm is produced with either a hydrophilic surface for optimal attachment, or with a hydrophobic surface for easy detachment of adherent cells. the use of a gas-permeable membrane in this way allows for a more precise control of partial levels of dissolved oxygen, carbon dioxide and hydrogen ions. The Heraeus type B 5060 EK/CO_2 incubator achieves accurate control of levels of CO_2, oxygen and *p*H.

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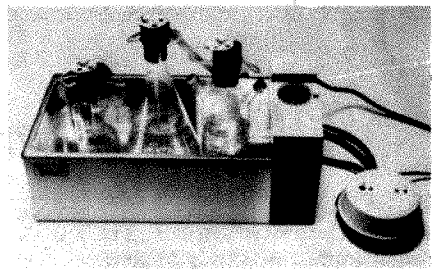
BELCO GLASS INC.

Stainless steel racks are available from Belco Glass Inc. in 23 standard configurations for all popular-size culture tubes, including 13, 16, 18, 20, 22, 25 and 38 mm. These racks, each designed to handle from 12 to 150 culture tubes, are made from heavy-gauge wire with turned-edge construction. Rust resistance and long life in carbon-dioxide incubators and water baths are assured by an electropolished finish. An extensive range of glassware for tissue culture is available from Belco. Two types of hanging-bar spinner flasks are produced, one fitted with central rubber stoppers and one with central plastic screw caps.

Circle No.95 on Reader Enquiry Card.



Belco culture-tube racks



Techne's air-driven stirrer

TECHNE

The 'classical' stirrer for suspension cell culture consisting of a suspended, rotating horizontal magnetic bar is not well suited for the suspension of microcarriers. For this application Techne produce a stirrer driven by a three-phase air supply (the pneumatic equivalent of three phase electricity). It consists of a vertical glass rod operated by a completely heatless, autoclavable, air motor mounted on the screw cap of a glass flask containing the medium and microcarriers; there are no rotating bearings in the flask. The flask can be stood in any water bath.

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BIO-RAD

Bio-Rad Bio-Carriers are an improved, high-strength polyacrylamide microcarrier developed to supply the need for an efficient and inexpensive method of culturing mamalian cells-including primary, diploid and established lines. In Bio-Carrier culture, anchorage-dependent cells grow on the surface of small, positively charged beads suspended in nutrient medium by gentle stirring or agitation. With Bio-Carriers, a single 1-litre spinner flask can be used to grow as many cells as 20 half-gallon roller bottles. Or, the beads can be added to flasks, Petri dishes or roller bottles for six- to sevenfold increase in surface area. Bio-Carriers conform to all parameters, defined over more than a decade of research, which promote optimal attachment and growth of a wide variety of cell lines. Bead charge is carefully controlled and evenly distributed; beads are narrowly sized (120–180μm) have a specific gravity of 1.04, which promotes homogeneous suspension, and are transparent for microscopic viewing of cells. Bio-Carriers are an improvement over previously available materials, because the cross-linked polyacrylamide is better able to withstand the forces of stirring and cell growth without breaking up. Cells can be cleanly separated from Bio-Carriers with no mixture of disintegrated bead fragments, a recurring problem with many other microcarriers. The inert polyacrylamide matrix also makes Bio-Carriers impervious to bacterial or enzyme attack.

Circle No. 93 on Reader Enquiry Card.

ASSAB LTD

Assab have had 10 years' experience in marketing their automatic CO_2 incubators in the UK and are now firmly established in the NHS and other fields. The product range embraces anhydric, cooling and CO_2 incubators, an anaerobic work station and dry air sterilisers. Within the last 3 years Assab have also introduced their system of controlled temperature installations. The system can be adapted to existing rooms or is available as a complete package including an insulated cubicle. The emphasis here is on control, with accuracies up to ± 0.2°C, and reliability. The system is inherently reliable but by duplication of controls a 'fail safe' situation can be achieved. Installations have already been carried out for the major pharmaceutical companies for uses ranging from incubation to package testing.

Circle No. 92 on Reader Enquiry Card.

BILLUPS-ROTHENBERG

The modular incubator chamber produced by Billups is a convenient and efficient alternative to conventional continuous flow and anaerobic incubators. The experimental apparatus is placed in the sealed chamber which is then purged with the required gas mixture and the system is kept in a controlled-temperature environment. As cultures can be set up in separate isolated cells it is economical and practical to vary culture conditions. Chamber and tray components are made from rugged non-toxic UV-resistant polycarbonate. The trays can accommodate Petri dishes, test tubes, tissue culture flasks, microtest plates and so on. The chambers may be stacked and interlocked for agitation or for storage.

Circle No. 91 on Reader Enquiry Card.

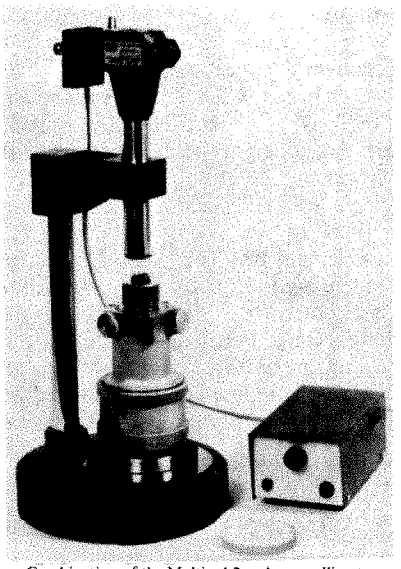
W.T. WINGENT

Cell types which do not need to adhere to a surface for their proliferation can be efficiently cultured in the waterjacketed culture spinner vessel produced by Wingent. Spinners are run by magnetic stirrer and a water circulator is required to supply the water jacket: one circulator can be used to supply several culture vessels. Each spinner can be fitted with four openings: one for continuous supply of the appropriate gas mixture; one as an outlet for pressure equalisation; one opening to take a glass tube to the bottom of the vessel for sampling; and one opening to permit addition of culture fluid as required. The Wingent vessel has been used successfully for many types of animal cell and human cells derived from normal and malignant tissue.

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newly on the market

Precision polishing machine. Metals Research. Using the Mark II polishing jig with the 'Multipol 2', together with the associated autocollimator system, laser rods are being routinely polished to a flatness better than $\lambda/10$ and parallel end-to-end within 2 arc s. The Multipol 2 is a precision polishing machine designed specifically for polishing metals, glass, semiconductors and soft optical crystals using either abrasive, etch or wet slurry polishing techniques. It can also be used to handle metallurgical, mineralogical and biological specimens using a wide variety of polishing media and a range of lap surfaces of up to 200 mm diameter for optimum surface finish. Using optical alignment provided by the autocollimator, the two faces of the laser rod are polished at 90° to the rod axis to a high degree of parallelism.
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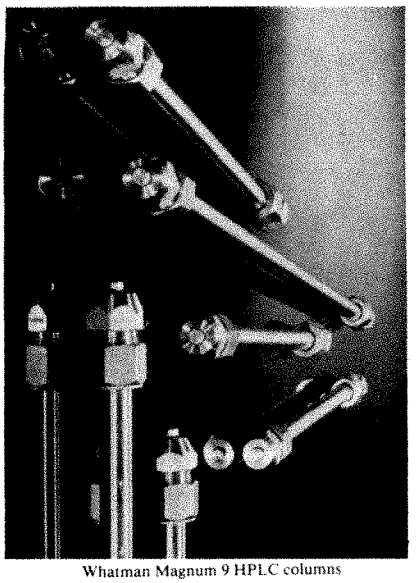
Combination of the Multipol 2 and autocollimator

Reagent for detection of reducing sugars. Pierce. The copper-bicinchoninate (Cu-BCA) reagent is used as a stable non-corrosive dye reagent for detecting sugars separated by ion-exchange of their borate complexes using an automated sugar chromatography system. A better peak resolution can be achieved than with the viscous sulphuric acid reagent. Reaction times less than 1 min and reaction coils with i.d. down to 0.3 mm were used. Owing to the high baseline stability of the detection system with the Cu-BCA reagents, the analyser can be run at higher sensitivity. The colour formation is linear over a detection range of more than two orders of magnitude. In routine work, amounts of 0.1–30 μg of different sugars have been determined.
Circle No. 65 on Reader Enquiry Card.

Shell freezer. FTS Systems. The Just-A-Tilt, manufactured by FTS Systems, will process up to four 1,200-ml freeze-drying flasks and shell-freeze 2.4 l of sample in under an hour. There are no flask-lift devices or rollers to adjust, even if samples are in different-sized containers, as all are rotated in a thin film of —40°C alcohol pumped from a mechanically refrigerated heat exchanger. Full alcohol volume is only 1.5 l. Shell-freezing flask angle is infinitely adjustable from front-mounted control between 0° (flat) and 16° (inclination) to present a more uniform wall thickness and faster, more even freeze-drying rates. Clear plastic top permits frost-free visibility of flask during processing.
Circle No. 66 on Reader Enquiry Card.

Ribonuclease H-1 from calf thymus. Enzo Biochem. The ribonuclease H-1 now available from Enzo Biochem was purified from calf thymus using polyethylene glycol CM-Sephadex, Cellex-CM, DEAE, and affinity chromatography. This enzyme can be effectively used in the digestion of the RNA moiety of the hybrid after reverse transcriptase assays; to avoid the de-amination of cytidine in the cDNA; in the removal of the poly (A) stretches found on the RNA messenger; as a restriction enzyme with trideoxynucleotides on the DNA matrix; it will preferentially act on the guanosine moiety.
Circle No. 67 on Reader Enquiry Card.

Pre-pleated filter papers. Schleicher and Schuell. To introduce the benefits of their pre-pleated filter papers, a three-pack sampling of three different grades of the filter paper is available free from Schleicher and Schuell. The pre-pleated filter paper affords twice the exposed surface area of ordinary filter paper that is hand-folded into quadrants and provides a significant time-saving by allowing solid-liquid separations to move faster. Since solids are more evenly dispersed over the paper's surface, clogging is reduced or eliminated. Widely used to recycle reagents in chemistry autoanalysers, S&S filter papers are burst-proof when used under normal laboratory conditions. They retain precipitates from course to fine in six standard grades and 10 standard diameters from 12.5 to 50.0 cm. The free three-pack includes a total of 75 pre-pleated filters, 25 each of three filtration speeds: fast, medium and slow. When ordering the free three pack, specify diameters: 12.5 cm, 18.5 cm or 24.0 cm.
Circle No. 68 on Reader Enquiry Card.



Whatman Magnum 9 HPLC columns

Preparative HPLC columns. Whatman. The new, modified Magnum 9 columns from Whatman permit yields of up to 2.5 g of highly purified compound. They can be fitted to any analytical liquid chromatograph and obviate the need for preparative instruments for laboratory scale preparations. An important benefit of the modified Magnum 9 columns is that they permit simple scale-up from corresponding Partisil analytical columns. Only the flow rate need be controlled to maintain an equivalent linear velocity in this scale-up. Modified Magnum 9 columns are 9.4 mm I.D. × 12.7 mm (½") O.D. × 50 cm long, 316 stainless steel with LDV outlet to fit any liquid chromatograph.
Circle No. 69 on Reader Enquiry Card.

Laboratory chest freezer. Forma Scientific has introduced a new 3 cu. ft laboratory chest freezer with an ultra-low temperature range of —75° in a warm +90°F ambient temperature; improved stainless steel interior construction; minimum of 5" foamed-in-place urethane insulation; built-in battery-operated ring back alarm system with self-charging nickel cadmium batteries; built-in indicating controller with automatic over-temperature safety; dual wheel castors for mobility; easy-opening counterbalanced lid. The freezer is available in both 120V, AC, 60 Hz and 220V, AC, 50 Hz models.
Circle No. 70 on Reader Enquiry Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Enquiry Card bound inside the cover.

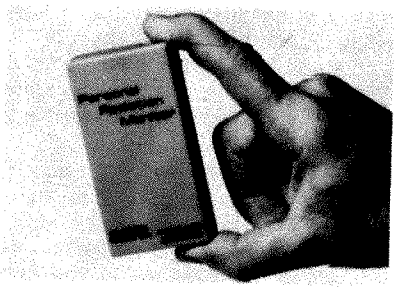
Pressure transmitter. Philips. A new range of compact industrial transmitters which measure pressure in gases and liquids from 1 bar up to 100 bar, is announced by Philips. The PR 9390 series of pressure transmitters operate according to the piezo-resistive principle and comprise a sensor with pressure-sensitive silicon crystal and an integral semi-conductor full bridge strain gauge network. A built-in amplifier produces a standard 0–20 mA load – independent output signal, proportional to the applied pressure and allowing direct connection of indicating, recording or other equipment. The transmitters require an unstabilised 24 V d.c. power supply and are supplied as standard with a 2-m long integral connection cable. The compact housing and pressure connections are made of a highly corrosion-resistant steel and are completely sealed.
Circle No. 71 on Reader Enquiry Card.

Angle head centrifuge. Gallenkamp. The compact angle head centrifuge now produced by Gallenkamp has a maximum capacity of 120 ml and the eight-place angle head will accept tube sizes up to 15 ml. A solid-state power controller enables the operator to adjust the speed of the centrifuge. Top speed is 5,000 r.p.m. and maximum RCF is 2,500*g*. Safety features of the centrifuge are that the lid must be properly closed before the centrifuge will start spinning and both the head and the motor are completely enclosed in a robust steel case. The self balancing action of the mounting makes critical balancing of tubes unnecessary.
Circle No. 72 on Reader Enquiry Card.



The Gallenkamp centrifuge

Digital field synchroniser. Marconi. the B3565 digital television field synchroniser is the latest addition to the range of equipment from Marconi Communication Systems. The equipment has the storage capacity of one television field as distinct from a frame synchroniser which stores two fields, thus reducing the cost and size to a minimum.
Circle No. 73 on Reader Enquiry Card.



The Andrex radiation monitor

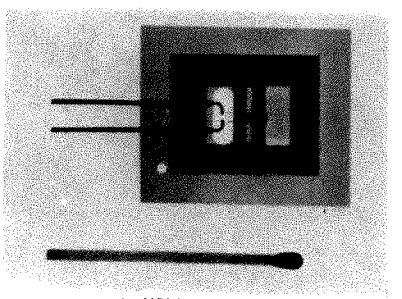
Pocket radiation monitor. Andrex Radiation Products has introduced a new version of its personal radiation monitor that is both lighter and smaller than previous models, but provides the same high level of performance. The monitor provides an instant audible warning of potential radiation hazards. The sensitivity setting is from 1 mR h^{-1} and the unit is activated by simply removing a disposable 'shipping plug'. Once activated the unit, powered by a 1.5 V battery, remains 'on' continuously, producing an intermittent reference bleep to confirm operation and battery condition. Bleep speed increases as radiation levels rise. With a weight of 80 g, the monitor can be clipped to a belt or slipped conveniently into a pocket.
Circle No. 74 on Reader Enquiry Card.

Triiodothyronine uptake kit. Amersham Corporation announce the availability of its new T_3 uptake (MAA) kit. The kit is designed to provide a rapid and convenient assessment of thyroid hormone binding capacity in human serum. The kit protocol depends on the distribution of ^{125}I-triiodothyronine between vacant binding sites on endogenous binding proteins and a secondary binder in the form of macro-aggregated albumin. Speed, accuracy and convenience are assured with a 50-μl sample, 10-min room temperature incubation and excellent correlation with the clinical picture. Kits containing sufficient reagents for 50, 100 and 400 single determinations are available.
Circle No. 75 on Reader Enquiry Card.

Coater for low-temperature sputtering. Edwards. Heat-sensitive specimens for scanning electron microscopy work can now be coated safely with the Edwards S150A, a new bench-top sputter coater. A new deposition system maintains very low specimen temperature. It incorporates an electrode design which suppresses high energy electrons which would otherwise lead to specimen heating. A two-magnet electron deflection system reduces secondary electron bombardment of the specimen and a large water-cooled specimen table absorbs heat and reduces plasma density. A carbon evaporation accessory can easily be fitted for depositing films onto specimens for X-ray microanalysis.
Circle No. 76 on Reader Enquiry Card.

Radiation measuring system. Radiation Components. The Diamentor-D radiation monitoring system from Radiatron Components enables instructors to use the system to demonstrate visually the extent of a patient's exposure to ionising radiation, allowing students to learn radiological techniques more quickly and thoroughly. Its accuracy in diagnosis and value as a teaching aid are derived from the fact that it measures the total useful output from the X-ray tube. The Diamentor-D system comprises a flat, transparent, rectangular ionisation chamber which is mounted on the light beam diaphragm or under couch tube. This is linked by a screened cable to a unit which displays the area exposure continuously in R cm^{-2}. It can also display screening time. The ionisation chamber is available in two sizes and can be easily fitted to any make of X-ray equipment. It has a filtering effect equivalent to 0.5 mm of aluminium.
Circle No. 77 on Reader Enquiry Card.

Strain gauges. HBM. The free grid strain gauge LF 30 for measuring strains at high temperatures has been developed by Hottinger Baldwin Messtechnik. The measuring grid consists of a platinum–tungsten alloy and is attached to a self-adhesive glass-fibre-reinforced Teflon backing strip, which is discarded during the mounting operation. The strain gauge is mounted with ceramic cement or by using the flame spraying process. The free grid strain gauges LF 30 are obtainable with measuring grids 3 mm and 6 mm long. They can be used at temperatures of up to 1,000°C depending on the mounting process and the type of stressing.
Circle No. 78 on Reader Enquiry Card.

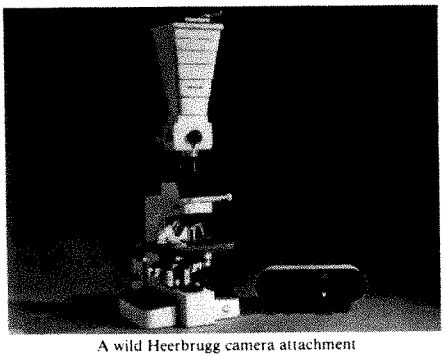


An HBM strain gauge

3-D Bench system. Oriel announces a new range of benches contained in a 28-page catalogue. The Oriel three-dimensional optical bench consists of a 3½ inch (8.89cm) diameter thick-walled aluminium tubing extruded with four symmetrical dove-tails. These are available in various lengths. A variety of connectors and fittings will connect these to each other to form very rigid structures in almost any configuration. Any standard sized Oriel carrier can be mounted on any of the four dove-tails. Almost any Oriel accessory can be mounted on these benches. Other instruments or lasers can be readily mounted using the flat-topped Oriel carriers.
Circle No. 79 on Reader Enquiry Card.

High-performance fluorescence spectrophotometer. The Perkin-Elmer Model 650-40 fluorescence spectrophotometer is the first high-performance instrument available with microprocessor control of ordinate and abscissa function. The optical system of the Model 650-40 uses concave gratings which are driven by stepper motors under microprocessor control. With a wavelength range of 200–850 nm, the instrument uses a reference photocell in monochromatic light to achieve a ratio recording system. Horizontal slit imaging allows enhanced sensitivity and smaller sampler volumes. All operational modes, with the exception of slit control, are selected from a keyboard containing both function and numerical keys. Selections from the keyboard include: scan speed, auto-zero, zero suppression, integration, concentration, response time and photomultiplier gain. A prescan mode function incorporated into the microprocessor allows either the excitation or emission monochromator to be scanned at 960 nm min^{-1} for automatic location of peak maximum.
Circle No. 80 on Reader Enquiry Card.

Camera attachments. Wild Heerbrugg. With three camera attachments by Wild Heerbrugg, photographs are now possible up to 4 in × 5 in. The 1.0× camera attachment, in addition to making possible the exposure of 4 in × 5 in or 9 cm × 12 cm cut film, also enables photographs to be taken on Polaroid cameras on 3¼ in × 4¼ in filmpack and 4 in × 5 in cut film. With the 0.8× camera attachment, 6 cm × 9 cm (cut or roll film), 4.5 cm × 6 cm and 6 cm × 6 cm photographs are possible.
Circle No. 81 on Reader Enquiry Card.



A wild Heerbrugg camera attachment

Electronic balances. Oertling introduce the Alpha 22 series of electronic balances. All models are alpha-numeric with a clear LED readout, and are dual range, with capacities of 2 kg to 0.1 g and 200 g to 0.01 g, with a fully subtractive tare. The balance is protected against overload, as 'OVER' appears on the display as a warning signal. The HC22/51 model has several extra features, including a store and recall facility. Up to eight weights can be stored for later recall. The operation of pressing the 'store' button also sets the reading to zero.
Circle No. 82 on Reader Enquiry Card.

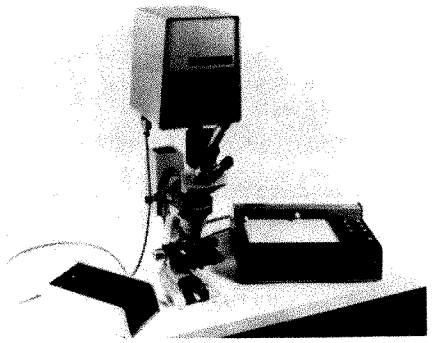
Electrophoresis power supply with digital readout. Buchler Instruments has introduced a junior version of its popular 3-1500 power supply to provide a reliable, lower voltage method for electrophoresis applications. This new unit, the DPS-350, uses push-button controls to select the appropriate output ranges and regulation modes. Constant voltage and constant current levels may be selected up to 350 V d.c. and 100 mA. This output is controlled by a 4-turn potentiometer and offers the user full burn-out protection. This unit will automatically switch over from one mode to another whenever the load demand is excessive in the original mode. The large LED display allows easy and precise readings from any reasonable distance within a lab.
Circle No. 83 on Reader Enquiry Card.

Particle and droplet sizer. Malvern. The ST1800 particle and droplet sizer has been introduced by Malvern Instruments. Based on laser diffraction scattering the equipment measures the size distribution of solid particles or liquid droplets over the size range 1–500 μm with an optional extension to 1,800 μm. Full sample to answer facilities are enabled by computer control, making the instrument suitable for use by semi-skilled operators if required. A hard copy print out of the size ranges in 15 size bands is presented.
Circle No. 84 on Reader Enquiry Card.

Custom chip for wire chambers. A new low-level amplifier/comparator introduced by LeCroy provides a compact, high-speed monolithic 16-pin DIP for low-level pulse counting. Especially suited for multiwire drift chamber and proportional chamber applications, the device offers a threshold of typically ± 200 μV and time slewing of 1.5 ns from 2× to 20× threshold. A single chip, the MVL100 pulse detector, contains a 100-MHz wideband amplifier, a discriminator, a monostable output timer, an ECL line driver, and a 50Ω linear driver. Each section is designed without compromising performance. Both the input and the output are fully differential and compatible with twisted-pair signal transmission.
Circle No. 85 on Reader Enquiry Card.

Refrigerated centrifuge. Du Pont. A low-speed refrigerated centrifuge with 6-l rotor capacity has been introduced by the Du Pont Company's 'Sorvall' Instruments Division. The Sorvall RC-3B refrigerated centrifuge can be used to handle large sample volumes, or up to 420 radioimmunoassay tubes in a single run at relative centrifugal forces to 5,136g Features of the RC-3B include imbalance detection and indicator, automatic door interlock with zero speed detection, automatic over-temperature detection, and open-door interlock to prevent rotor start-up.
Circle No. 86 on Reader Enquiry Card.

Microspectrophotometer. Anaspec. A new microspectrophotometer is available from Anaspec, capable of obtaining spectra from samples or objects as small as 10 μm. The system is essentially a miniature photometer that will interface directly to the camera port of any laboratory microscope. A unique feature of the system is its microprocessor which utilises programmed 'PROMS' for ABS/TRANS conversion. The microprocessor will store both the base line 100%T and spectra for spectra normalisation, such that the system is capable of double-beam ratiometric operation.
Circle No. 87 on Reader Enquiry Card.



The Anaspec microspectrophotometer

Chromatography column eluate concentrator. ChemLab. With the new column concentrator from ChemLab which uses molecular retentive membranes, it is possible to increase the concentration of a compound in the eluate from a chromatography column by a factor of 3 or 4 and sometimes by as much as 10 times. The concentrator consists of two perspex blocks into which grooves in spiral form are accurately machined. A membrane is placed between the upper and lower blocks so that low molecular weight compounds pass through the membrane to the lower chamber, while the eluate from the column is passed through the upper groove, and during the passage of the liquid through this groove a concentration of the high molecular weight compounds takes place. The increase in concentration can be varied by a control valve which increases or decreases the back pressure in the line of the eluate from the column. The concentrator is designed for operation with chromatography columns working at flow rates of 10–200 ml h^{-1}.
Circle No. 88 on Reader Enquiry Card.

Brochure on ultra-low temperature freezers. Forma Scientific has published a new brochure detailing the company's entire ultra-low temperature freezer product line including chest, upright and walk-in models. The brochure includes information on the company's newest 3 cu. ft chest freezer, along with information on simulated power failure tests, blood storage freezers, safety back-up systems and inventory system selection.
Circle No. 89 on Reader Enquiry Card.

Recent scientific and technical books

Mathematics

AFIFI, A. D., and AZEN, S.P. Statistical Analysis : a Computer Oriented Approach. Second edition Pp.xx + 442. ISBN-0-12-044460-7. (New York and London: Academic Press, 1979.) $19.50.

BARNETT, Stephen, Matrix Methods for Engineers and Scientists. Pp.xi + 185. ISBN-0-07-084084-9. (London and New York: McGraw-Hill Book Company (UK), Ltd., 1979.) £3.95.

BEINEKE, Lowell W., and WILSON, Robin J. (edited by). Selected Topics in Graph Theory. Pp.xii + 451. ISBN-0-12-086250-6. (London and New York : Academic Press, 1978.) £21.50; $44.50.

GOODMAN, A. W., and RATTI, J. S. Finite Mathematics with Applications. Third edition. Pp.xiv + 584. ISBN-0-02-344760-5. (New York : MacMillan Publishing Co., Inc.; London: Collier Macmillan Publishers, 1979.) £11.95.

OYSTAEYEN, F. Van, and VERSCHOREN, A. Reflectors and Localization: Application to Sheaf Theory. (Pure and Applied Mathematics: a Program of Monographs, Textbooks, and Lecture Notes, Vol.41.) Pp.vii + 166. ISBN-0-8247-6844-2. (New York and Basel: Marcel Dekker, Inc., 1979.) Sw.fr.54.

PUTERMAN, Martin L. (edited by). Dynamic Programming and Its Applications. (Proceedings of the International Conference, University of British Columbia, Vancouver, British Columbia, Canada, April 14-16, 1977.) Pp.xv + 410. ISBN-0-12-568150-X. (New York and London: Academic Press, 1978.) $19.50.

ROTA, Gian-Carlo (edited by). Studies in Algebraic Topology. (Advances in Mathematics, Supplementary Studies, Vol.5.) Pp.xi + 263. ISBN-0-12-599152-5. (New York and London: Academic Press, 1979.) $28.

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WOLF, Kurt Bernardo. Integral Transforms in Science and Engineering. (Mathematical Concepts and Methods in Science and Engineering, Vol. 11.) Pp.xiii + 489. ISBN-0-306-39251-8. (New York and London: Plenum Press, 1979.) $32.50.

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DUNCOMBE, Raynor L. (ed.) Dynamics of the Solar System. (Proceedings of the 81st Symposium of the International Astronomical Union held in Tokyo, Japan, 23-26 May, 1978.) Pp.xiv + 330. ISBN-90-277-0976-9. (Dordrecht, Boston, Mass. and London: D. Reidel Publishing Company, 1979.) Dfl.85; $44.50.

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TASSOUL, Jean-Louis. Theory of Rotating Stars. (Princeton Series in Astrophysics.) Pp.xiii + 506. ISBN-0-691-08211-1. (Princeton, NJ. and Guildford: Princeton University Press, 1978.) Cloth £25; Paper £9.40.

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VAN SCHOONEVELF, Cornelis (ed.) Image Formation From Coherence Functions in Astronomy. (Proceedings of IAU COLLOQUIUM No. 49 on the Formation of Images from Spatial Coherence Functions in Astronomy, held at Groningen, The Netherlands, 10-12 August 1978.) (Astrophysics and Space Science Library, Vol. 76, Proceedings) Pp.x + 337. ISBN-90-277-0987-4. (Dordrecht, Boston, Mass. and London: D. Reidel Publishing Company, 1979.) Dfl.75: $39.50.

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APPOINTMENTS VACANT

UNIVERSITY OF ABERDEEN
DEPARTMENT OF MICROBIOLOGY

Applications are invited for the following posts:

POST-DOCTORAL RESEARCH FELLOW

A post-doctoral microbiologist is required to join a major new project on corrosion studies on North Sea Oil structures, financed by the SRC Marine Technology Directorate. The research group will work in close collaboration with marine biologists and metallurgists in the University and Robert Gordons Institute of Technology, and in association with the major oil and service companies.

Appointment will be from 1 October 1979, and will be for one year in the first instance with possible extension for a second year. Salary within Range 1A, £4,910 to £5,770, with appropriate placing.

RESEARCH ASSISTANT

To work with Dr I R Booth and Dr W A Hamilton on the kinetics of ion-linked solute transport in bacteria, financed by SRC.

RESEARCH ASSISTANT

To work with Dr J I Prosser on the effects of surface and pH on the dynamics of nutrification in the soil, financed by NERC.

Salary in each case within Range 1B, £3,775 to £4,333, with appropriate placing. Appointment will be from 1 October 1979, for 1 year in the first instance with possible extension up to a maximum of three years. It will be possible for the successful candidates to register for a PhD.

Telephone The Secretary, The University, Aberdeen (0224 40241) for further particulars. Applications (2 copies) to The Secretary should be lodged by 10 August 1979. 2584(A)

COMMONWEALTH AGRICULTURAL BUREAUX

Vacancy for
SCIENTIFIC INFORMATION OFFICER

(Full-time or Part-time) at National Institute of Agricultural Engineering, Wrest Park, Silsoe, Bedford MK45 4HS, UK

Duties of Post: The scrutiny of technical literature, selection and preparation of abstracts on all subjects related to agricultural and horticultural engineering (this may involve visits to outside libraries), indexing, proof-reading, and dealing with technical enquiries.

Qualifications: Applicants should have a knowledge of both engineering and foreign languages. They should have a degree or similar qualification in engineering or a relevant subject and a high competence in at least one foreign language, or a foreign language degree with a sound background knowledge of engineering. The ability to write clear concise English is essential.

Salary: In the scale £2,839 to £5,448 (under review) plus a compensatory allowance to offset personal contribution to superannuation. Starting salary according to qualifications and experience. Promotion to higher scales on merit.

Application forms and full particulars from Executive Director, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN. Closing date for applications 15 August 1979. 2588(A)

UNIVERSITY OF BATH

POSTDOCTORAL RESEARCH IN BIOCHEMISTRY

Applications are invited for the above post to work with Professor P D J Weitzman. The project is supported by the SRC and is concerned with structure-function relationships in variant forms of the enzymes citrate synthase and succinate thiokinase. Previous experience in enzymology and/or protein chemistry is essential.

The post is available as soon as possible, tenable for two-and-a-half-years. Salary in the range £4,333 to £4,910 per annum. Reference number 79/165.

GRADUATE RESEARCH OFFICER IN BIOCHEMISTRY

Applications are invited for the above post to work with Professor P D J Weitzman and Dr M J Danson on a project supported by the SRC and concerned with the oligomeric, catalytic and regulatory properties of citrate synthase variants. The appointee will be engaged in enzyme purification and a full range of protein chemical procedures. Candidates should have a good degree; previous relevant experience will be an advantage. The post is tenable for three years from 1st October, 1979. Starting salary in the range £3,775 to £4,333 per annum. Reference number 79/166N.

Application forms from the Personnel Officer, University of Bath, BA2 7AY, quoting appropriate reference numbers. Closing date will be 3rd August 1979. 2621(A)

KENYATTA UNIVERSITY COLLEGE KENYA

Applications are invited for the post of

LECTURER (ECOLOGIST) in the
DEPARTMENT OF BOTANY

Applicants must have a PhD in Botany wi specialization in either Terrestrial Aquatic Plant Ecology. They should ha had several years teaching experience University level, while teaching secondary school level will be an add advantage. The appointee will be expect to teach in the B.Ed undergradua programme and to supervise post-gradua students. He/she will be expected to initia and conduct research in the stated areas specialization. Salary scale: Lectur £2,016 to £3,312 pa (K£1 = £1.24 sterling The British Government may supplem salary in range £4,278 to £4,818 (sterling) for married appointee or £2,7 to £3,156 pa (sterling) for single appoint (reviewed annually and normally free fro tax) and provide childrens' educatic allowances and holiday visit passage Family passages; SSSF or FSSU subsidized housing; non-contributo medical aid scheme; baggage allowance.

Detailed applications (2 copies) wi curriculum vitae and naming three refere to be sent direct to the Registrar, Kenyat University College, P O Box 4384 Nairobi, Kenya by 15 August 197 Applicants resident in the UK should al send one copy to Inter University Counc 90/91 Tottenham Court Road, Londo W1P 0DT. Further details may be obtain from either address. 2603(A)

EXCERPTA MEDICA

is one of the largest scientific information services in the world, adding 230,000 items each year to its database.

We wish to recruit a

PROGRAMMER with 2 years' experience, who is also a

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Further details from Prof. G.A. Somerfield, Excerpta Medica B.V., P.O. Box 1126, 1000 BC Amsterdam — The Netherlands.

ELSEVIER SCIENCE DIVISION

W232(A)

UNIVERSITY OF DUNDEE

POSTDOCTORAL BIOCHEMIST

Applications are invited for a SRC grant financed postdoctoral fellowship to work ir collaboration with Dr D G Nicholls on a project whose aim is to establish the molecular mechanism by which noradrenaline regulates the exothermic metabolism of isolated brown adipose cells. For reviews see Biochem. Soc. Trans. (1977) 908; New Scientist 13/4/78. The Neurochemistry Laboratory is located in the Department of Phychiatry, and has close tie with the Department of Biochemistry.

The post is available for up to two year from an early date to be agreed.

Salary within the range £4,232 to £4,776 (t be reviewed from October 1979).

Informal enquiries may be made t Dr D G Nicholls, Neurochemistr Laboratory, Department of Psychiatry Ninewells Medical School, University o Dundee. Applications, quoting referenc EST/61/79 J and containing the names o two referees should be lodged with Th Secretary, The University, Dundee DD1 4HN as soon as possible. 2572(A)

THE OPEN UNIVERSITY

Graduate Research Assistant in Physical Organic Chemistry

Applications are invited for the above post to work with Dr P G Taylor at the Open University for a period of three years commencing 1st October 1979, or as soon as possible thereafter.

The Research Assistant will participate in a research programme concerned with the study of the mechanism of Friedel-Crafts type reactions and in particular the cyclisations of substituted 2 (2-Naphthalenylmethyl) benzoic acids.

Candidates should be 23 years or under on October 1st 1979, and possess, or be currently completing, an honours degree in Chemistry (a I or IIA is preferable but not essential). The successful candidate will be encouraged to register for a higher research degree (BPhil, MPhil, or PhD).

The appointment will be made at the first point on the Graduate Research Assistant 1B scale and the commencing salary will be £3,689 (under review) plus U.S.S.

Application forms and further particulars are available from The Recruitment Office (JD3934/1), The Open University, P O Box 75, Walton Hall, Milton Keynes, MK7 6AL or telephone Milton Keynes 63404: there is a 24 hour answering service on 63868. Closing date for applications: 2nd August. 2558(A)

UNIVERSITY OF BRISTOL

DEPARTMENT OF PHYSICS

pplications are invited for a Science esearch Council supported

POSTDOCTORAL RESEARCH ASSISTANT

o work on the theory of tunnelling states in uperconducting metallic glasses. amiliarity with field theoretical chniques of solid state physics is sirable. The project is funded for three ars commencing as near after October 1st 79 as is practicable. The salary scale is ,689 to £5,321 pa depending on alifications. (Scale subject to revision as om 1st October, 1979.)

Applicants should write to B L Gyorffy, niversity of Bristol, H H Wills Physics aboratory, Bristol BS8 1TL, before ctober 1st, 1979. 2620(A)

UNIVERSITY OF BATH

POSTDOCTORAL RESEARCH
Plant Sciences

plications are invited for a two year pointment funded by the SRC to work h Dr A D Dodge in the School of ological Sciences. The work is in the area plant membrane damage with particular erence to singlet oxygen induced oxidation of isolated chloroplasts.

Candidates should be or expect shortly be postdoctoral; experience of lipid alysis would be an advantage. Starting ary in the range up to £4,910 per annum. Application forms from the Personnel ficer, University of Bath, Bath, BA2 Y, quoting reference number 79/163N. sing date will be 3rd August 1979. 2597(A)

CSIRO AUSTRALIA

Research Staff
Division of Human Nutrition
Adelaide South Australia

CSIRO has a broad charter for the conduct of scientific and technological research for the benefit of the Australian community; it has approximately 7000 employees, located in Divisions and Sections throughout the country, of whom 2200 are professional scientists.

GENERAL: The Division is conducting multi-disciplinary research into nutritional factors, biological mechanisms, and disease processes. Its programs comprise experimental studies in inorganic nutrition (micronutrients) and in metabolism and digestion (macronutrients), and epidemiological and behavioural studies in human populations. Appointees are being sought in the program on Metabolism and Digestion which comprises (1) Diet & Metabolic Regulation, (2) Digestion & Lipid Metabolism, (3) Protein Turnover. The projects examine the role of dietary and environmental factors on metabolic disorders in man. Experimental models include, mice, rats (normal & genetically obese), marmosets and pigs. Studies with isolated liver and fat cells are in progress; tissue culture techniques are established.

FIELD: *Diet and Metabolic Regulation*. This group is examining the mechanisms associated with the efficiency of utilization of nutrients, hormonal control relevant to overnutrition and obesity and metabolic disorders associated with hormonal imbalances. Three appointments are proposed in this area, including one to replace the senior scientist who will retire in March 1980.

1. Endocrinologist — Biochemist — REF 250/271

DUTIES: To initiate research in the field described above. Collaborate with other research workers at the Division and at hospitals and medical schools.

QUALIFICATIONS: A PhD or MD degree or equivalent qualifications with research experience in endocrinology and biochemistry or clinical biochemistry and evidence of ability to lead a research team. A background in metabolic endocrinology with a bias towards mechanistic studies on peptide hormones would be an advantage.

SALARY: Senior Research Scientist (SRS) or Principal Research Scientist (PRS). Appointment of an outstanding candidate at a higher level would be considered.

2. Biochemist — REF 250/272

DUTIES: Develop research on the efficiency of utilization of nutrients with particular reference to carbohydrate metabolism and lipogenesis.

QUALIFICATIONS: A PhD degree or equivalent qualifications in biochemistry with appropriate postdoctoral experience. A background of isotopic labelling techniques in the study of intermediary metabolism and energy-dissipating cycles. Experience in the preparation and use of isolated cells and perfused organs.

SALARY: Research Scientist or Senior Research Scientist.

3. Biochemist (Postdoctoral Research Fellow) — REF 250/273

DUTIES: Research in the area of Metabolic Regulation, most likely in association with the appointee to Position 1.

QUALIFICATIONS: A PhD degree in biochemistry or equivalent qualifications.

SALARY: Research Scientist

FIELD: *Digestion and Lipid Metabolism*

4. Biochemist — Physiologist — Ref 250/274

DUTIES: Investigation of nutritional and physiological factors associated with metabolism of lipids relevant to atherogenesis and obesity.

QUALIFICATIONS: A PhD degree or equivalent qualifications. Formal training in biochemistry and physiology. Several years' postdoctoral research experience in lipid metabolism, together with a background in whole animal studies and knowledge of organ perfusion methods. An appreciation of epidemiological research is desirable.

SALARY: Research Scientist or Senior Research Scientist.

TENURE:
Positions 1, 2 and 4 — indefinite with superannuation.
Position 3 — a fixed-term of two years.
Note — Positions will not be available before April 1980.

SALARY:

Research Scientist	$A15,422 to $A18,904 pa
Senior Research Scientist	$A19,572 to $A22,405 pa
Principal Research Scientist	$A23,247 to $A26,368 pa
Senior Principal Research Scientist	$A27,790 to $A30,530 pa

Applications in duplicate, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting the appropriate reference number(s) should reach: The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, London WC2R 3EH by 18th August, 1979. Applications in USA and Canada should be sent to: The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue NW, Washington DC 20036 2612(A)

UNIVERSITY OF GLASGOW

ADMINISTRATIVE ASSISTANT

INSTITUTE OF VIROLOGY

READVERTISEMENT

Applications are invited from university graduates for the post of Administrative Assistant in the Institute of Virology. Salary will be on Grade 1B of the scales for Administrative Staff (£3,775 to £6,355 from 1st October, 1979), with placing according to qualifications and experience. A degree in science, preferably biological, is desirable but not essential.

Further particulars may be obtained from the Secretary of the University Court (Room 18), University of Glasgow, Glasgow G12 8QQ, with whom applications (8 copies), giving the names and addresses of three referees, should be lodged on or before 3rd August, 1979. In reply please quote Ref. No. 4434/1M. 2600(A)

UNIVERSITY OF DURHAM

DEPARTMENT OF BOTANY

Applications are invited from Botanists, Biologists and Biochemists for several posts of

POSTDOCTORAL RESEARCH ASSISTANT

for two years from 1st September, 1979 or later by arrangement.

For one post, plant physiology or histochemical experience is required and for another, knowledge of recombinant DNA technology.

Initial salary £4,232 or £4,505 (£4,333 per annum and £5,622 per annum from 1st October 1979) within Range 1A plus superannuation.

Applications (3 copies) naming three referees should be sent by 27 July 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. 2557(A)

UNIVERSITY OF BATH

SCHOOL OF PHARMACY & PHARMACOLOGY

RESEARCH OFFICERS IN MEDICINAL CHEMISTR

Applications are invited for the above pos for work with Professor R T Parfitt on th synthesis of novel analgesics related t morphine, and tropanes with centr nervous activity. Candidates should have PhD Degree in Medicinal Chemistry Organic Chemistry and have experience synthetic organic chemistry.

One post is tenable for a period of thr years from 1st October 1979, the other for period of 2 years.

Starting salary will be in the range up t £4,910 per annum.

Further particulars and applicatio forms are available from the Personn Officer, University of Bath, Bath, BA 7AY, quoting reference numb 79/116RN. Closing date will be 6th Augu 1979. 2634(A)

CSIRO AUSTRALIA

Research Scientist
Division of Fisheries & Oceanography Sydney N.S.W.

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees — 2,400 of whom are research and professional scientists — located in Divisions and Sections throughout Australia.

FIELD: *Physical Oceanography*

GENERAL: The Division is Australia's principle marine research institution with over 80 scientists investigating the physical, chemical and biological features, including fisheries, of the seas around Australia. Headquarters of the Division are in Sydney with regional laboratories in Perth and Brisbane. On-line access to a CYBER 76 computer is available. Sea-going facilities include two medium sized chartered vessels and several smaller vessels. The appointee will be located in Sydney.

DUTIES: As Head of the Physical Oceanography Group of ten scientists, the appointee will be a senior physicist who will be responsible to the Chief of the Division for the overall planning, organization and execution of the Division's programs in physical oceanography, and will be expected to ensure that these are closely co-ordinated with work in chemical and biological oceanography and in fisheries.

The appointee will also initiate and personally carry out research in physical oceanography in a field to be agreed on with the Chief of the Division.

QUALIFICATIONS: For such a senior appointment, a person with extensive research experience, and with managerial ability is sought. Minimum qualifications are a PhD or equivalent in an appropriate branch of science (physics, mathematics or engineering), together with considerable experience most of which should be in physical oceanography. It would be an advantage if this experience were in one of the fields presently regarded as directly relevant to the Division's work, namely dynamics of mesoscale water movements and dynamics of circulation over continental shelves and slopes. Ability to work at sea is essential.

SALARY: Dependent upon experience, but within the ranges:
Principal Research Scientist: $A23,247-$A26,368 pa
OR Senior Principal Research Scientist: $A27,790-$A30,530 pa

TENURE: Indefinite with superannuation.

Applications in duplicate, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting reference number 320/626 should reach The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 18th August, 1979.

Applications in USA and Canada should be sent to The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, NW, Washington, DC 20036, USA.

2609(A)

FLOW LABORATORIES LIMITED

Flow Laboratories Limited are looking for the following highly motivated personnel to join their Research Staff at Irvine.

R & D OFFICER

This person will have a Doctorate in Biochemistry, Cell Biology, or Microbiology and will be familiar with cell culture, protein purification and protein labelling techniques.

R & D ASSISTANT

This person will be qualified in the fields of Biochemistry, Genetics or Immunology and be familiar with enzymatic and radioisotope techniques. Experience in karyology or hybridisation would be an advantage.

Apply in writing, giving brief career details, to:
Mrs E McConnell, Personnel Secretary,
Flow Laboratories Limited, PO Box 17, Second Avenue,
Industrial Estate, Irvine KA12 8NB

2635(A)

UNIVERSITY OF GLASGOW
DEPARTMENT OF ZOOLOGY
RESEARCH ASSISTANT

Applications are invited for the above post, to work with Dr P Calow on a programme concerned with ageing in flatworms. Applicants should have a good honours degree in zoology or biology. Registration for a higher degree may be possible. Tenure is for 3 years and is subject to the standard conditions of SRC. Salary will be within Range 1B of the scales for Research and Analogous Staff (£3,689 to £4,232). The post is superannuated.

Further details from, and applications (a curriculum vitae plus names and addresses of two referees) to: The Secretary, Department of Zoology, University of Glasgow, Glasgow G12 8QQ. Closing date 4th August, 1979. In reply please quote Ref No 4489M. 2601(A)

SOIL MICROBIOLOGIST

Applications are invited for the above post for multidisciplinary effort to develop grass/legume pastures under acid soil conditions. Responsibilities include the isolation and evaluation of rhizobial strains for promising legume germplasm and the development of inoculation technology for pasture management. Studies on survival, movement and competitive abilities of introduced rhizobia in acid soils are envisaged. The applicant should have a PhD in microbiology. A working knowledge of Spanish would be desirable. The position is located at CIAT headquarters in Cali, Colombia.

Send application with curriculum vitae and three references to: Dr Gustavo A. Nores, Director for Land Resources Research, Centro Internacional de Agricultura Tropical, Apartado Aéreo 6713, Cali, Colombia. Applications required by October 15. W245(A)

COMMONWEALTH AGRICULTURAL BUREAUX
Vacancy for
SCIENTIFIC INFORMATION OFFICER
at the
Commonwealth Bureau of Nutrition,
Rowett Research Institute,
Bucksburn, Aberdeen AB2 9SB.

Duties: The scrutiny of the scientific literature, selection of papers and preparation of abstracts in concise English for publication in *Nutrition Abstracts and Reviews*.

Qualifications: An appropriate degree or its equivalent in agriculture or a related biological science. A reading knowledge of a European language is required and preference will be given to a candidate with ability in a Slav language or languages. The ability to write clear concise English is essential.

Salary: In the scale £2,839 to £5,448 (currently under review) plus a compensatory allowance (taxable but not superannuable) of 3½% to offset personal contribution to CAB Superannuation Scheme. Starting salary according to qualifications, experience and age. Promotion to higher scales on merit.

Application forms and full particulars from Executive Director, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN. Closing date for applications 6th August, 1979.

2577(A)

THE UNIVERSITY OF MANCHESTER
TECHNICIAN (GRADE 5)
DEPARTMENT OF SURGERY
Withington Hospital

required to work in multi-disciplinary laboratory at the University Hospital of South Manchester, in the University Department of Surgery. The Department's research is in gastroenterology, haemodynamics and computing.

Applicants should have HNC, degree or equivalent qualification, preferably in Physics. Salary scale £3,474 to £4,056 pa.

Applications should be sent to Professor R A Sellwood, Department of Surgery, University Hospital of South Manchester, Nell Lane, West Didsbury, Manchester M20 8LR. 2608(A)

HERIOT-WATT UNIVERSITY RICCARTON
MARINE SCIENCE UNIT
INSTITUTE OF OFFSHORE ENGINEERING
RESEARCH ASSOCIATE

Applications are invited for the abov post. The successful applicant woul join a team investigating marin benthic populations unde programmes supported by industri contract and research grants.

Applicants should have an Honou degree in Marine Biology, Biology c Zoology with an interest in marin invertebrates.

Salary scale £3,689 to £5,321 p (under review).

Application forms and furthe particulars are available from the Staf Officer, Chambers Street, Edinburg EH1 1HX.

Informal enquiries for furthe details may be made direct to Miss J Appelbee, Marine Science Unit, Te No 031 449 5111.

2571(A)

MANCHESTER AREA HEALTH AUTHORITY (T CENTRAL DISTRICT
RESEARCH SCIENTIST

University Department of Clin Haematology, Manchester Ro Infirmary. The successful applicant have extensive experience protein/organic chemistry and b scientist of exceptional abilities who match the requirements of this se research post attached to the Su Regional Haemophilia Centre.

The aims of the post are to enhance academic and practical development haemostatic research with special refere to the biochemistry of Factor VII relation to function.

Minimal requirements are 1st-2nd c honours degree, F.I.M.L.S. or equiva and ideally a number of years prac laboratory experience. Appointmen Chief Medical Laboratory Scien Officer £5,472 to £6,192 or Se Scientific Officer £5,451 to £6,837. F salaries under review.

Further information from Mr K H Clinical Haematology, 061 273 3 Applications with curriculum vitae references to Dr I W Delamore at the ab address by 14th September 1979.

2576(A

Head of Toxicology

Continental Europe to c.£25,000 + benefits

This is an attractive and challenging appointment in a leading international company with a considerable research involvement in ethical pharmaceuticals.

The post is unusual in that the Toxicology Department concentrates on preliminary safety studies for new drugs, and then follows up—in-house—any unusual or unexpected responses which have been observed in the more routine and longer-term studies, which are contracted out.

The Department comprises some six graduates and a strong supporting staff. In addition to his/her managerial responsibilities for the effective running of the Department, the successful candidate will be directly involved in:

- Maintenance of a strong working relationship with contract research organisations in the UK and Europe, including regular liaison visits.
- Keeping abreast of developments in toxicology, regulatory and GLP requirements.
- Shaping research policy as a member of the Research Management Group.

The person we seek for this appointment will probably be in his or her 30s, and must have several years' broadly based experience in the toxicity testing of pharmaceuticals, in a senior position either within industry or in contract research. In addition, good management, planning and communication skills are of paramount importance.

Existing linguistic ability—other than in English—is not called for: English is widely spoken in the company and there are an increasing number of English employees. Applicants should, however, welcome the opportunity to broaden their experience by living on the Continent and learning another language.

The starting remuneration level will be around £25,000 (according to qualifications and experience) which, together with the other benefits of service, should provide a standard of living equivalent to about £17,500 in the UK. Generous removal expenses will be paid, including provision of a substantial tax free allowance to help settling into a new home.

Please write in confidence for an application form, or telephone if you would like to discuss the appointment further before applying:

Talentmark
Leaders in Health Care Recruitment

James A Edwards, Talentmark Limited
King House, 5-11 Westbourne Grove,
London W2 4UA. 01-229 2266 (24 hours).

2593A

BEATSON INSTITUTE FOR CANCER RESEARCH
Glasgow

POST DOCTORAL RESEARCH FELLOW

pplications are invited from post-ctoral scientists to collaborate on a oject involving a new approach to the lation and study of erythroleukaemia-ducing viruses in the mouse. This MRC oject will be associated with an ablished group involved in the analysis transformation and differentiation. iis position will provide ample portunity for gaining experience in cell d molecular biology and making ogress in an exciting area of research. chnical assistance will be provided.
The appointment is for a period of three ars from October 1979 with an initial ary of up to £5,129 on the University search Grade 1A, for someone with the propriate experience and qualifications. pplications enclosing a curriculum vitae ould be sent to Dr I B Pragnell from om further information can be gained. 2631(A)

UNIVERSITY OF DUNDEE

pplications, from candidates with interests in th biology and mathematics and with Honours gree qualifications in one or other of these lds, are invited for appointment as a

RESEARCH ASSISTANT

work with Dr I H M Smart in the Department of atomy on a project involving mathematical delling of the generation of cell populations in mammalian brain.
The appointment is financed from an external nt and is available for up to three years at a ry of £3,689 (to be reviewed in October 1979). pplications by curriculum vitae, quoting rence EST/64/79J and giving the names of referees, should be sent as soon as possible to Secretary, The University, Dundee DD1 N. 2566(A)

UNIVERSITY OF BRISTOL
SCHOOL OF CHEMISTRY, ORGANIC GEOCHEMISTRY UNIT

RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant (Grade 1B) to assist in the application and development of computerised gas chromatography-mass spectrometry and related techniques to the analysis of organic compounds in sediments and petroleums. Instrumentation includes Finnigan 4000, MS 30 and CH-7 spectrometers interfaced to an INCOS 2300 data system. Applicants should have a qualification in chemistry or physics and an interest in instrumentation. Previous experience in mass spectrometry and/or gas chromatography would be an advantage.
This post is funded by the Natural Environment Research Council (starting salary £3689 - £4232 per annum).
Please send applications with details and the names of two referees to Professor G. Eglinton, University of Bristol, School of Chemistry, Cantock's Close, Bristol BS8 1TS. 2490(A)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF VETERINARY PHYSIOLOGY AND PHARMACOLOGY

Applications are invited for the post of Lecturer in the Department of Veterinary Physiology and Pharmacology.
Initial salary on the scale £4,323 to £8,452 per annum.
Applications, together with the names of three referees, should be received not later than 9th August, 1979, by the Registrar, The University, P O Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/702/N. 2570(A)

CROP PHYSIOLOGIST

The Centro Internacional de Agricultura Tropical (CIAT), an international agricultural research center based in Colombia, South America, has an active program of crop research in *Phaseolus* species with emphasis on *P. vulgaris*. An interdisciplinary team concentrates its activities on developing new technology for this crop, principally for Latin America, but with increasing emphasis outside of the Western Hemisphere. Applications are invited to fill the position of Crop Physiologist within the team. The physiologist has responsibility for identifying physiological limits to yield and adaptation and in the screening of germplasm for characters likely to lead to breeding progress. Physiological research has been conducted for over four years and a strong base has already been established. The applicant should have a PhD in crop physiology and experience in an active crop improvement program. A working knowledge of the Spanish language would be preferred. The position is located at CIAT headquarters in Cali, Colombia.
A letter of application and curriculum vitae with names of three referees should be forwarded to: Dr Douglas R. Laing, Director for Crops Research, CIAT, Apartado Aéreo 6713, Cali, Colombia. Further information available on request. Applications must be sent to arrive by September 30. W244(A)

ANIMAL TECHNICIAN

A vacancy exists for an animal technician in the Cardiothoracic Research Laboratories at the Cardiothoracic Institute. Applicant should have A.I.A.T. Salary on Animal Technician Scale £2,883 to £3,192 plus LW.
Apply to the Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP stating age, qualifications and experience and giving the names of two referees. 2590(A)

UNIVERSITY OF EDINBURGH
Mitochondrial Genome Expression

POSTDOCTORAL RESEARCH FELLOW

required to join a group led by Dr C J Leaver working on mitochondrial genome expression during plant development. Previous experience in the field of mitochondrial biogenesis or function together with an interest in membrane protein isolation and purification would be an advantage but not a necessity. The successful candidate will also be required to use various techniques involved in nucleic acid isolation and *in vitro* translation already in use in the laboratory.
This post is supported by the SRC and is available from 1st October 1979 for three years but a suitable applicant may start at a later date. Salary on the 1A Scale for research staff, plus USS benefits.
Applications with curriculum vitae and naming two referees should be sent to Dr C J Leaver, Department of Botany, The King's Buildings, Mayfield Road, Edinburgh EH9 3JH, Scotland, as soon as possible. Please quote reference 5037. 2599(A)

TECHNICIAN Grade 3, required to assist in research on the actions of catecholamines on liver and muscle cell membranes. A biological background is essential and experience of one or more of the following techniques would be an advantage: organ bath pharmacology, electrophysiological recording, cell culture. The post offers the opportunity to learn a wide range of techniques. Salary in range £3,455 to £3,860 inclusive of London Weighting. Five weeks' annual paid holiday plus statutory and customary days giving one week at Christmas and Easter. Application form from Personnel Officer (Technical Staff FE11) University College London, Gower St WC1E 6BT. 2626(A)

Royal Observatory, Edinburgh

HIGHER SCIENTIFIC OFFICERS

(INFRARED ASTRONOMERS)

There are vacancies at the Royal Observatory, Edinburgh for astronomers experienced in infrared techniques. The successful applicants will become members of the UK Infrared Telescope Unit (UKIRT), one of the three National Facilities run by the Royal Observatory, the others being the UK Schmidt Telescope in Australia and the COSMOS Measuring Machine in Edinburgh.

Initially, the successful candidates will be allocated to the support group to monitor the construction of common-user instruments for UKIRT and maintain contacts between instrument builders, the UK astronomical community and the Unit in Hawaii. Both short term visits and postings of about three years to Hawaii are likely to be involved in the longer term.

The successful candidates will be expected to spend half their time on astronomical research, for which there are excellent facilities in Edinburgh. Applicants should have good degrees in physics or astronomy, extensive experience of relevant astronomical observing and instrumentation, and either a PhD or evidence of independent astronomical research.

Because the telescope is situated at high altitude a special medical examination is given to all members of the UKIRT operating team. Success in that examination will be a pre-requisite for appointment to this post.

Salary will be in the range £4,101 to £5,448 (under review) depending on age and experience.

Further details and application forms from the Personnel Officer, Royal Observatory, Blackford Hill, Edinburgh EH9 3HJ. Tel: 031 667 3321.

CLOSING DATE: 2 AUGUST 1979. 2605(A)

CSIRO AUSTRALIA

Research Scientist
Division of Protein Chemistry
Melbourne

CSIRO has a broad charter for research into primary and secondary industry area. The Organization has approximately 7,000 employees — 2,400 of whom are research and professional scientists located in Divisions and Sections throughout Australia.

FIELD: *Biochemistry*

GENERAL: The Division has a staff of over 120, approximately half of whom are professional. The Division's Research Programme includes a group, working on Hide, Skin and Leather research, which has close ties with the Leather Industry.

DUTIES: To study the effect on the properties of hides, skins and leather produced by changes at the molecular level, either naturally occurring or externally induced, in the nature of the collagenous and other components of the hide and skin.

QUALIFICATIONS: A PhD with a biochemistry background. Experience in protein chemistry and/or hide and skin properties an advantage.

SALARY: Research Scientist/Senior Research Scientist $A15,422 to $A22,405 pa.

TENURE: Indefinite with superannuation.

Applications in duplicate, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting reference number 462/447 should reach The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 18th August, 1979.

Applications in USA and Canada should be sent to The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, NW, Washington DC 20036, USA. 2610(A)

CHAIRMAN
of the
DEPARTMENT OF PHYSIOLOGY
WAYNE STATE UNIVERSITY
SCHOOL OF MEDICINE

Creative and energetic leader to assume the chair of this large and diverse department July 1, 1980. More than 20 faculty members, large and modern research and teaching facilities. Applicants should have an established and productive research record, experience in teaching and administration, to be able to assume responsibility for the medical, allied medical and graduate teaching programmes of the department. Sex, race, religion or national origin will not be factors in the selection process.

Interested persons should reply by August 1st 1979 to:

Dr Bernard H. Marks,
Department of Pharmacology,
Wayne State University,
School of Medicine,
Detroit, Michigan 48201.

W227(A)

UNIVERSITY OF MANCHESTER

EXPERIMENTAL OFFICER IN PHYSICS

Applications invited from graduates in Physics or Computer Science, or others with appropriate experience, for this post to co-ordinate and develop software on a network of mini-computers, and data aquisition systems connected to the SRC computer network. The systems are at present being installed and are supported by the SRC and the University. (This post, however, is established on University funds and not by a fixed term SRC grant). The person appointed will liaise directly with SRC computer personnel and staff of the Physics Department. Applicants should have experience in computing, preferably with PDP11 systems, and be keen to develop with the job and broaden their experience in the rapidly developing field of computer networks and on-line computer systems. Salary scale £4,333 to £7,521 pa, USS.

Details and application forms (returnable by August 3rd) from the Registrar, The University, Manchester M13 9PL. Quote ref: 172/79/Na.

2615(A)

UNIVERSITY OF BRISTOL
DEPARTMENT OF THEORETICAL CHEMISTRY

Theoretical studies of Electron-molecule collisions (Dissociative recombination processes)

Applications are invited for a

Science Research Council-supported Post-Doctoral Research Assistant

to work on theoretical calculations of electron-molecule collision processes of the type $e + AB^+ \rightarrow AB^* \rightarrow A + B$, with particular emphasis upon non-adiabatic (curve crossing) effects. The project is initially funded for 21 to 23 months (depending upon initial salary), with prospect of further extension. The work will also include co-operation with Professor P.G. Burke's research group at Daresbury Laboratory.

Applicants should write as soon as possible to Dr J Gerratt, Department of Theoretical Chemistry, University of Bristol, Bristol BS8 1TS, enclosing curriculum vitae and names and addresses of two referees. 2596(A)

SCOTT POLAR RESEARCH INSTITUTE
University of Cambridge

RESEARCH ASSISTANT ANTARCTIC GEOPHYSICS

The Scott Polar Research Institute ha completed glaciological and geophysica surveys over 50% of ice-covered Antarctic (7.5 M km^2). It is intended to consolida the scientific results of this work in a majo map and text folio.

We seek a research assistant for minimum period of two years to tak responsibility for compilation of materi for this folio including, where necessar some original analysis of data. Candidate should have a good Honours Degree in Physical or Earth Science subject (Physic Geophysics, Geology etc.). In additio experience in computer data-manageme and cartography would be advantageou Salary conforms to University age-relate pay scales (eg 25 years £4,505 [unde review]).

Applications, accompanied by fu curriculum vitae and names of two refere should be sent to The Director, Scott Pol Research Institute, Lensfield Roa Cambridge CB2 1ER (0223-66499).

2587(A)

EUROPEAN JOURNAL OF BIOCHEMISTRY

Applications are invited from graduates in biochemistry, PhD preferably, for the post of

EDITORIAL SECRETARY

at the journal's office in Liège.

Salary will be around £5,800 net per annum, or more according to special experience. Applicants should preferably be nationals of the member states of the European Economic Community.

Applications, which should include the name of at least one referee and complete curriculum vitae, should be sent as soon as possible to Professor Claude Liébecq, 69/054 Boulevard de la Constitution, B-4020 Liège, Belgium.

2533(A)

BIOCHEMIST AND BIOPHYSICIST

Postdoctoral positions available immediately to investigate (1) regulation and mechanism of gene expression, (2) nucleaic acid-protein interactions.

Send c.v. and 3 reference letters to Professor C.W. Wu, Dept of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461. An equal opportunity employer. W238(A)

LIPOPROTEIN BIOCHEMIST

UTAH

one year postdoctoral on lipoproteins solid-liquid interfaces. Some backgrou with complement and immunoglobuli desirable. Contact J.D. Andrade, Dept. Bioengineering, University of Utah, Sa Lake City, Utah 84112. (801) 581-850 Position available September 1, 1979. A Equal Opportunity/Affirmative Actic Employer. W239(A)

START A UNIQUE PHARMACEUTICAL PLANT

A major factor in the fight against the diseases of poverty in rural Bangladesh is the steady supply at low cost of essential drugs and medicines. Expensive imported products are available to a favoured few in urban areas.

Last year the World Health Organisation encouraged the creation of in-country pharmaceutical production units as one solution. That hope is now turning to reality at a community health project 22 miles from Dacca.

We have been asked to find two pharmacists — one for quality control, the other for planning and production — and a maintenance engineer to help establish and run a pharmaceutical production unit. It aims to supply 20% of the drug requirement for rural Bangladesh.

You will need at least three years pharmaceutical production experience and the ability to pass on your skills to local staff. You will receive the local salary, free accommodation and travel to and from Bangladesh.

Write to Jane Mackay or Ed Murray, Room M, Voluntary Service Overseas, 9 Belgrave Square, London SW1X 8PW. 2583(A)

SANDOZ RESEARCH INSTITUTE
in Vienna, Austria

is one of three international research centres of the Swiss based pharmaceutical company.

In the **Immunobiology Department** of this institute a senior staff position for a

VIROLOGIST

is available.

The person we are seeking should have a PhD or equivalent degree with specialization in animal or medical virology. Experience in molecular virology and virus genetics would be of advantage.

The successful candidate will join an active research group working on **influenza and other respiratory viruses**. The work will lead to practical applications in the field of viral vaccines, but emphasis is laid on basic research as well.

A knowledge of German is not a prerequisite, but would be advantageous.

Research facilities and working conditions are excellent. Salary will be negotiable, commensurate with experience and qualifications. Other benefits include a non-contributory pension scheme and full assistance toward moving expenses.

Please send curriculum vitae, list of publications and the names of referees to

SANDOZ

Dr. N. Zacherl, Personnel Manager,
SANDOZ Forschungsinstitut Wien,
Brunner Straße 59
A-1235 Vienna, Austria W242(A)

SIMON FRASER UNIVERSITY
Burnaby, British Columbia
Canada
DEPARTMENT OF BIOLOGICAL SCIENCES
ENTOMOLOGIST

pplications are invited for a tenure-track sition as an Assistant Professor in tomology. The successful applicant will expected to develop a strong research ogram and to participate in the teaching ogram at both the undergraduate and aduate level including the Master of Pest anagement Program. Candidates must ve a PhD degree in entomology or a ated subject. The position is available mediately, subject to budgetary proval. The initial appointment will be a period of 2 years, renewable. The ary scale is under revision; the current ary base for the Assistant Professor rank $18,604 per annum.

Applications should include an up-to-e curriculum vitae, a brief statement of earch interests and goals, and reprints of olished research. Applicants should uest a confidential assessment of their earch and teaching ability from three erees, to be forwarded directly to M Mackauer, Chairman, Dept of ological Sciences, Simon Fraser iversity, Burnaby, BC, Canada, A 1S6. Deadline for receipt of lications is 15 September 1979 or when ition filled. W240(A)

UNIVERSITY OF OXFORD
DEPARTMENT OF CLINICAL BIOCHEMISTRY
TECHNICIAN

iired for research into regulation of the uvate dehydrogenase complex. The t is available initially for two years from ober 1. Applicants should have a degree iochemistry (or a related subject) or ess suitable laboratory experience. ting salary up to £4,047 per annum. pplications including the names of two rees to Professor P. J. Randle, artment of Clinical Biochemistry, n Radcliffe Hospital, Headington, rd, OX3 9DU. 2578(A)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF ZOOLOGY
FIELD TECHNICIAN (GRADE 3)

The post involves assistance with field studies, especially on freshwater fish, although laboratory work is also involved. Candidates must be willing to work long hours, often in adverse weather conditions. Experience with small boats and engines and an interest in fish an advantage.

Candidates must hold current driving licence, possess ONC or equivalent and have three years laboratory experience.

Salary in range £2,931 to £3,336 p.a.

Application forms may be obtained from The Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref: RV/699/N. 2560(A)

UNIVERSITY OF GLASGOW
DEPARTMENT OF BIOCHEMISTRY
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the position of Postdoctoral Research Assistant to study "The Translational Control of Rat Skeletal Muscle Protein Synthesis by Insulin". The appointment, which is from October 1st 1979, will be supported by the British Diabetic Association for a maximum of three years, and the starting salary will be £4,333 per annum on Range 1A of the salary scales for Research and Analogous Staff. The appropriate superannuation scheme will apply.

Applications, with curriculum vitae and the names of two referees should be sent to Dr D P Leader, Department of Biochemistry, University of Glasgow G12 8QQ. In reply please quote Ref No 4488M. 2563(A)

Royal Observatory, Edinburgh
PROFESSIONAL AND TECHNOLOGY OFFICER
GRADE 1

The Technology Unit at the Royal Observatory has a vacancy for an engineer at PTO I level. The Unit is responsible for research, development and manufacture of a wide range of instrumentation for the National Facilities run by the Observatory, which include the 1.2 metre Schmidt Telescope in Australia, the 3.8 metre Infrared Telescope in Hawaii and the COSMOS Measuring Machine at Edinburgh.

The successful candidate will be responsible for applications of infrared and optical solid-state and vidicon-type detectors to the recording of extremely low levels of radiation. Initially he/she will be involved in the development and construction of a cooled 10 micron array spectrometer, operating with As doped silicon diodes.

The applicant should be a fully qualified engineer; possession of an advanced degree may be an advantage. Applicants will be required to describe successful results with solid-state or vidicon-type systems which they have developed.

Starting salary will be in the range £5,739 to £7,064 depending on age and experience. A non-contributory superannuation scheme is included.

SRC

Further details and application forms from The Personnel Officer, Royal Observatory, Blackford Hill, Edinburgh EH9 3HJ. Tel: 031 667 3321.
CLOSING DATE: 2 AUGUST 1979.

2604(A)

Overseas Opportunities in Agriculture & Research

The Kuwait Institute for Scientific Research, a rapidly expanding institute for applied science and technology, is seeking professionals interested in challenging careers in agriculture and research.

Range Specialist

You should have an M.SC in range management; a minimum of three years' experience in range improvement practices and sheep production; and a knowledge of range machinery and equipment.

Researcher

You should have a Ph. D. in agronomy or equivalent; a minimum of four years' experience in forage production management of pasture harvested crops and nurseries; and academic training or experience in arid region development. Knowledge of irrigation systems is desirable.

Kuwait Institute offers attractive salaries commensurate withh qualifications and experience, furnished housing, liberal fringe benefits including round trip air tickets, and free medical benefits.

Please airmail your resume to the Personnel Manager, Kuwait Institute for Scientific Research, P.O. Box 24485, Safat, Kuwait, State of Kuwait, no later than one month from the date of this publication.

Kuwait Institute for Scientific Research

W234(A)

TECHNICIAN

A Technician is required to assist in a programme of work on neurotoxicity. Training in biochemistry is essential but some experience in physiological techniques would be an advantage. Qualifications: Degree HNC or equivalent. Salary on scale of £3,261 to £4,680 plus London Weighting of £354.

Apply in writing giving curriculum vitae to Mr V. H. Parker, Toxicology Unit, MRC Laboratories, Woodmansterne Road, Carshalton, Surrey.

MRC Medical Research Council

2598(A)

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOLOGICAL SCIENCES

There is a vacancy for a

RESEARCH ASSISTANT
(Plant Physiology)

to work with Dr Jonathan Weyers on a project concerned with stomatal movements during water stress. The work will involve the isolation of leaf epidermis cell protoplasts and investigation of their reactions to the plant hormone abscissic acid.

The appointment, supported by an SRC grant, is available for three years from 1 October 1979 at an initial salary (under review) of £3,775. The successful applicant may be able to register for a PhD.

Applications (by curriculum vitae containing the names of two referees and quoting REF: EST/73/79J) should be lodged as soon as possible with The Secretary, The University, Dundee, DD1 4HN. 2573(A)

UMIST
LECTURER IN ORGANIC CHEMISTRY

Applications are invited from candidates of either sex for a post of Lecturer in Organic Chemistry. The Department of Chemistry has a full teaching programme in Organic Chemistry for Honours and Ordinary degrees of the University of Manchester, and has excellent analytical, spectroscopic and synthesis services for research. Applicants should have an interest in the biological applications of organic synthesis.

Salary will be according to age and experience on the scale £4,232 to £8,452. The appointment will begin as soon as possible.

Application forms and further particulars can be obtained from the Registrar, UMIST, P O Box 88, Manchester M60 1QD, and should be returned by 30th September 1979 at the latest. Please quote reference CH/132/AI. 2559(A)

UNIVERSITY OF NEW SOUTH WALES
Sydney, Australia

National Health and Medical Research Council (NH & MRC) SENIOR RESEARCH OFFICER
SCHOOL OF PHYSIOLOGY & PHARMACOLOGY

Applications are invited for appointment to a postdoctoral position commencing October 1979, to work on synaptic effects of anaesthetics using voltage clamp and noise analysis techniques. Position available initially to 31 December 1980.

Further information is available from Professor Peter W. Gage, School of Physiology and Pharmacology, University of New South Wales, Kensington, NSW Australia 2033. Salary per annum: $A15,786 range $A20,737. Commencing salary according to qualifications and experience. Applications should be submitted immediately.

Information about method of application can be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 2602(A)

PRE AND POSTDOCTORAL POSITIONS

are available for individuals with microbiology background for studies on the protein synthesising system with special emphasis on ribosomes — topography, biogenesis and function. Teaching opportunities are available. Reply with résumé to: Dr Bruce H. Sells, Professor and Director, Laboratories of Molecular Biology, Faculty of Medicine, Health Sciences Centre, Memorial University of Newfoundland, St John's, Newfoundland A1B 3V6, Canada. W235(A)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
University of London

TECHNICIAN

Two Technicians required to work in Department of Biochemistry. Salary on Whitley Council scale according to age and experience. Further details and application forms from the School Secretary, R.F.H.S.M., 8 Hunter Street, London WC1N 1BP, or tel. 01 837 5385 ext. 54. Closing date 3rd August, 1979. 2555(A)

THE UNIVERSITY OF MANCHESTER

TECHNICIAN (GRADE 3)
DEPARTMENT OF MEDICINE
Hope Hospital

The successful applicant will work under the direction of Dr D C Anderson, Senior Lecturer in Medicine (Endocrinology) on a research project examining the action and interaction of hormones and vitamin D metabolites in bone. This involves *in vivo* and *in vitro* experiments and techniques including tissue and cell culture, subcellular fractionation, light and electron microscopy and receptor and saturation analysis techniques. Previous experience in some or all of these techniques is desirable but not essential. It is hoped that the successful applicant would want to become actively involved in the research. Starting date will be mid-September. Salary scale £2,931 to £3,336 pa.

Applications should be addressed to Dr D C Anderson, Department of Medicine, Hope Hospital, Eccles Old Road, Salford, M6 8HD. 2607(A)

BIRKBECK COLLEGE (University of London)
DEPARTMENT OF ZOOLOGY

MSc IN COMPARATIVE PHYSIOLOGY

A two-year part-time evening course suitable for zoology graduates wishing to specialise in the comparative aspects of the adaptations of animals to their environment. Topics covered include: osmotic and ionic regulation; respiratory & circulatory physiology; endocrinology; neurophysiology. Students are introduced to a wide range of modern physiological techniques. For further details contact the Departmental Secretary, Department of Zoology, Birkbeck College, Malet Street, London WC1E 7HX. 2581(A)

UNIVERSITY OF MANCHESTER
DEPARTMENT OF PHYSICS

Applications are invited for the post of

LECTURER

in the Department of Physics. The successful candidate will be expected to carry out experimental work within the Atomic, Molecular and Polymer Physics Group of the Department. Some preference will be given to candidates intending to pursue studies in the field of coherent interactions or laser spectroscopy, but candidates having other interests which are related to those of the Group will also be considered. The appointment is from October 1st, 1979 or as soon as possible thereafter at an initial salary normally within the range £4,33 to £5,199 p.a. Further particulars and application forms are obtainable from the Registrar, the University, Manchester M13 9PL, to whom completed forms should be returned by August 3rd. Quote ref. 147/79/N. 2526(A)

UNIVERSITY OF BRISTOL
DEPARTMENT OF ZOOLOGY

POSTDOCTORAL POSITIC NEUROPHYSIOLOGY

To join a group investigating spinal c neurones co-ordinating swimming amphibian embryos. Experience v intracellular microelectrode recording, horseradish peroxidaze technique computer modelling of neuronal netwo is needed.

The post can start after 1st October, I at a salary of £4,382 pa (subject to revis from the 1st October, 1979). Applicati with a curriculum vitae and two referee Dr Alan Roberts, Department of Zoolc University of Bristol, Woodland Rc Bristol BS8 1UG, as soon as possible. 2575(A

WELSH NATIONAL SCHOOL OF MEDICINE
(Univeristy of Wales)
DEPARTMENT OF MEDICINE
RESEARCH OFFICER

Applications are invited for the above appointment with Dr R Marks, Reader and Consultant in Dermatology, Welsh National School of Medicine, on a collaborative project with the Department of Biochemistry at University College, Cardiff to identify serum component(s) of possible importance in wound healing. The work will involve assay systems, organ culture and histometric techniques.

Candidates should have a degree in Biochemistry or other biological discipline and experience with histological procedures and tissue-culture systems would be advantageous.

The appointment, which will be for one year in the first instance (renewable for a second year) is financed by a grant from the Medical Research Council. Starting salary up to £4,232 per annum (under review).

Applications in the form of a curriculum vitae, with the names and addresses of two referees, should be sent to the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff 3 weeks from the date of appearance of this advertisement (quoting ref. No. M6/53). Further information from Dr R Marks (0222/755944 ext. 2885). 2552(A)

PORTSMOUTH POLYTECHNIC
DEPARTMENT OF BIOLOGICAL SCIENCES
RESEARCH ASSISTANT

Applications are invited from final year students and graduates wishing to undertake research for a higher degree.

Title: Ecological studies of enterotoxin production by staphylococcal isolates from meat. Supervisor: Dr E May. Salary: £2,589 to £2,643 pa (under review). Contract is initially for 2 years.

Application forms and further particulars obtainable from the Staff Office, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth PO1 2QQ, to whom completed applications should be returned by 3rd August, 1979. Please quote reference number B45. 2624(A)

POSTDOCTORAL POSITION

Available September 1979, for research on the transport and metabolism of purines by malaria infected red cells. Experience in nucleic acid chemistry and/or transport preferred.

Send résumé and three references to: Dr Irwin W. Sherman, Department of Biology, University of California, Riverside, Calif. 92521. An Equal Opportunity/Affirmative Action Employer. W241(A)

POSTDOCTORAL FELLOW

An immediate opening is available for a recent PhD biochemist with experience in 1) *in vitro* translational systems and/or 2) purification and characterization of phosphatases and phosphokinases. The person chosen will have the opportunity to interact with several interdisciplinary groups and the fellowship will run for two to three years. All interested candidates should send a resumé and three names of referees to:

Dr George Thomas
Friedrich Miescher-Institut
P O Box 273
CH-4002 Basel,
Switzerland W230(A)

UNIVERSITY COLLEGE LONDON
DEPARTMENT OF PHARMACOLOGY
TECHNICIAN VACANCIES

Technician Grade 5 required to work on MRC supported programme of work on peripheral synaptic mechanisms. The post requires some experience of electrophysiological techniques and will involve the construction, testing and maintenance of electrical equipment. The laboratory is equipped with a PDP 11/40 computer and some knowledge of this type of equipment will be an advantage. Salary in range £3,998 to £4,106 inclusive of London Weighting.

Technician Grade 3 required for biochemical and pharmacological work on peripheral synaptic mechanisms, supported by the Medical Research Council. The programme involves about six scientific staff and a variety of experimental techniques, including tissue culture, microelectrode recording, radiolabelling experiments and biological assays. Salary in range £3,455 to £3,533 inclusive of London Weighting. Five weeks' annual paid holiday plus statutory and customary days giving one week at Christmas and Easter. Application form from Personnel Officer (Technical Staff FE10) University College London, Gower St, WC1E 6BT. 2627(A)

THE UNIVERSITY OF MANCHESTER
TECHNICIAN (GRADE 5)
DEPARTMENT OF DIAGNOSTIC RADIOLOGY

The Department has three radiographic suites, one of which contains the EMI Whole Body Scanner. Current research is concerned with the development of new non-invasive techniques employing conventional radiographic equipment and computed tomographic scanning. Minor surgical procedures, isotope techniques and animal care are important aspects of the work. The post offers considerable scope for initiative. Radiographic experience is unnecessary but previous animal experience would be desirable. Salary scale £3,474 to £4,056 pa.

Applications should be addressed to Professor Ian Isherwood, Department of Diagnostic Radiology, Stopford Medical School, University of Manchester, Oxford Road, Manchester M13 9PT. 2606(A)

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOCHEMISTRY
RESEARCH TECHNICIAN

A vacancy exists for a technician to join a research group led by Dr Philip Cohen in the above Department and engaged in a study of the role of protein phosphorylation in the nervous and hormonal control of intermediary metabolism.

The appointment is financed from an MRC programme grant and is from 1 October 1979. Candidates with an appropriate formal qualification (eg ONC Biochemistry) and three years' relevant practical experience will be eligible for appointment at a salary of up to £3,009 per annum. The possibility of a suitable candidate being appointed at either a more senior or more junior grade is not excluded.

Application forms are available from and should be returned as soon as possible to the Establishments Office, The University, Dundee DD1 4HN. Please quote Reference EST/141/79J. 2565(A)

CSIRO AUSTRALIA

Postdoctoral Research Fellow
Division of Fisheries & Oceanography
Perth

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees — 2,400 of who are research and professional scientists — located in Divisions and Sections throughout Australia.

FIELD: *Marine Population Ecologist.*

GENERAL: The Division is Australia's principal marine research institution with more than 80 scientists investigating the physical, chemical and biological features, including fisheries, of the oceans around Australia. Headquarters of the Division are at Sydney with regional laboratories in Perth, Brisbane and a field laboratory at Karumba. The appointee will be located in Perth.

DUTIES: To develop a programme that would integrate with ongoing studies of food web dynamics, rock lobster distribution and larval settlement, macroalgal and seagrass growth and distribution on limestone and coral reefs along the coast of Western Australia. Research interests should be in population dynamics and field experimentation. Experience in nearshore and coral reef ecosystems would be advantageous as would taxonomic knowledge of macroalgae on invertebrates.

QUALIFICATIONS: A PhD or equivalent qualifications in a relevant field, preferably with experience in a marine environment. Ability to work efficiently at sea is essential.

SALARY: Research Scientist or Senior Research Scientist $A15,422 to $A22,405.

TENURE: Fixed term appointment of 3 to 5 years depending upon experience.

Applications in duplicate, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting reference number 320/632 should reach The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 18th August 1979.

Applications in USA and Canada should be sent to The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, NW, Washington DC 20036, USA. 2611(A)

OXFORD UNIVERSITY
Sir William Dunn School of Pathology
NUCLEIC ACID BIOCHEMIST

Applications are invited for a post-doctoral research position, funded by the Cancer Research Campaign. The research concerns the preparation and transcription of superhelical templates derived from the nuclear DNA of human cells. Experience in enzyme purification (for the preparation of DNA gyrase and the relaxing enzyme) or the assay of specific transcripts is desirable, but not essential.

The appointment, starting as soon as possible after 1st October 1979, is likely to be for 2 years. The salary will be at an appropriate point on the University 1A scale, £4,232 to £7,145.

Applications, including curriculum vitae and the names and addresses of 2 referees, should be sent as soon as possible to Dr P R Cook, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE. 2625(A)

UNIVERSITY OF OXFORD IN ASSOCIATION WITH ST. HUGH'S COLLEGE
LECTURERSHIP IN ASTRONOMY

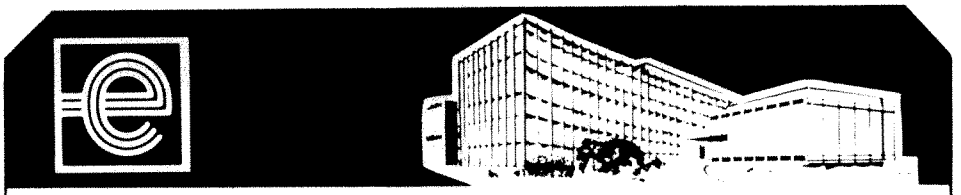
Applications are invited, from men and women candidates, for a University Lecturership in Astronomy, in the Department of Astrophysics, to be filled from 1 January 1980 or as soon as possible thereafter.

Preference will be given to applicants with a broad knowledge of astrophysics whose research interests lie in the field of optical astronomy (including the ultraviolet and infrared regions).

The stipend will be according to age on the scale £4,232 to £9,000 (under review) with superannuation.

The lecturership may be held with a tutorial fellowship in physics at St. Hugh's College and a linked lecturership at St. Edmund Hall. Applicants should state whether they wish to be considered for the college posts.

Further details of the university and college posts may be obtained from Professor D.E. Blackwell, Department of Astrophysics, South Park Road, Oxford, OX1 3RQ, to whom applications (one typed copy) should be sent before 15 October, 1979. Applications should contain a statement of qualifications, a list of publications, an outline of a proposed research programme and the names of three referees. 2595(A)



TECHNICAL OFFICER
(COMPUTER APPLICATIONS)
Applied Physics Division

MATHEMATICS SECTION

The successful candidate will join a team in the Mathematics Section whose work covers a range of research areas in addition to providing a mathematical service within CERL. He/she will work closely with Research Officers engaged on a number of problems for which numerical and computation solution plays a large and important role. In addition he/she would contribute to the mathematical and computational service provided by the Section.

Applicants should ideally possess an HNC or equivalent qualification and at least two years experience of computer programming. The duties of the post require ideally a good understanding of mathematics and numerical analysis, together with a knowledge of a range of computer languages and modern programming techniques.

The appointment will be made within a salary range of £4110 to £6035 (currently under review) plus a payment under a Self-Financing Productivity Scheme of between £10 and £15 per month.

The Laboratories are situated in a pleasant part of Surrey and offer attractive Conditions of Service, and facilities for the total of 800 Research and Support Staff engaged in a broad spectrum of research into the materials, technologies and plant performance problems of the Central Electricity Generating Board.

Application forms are obtainable from the Head of Personnel Development & Services, Central Electricity Research Laboratories, Kelvin Avenue, Leatherhead, Surrey KT22 7SE or telephone Leatherhead 74488 Ext. 363, quoting reference number **RL/37/N.** Closing date is: **Friday 3rd August 1979.**

CENTRAL ELECTRICITY RESEARCH LABORATORIES

2642(A)

BRUNEL UNIVERSITY

SCHOOL OF CHEMISTRY

POSTDOCTORAL RESEARCH

CATALYST PREPARATION AND CHARACTERISATION

Applications are invited from suitably qualified candidates for a postdoctoral research appointment on a 2 year SRC-supported programme which will be available from 1 October 1979 or as soon as possible thereafter. The programme, which is supervised by Dr Paul A. Sermon, concerns novel methods of preparing and characterising supported catalysts. A wide variety of techniques is available for catalyst characterisation and testing, including S EM, XPS, YRD and adsorptive and catalytic probes and spectroscopic techniques. Starting salary would be £4,952 per annum but this is under review. Candidates should send a detailed curriculum vitae to Establishment Secretary, Brunel University, Uxbridge, Middx UB8 3PH by 1 August 1979.

2641(A)

UNIVERSITY OF CAMBRIDGE

CAVENDISH LABORATORY

The Metal Physics Group in the Cavendish Laboratory wish to fill a new post of Senior Assistant in Research. The post is tenable for up to five years, and is renewable after that period; the salary is in the range £5,048 to £6,627 per annum (under review) and carries U.S.S. benefits.

The main duties of the post are to undertake the development of new techniques of electron microscopy and analysis and the application of these techniques to solid state physics and to materials science, including the design and commissioning of instrumentation such as electron spectrometers, electron and photon detectors and related electronics.

The successful applicant will have responsibility for research projects and the training of research students as well as the pursuit of his own research.

Applications should be sent to the Secretary, Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE before 1 September, accompanied by the names of two referees and a curriculum vitae. 2591(A)

IMPERIAL COLLEGE

POSTDOCTORAL RESEARCH BIOCHEMIST

Applications are invited for an SRC financed research assistantship to continue a project on ergot alkaloid biosynthesis using a fungal cell-free enzyme system. The post is available for one year from 1 October 1979 at a salary of £4,762 (under review). Candidates should write to Dr P G Mantle, Biochemistry Department, Imperial College, London SW7 2AZ. 2632(A)

BIRKBECK COLLEGE
(University of London)

DEPARTMENT OF ZOOLOGY

MSc IN ENTOMOLOGY

This two-year, part-time evening course is designed primarily for entomologists but also allows teachers and others to extend their training in a scientific discipline and to become conversant with the tools of current research. Topics covered include: sensory and neuromuscular physiology, respiratory physiology, osmotic and ionic regulation, insect genetics, taxonomy and economic entomology. For further details contact the Departmental Secretary, Department of Zoology, Birkbeck College, Malet Street, London, WC1E 7HX.

2582(A)

UNIVERSITY OF NAIROBI
(Kenya)

Applications are invited for the following posts in the

DEPARTMENT OF FORESTRY:

LECTURER IN FOREST PROTECTION

Applicants should preferably be holders of a PhD in Forestry with specialisation in Forest Pathology. Additional training in Wildlife Management and Fire Contro will be advantageous. The appointee wil participate in teaching undergraduate anc post-graduate courses and will undertake research in his/her field of specialisation.

LECTURER IN FOREST ECOLOGY

Applicants should preferably be holders of a PhD in Forestry or Botany with emphasis on forest ecology. Training and research experience in arid land afforestation, quantitative classificatior of forest types and regional dendrology will be advantageous. The appointee wil participate in teaching undergraduate anc post-graduate courses and undertake research in his/her field of specialisation

LECTURER IN WOOD UTILISATION

Applicants should preferably be holder of a PhD in Forestry with specialisatior in identification, structure and propertie of common softwoods and hardwoods The appointee will participate in teachin undergraduate and post-graduate course and will undertake research in his/he field of specialisation.

Salary scale: K£2016 to K£3312 p.a (K£1 = £1.22 sterling). The Britis Government may supplement salaries i range £4278 to £4818 p.a. (sterling) fo married appointees and £2730 to £3156 p.a (sterling) for single appointees (reviewe annually and normally free from tax) asn provide children's education allowance and holiday visit passages. Famil passages; FSSU or SSSF; non contributory medical scheme; subsidise housing/housing allowance.

Detailed applications (2 copies) wit curriculum vitae and naming 3 referees t be send direct to Registrar (Recruitmer and Training), University of Nairob P.O. Box 30197, Nairobi, kenya by 2 August 1979. Applicants resident in th UK should also send one copy to Inte University Council, 90/91 Tottenhar Court Road, London, W1P 0DT. Furthe details may be obtained from eithe address. 2640(A)

UNIVERSITY OF CAMBRIDGE

CAVENDISH LABORATORY

Applications are invited from suitab qualified physicists, chemists metallurgists for a post doctoral resear post jointly funded by ICI and the SR under the Collaborative Award Schem The work is concerned with the structu and composition of very highly dispers supported metal catalysts of enhanc activity and will provide an insight into t application of advanced resear techniques to systems of direct industr interest. Experience in electron microsco and related techniques is required.

The appointment will be for two ye from October 1979, based in Cambrid but with about 10% of the period at Corporate Laboratory of ICI at Runco Salary is in the range £4,282 to £5,048 annum (under review). Applicatio should be addressed to the Secreta Cavendish Laboratory, Madingley Ro Cambridge CB3 0HE. 2592(A)

UNIVERSITY OF NAIROBI
(Kenya)

Applications are invited for (4 posts)

SENIOR LECTURESHIPS
in the
DEPARTMENT OF ZOOLOGY

The specialisms required are as follows: (a) medical entomologist; (b) vertebrate ecologist; (c) fish biologist; and (d) physiologist, cell biologist, entomologist or parasitologist.

Applicants must have considerable post-doctoral experience in teaching and/or research. Where relevant, tropical and especially African experience will be an advantage. The appointees will be expected to teach both undergraduate and graduate students and to conduct their own research.

Salary scale: K£2988 to K£3984 p.a. (K£1 = £1.22 sterling). The British Government may supplement salaries by £5430 p.a. (sterling) for married apointees and £3648 p.a. (sterling) for single appointees (reviewed annually and normally free from tax) and provide children's education allowances and holiday visit passages. Family passages; SSSF or FSSU; non-contributory medical scheme; subsidised housing/housing allowance.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Registrar (recruitment and Training), University of Nairobi, P.O. Box 30197, Nairobi, Kenya by 21 August 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 2638(A)

SENIOR TECHNICIAN/ TECHNICIAN

required to assist the Biological Hazard Safety Officer on all matters relating to Safety in the Fund's Laboratories. Microbiological and virological experience essential.

Level of appointment will depend on qualifications and experience. Salary range £3,600 to £6,100. For further information and an application form please telephone Miss S M Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, WC2 on 242 0200 ext. 305. 2585(A)

UNIVERSITY COLLEGE LONDON
DEPARTMENT OF PHARMACOLOGY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a two-year, MRC-supported appointment to work on the effects of catecholamines and adenine nucleotides on the membranes of liver, fat and smooth muscle cells. Electrophysiological or biochemical experience required.

Applications with curriculum vitae and the names of two referees to Dr D H Jenkinson, Dept of Pharmacology, University College London, Gower St, London WC1E 6BT from whom further details may be obtained. Closing date 9th August 1979. 2580(A)

AUSTRALIAN NATIONAL UNIVERSITY
Research School of Physical Sciences
DEPARTMENT OF SOLID STATE PHYSICS

FELLOW/ SENIOR FELLOW

The University wishes to make a tenured appointment at the level of Fellow or Senior Fellow to extend and enhance research in the Department of Solid State Physics, Research School of Physical Sciences. The Department is well equipped for studies in all aspects of solid state spectroscopy (EPR, Mossbauer, ultraviolet, visible and infra-red) and in cryogenics and thermal properties of condensed matter. There are excellent facilities for materials preparation and supporting mechanical and electrical workshops. The senior solid state experimentalist appointed will have broad interest in an area of research central to modern solid state physics, and will be expected to make appropriate use of existing facilities with, if necessary, some modest providision for additional specialised equipment. An ability to foster links with researchers elsewhere in the University, and with other Australian solid state physicists will be important.

Closing date: 31 August 1979.

Salaries: Salary will be in accordance with qualifications and experience within the range: Senior Fellow $A24,686 to $A28,773; Fellow $A18,401 to $A24,652, Present exchange rate is $A1 = 52p.

Term of Appointment: Fellow/Senior Fellow — five years in the first instance with the possibility of reappointment to age 65.

Other Conditions: Reasonable appointment expenses are paid. Superannuation benefits are available for applicants who are eligible to contribute. Assistance with finding accommodation is provided for an appointee from outside Canberra. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should first obtain further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 2637(A)

TECHNICIAN/ RESEARCH OFFICER

to assist our Chemistry Laboratory with projects involving the relationships between chemical structure and cancer producing activity. Degree/HNC. Experience of synthetic organic chemistry preferred. Salary range £3,600 to £5,250. For application form write or telephone Miss S M Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, WC2 on 242 0200 ext 305. 2618(A)

BIOCHEMICAL VIROLOGY

A junior or senior postdoctoral position is available to study biochemical aspects of influenza virus replication and antigenic variation in influenza viruses. Successful candidates must have strong backgrounds in biochemistry. Send curriculum vitae and a statement of career goals to Dr R G Webster, Division of Virology, St Jude Children's Research Hospital, P O Box 318, Memphis, Tn. 38101. An Equal Opportunity/Affirmative Action Employer. W236(A)

FELLOWSHIPS

UNIVERSITY OF YORK

ARCHAEOZOOLOGICAL RESEARCH FELLOWSHIP

Applications are invited for this post in the Environmental Archaeology Unit of the Department of Biology. The appointment is for three years from 1 October 1979 or later, on Range 1A, and is to study multivariate changes in bones of pigs and cattle from the Anglo-Scandinavian to early medieval period in York.

Three copies of applications and curricula vitae, naming three referees, should be sent by 1 September 1979 to the Registrar, University of York, Heslington, York, YO1 5DD, from whom further particulars may be obtained. Please quote reference number 11/6109. 2630(E)

DEPARTMENT OF EDUCATION
Dublin

POST-DOCTORAL RESEARCH FELLOWSHIPS 1979-80

The closing date for the receipt of applications for post-doctoral research fellowships 1979-80 awarded by the Department of Education has been extended to 20 July, 1979.

Research fellowships in Science (excluding Medical and Social Science) and Engineering may be awarded to sufficiently meritorious candidates. Fellowships will be tenable for a period of two years at any of the following institutions: University College, Dublin; University College, Cork; University College, Galway; Trinity College, Dublin; An Foras Talúntais (The Agricultural Institute); The Institute for Industrial Research and Standards.

Applicants should hold the degree of PhD or have equivalent research or industrial experience. Candidates should not be more than 30 years of age but preferably under 28 years of age.

Further particulars of the scheme including application forms may be obtained from the Department of Education, Headquarters (Section 3), Marlboro Street, Dublin 1. Applications for fellowships should be forwarded so as to arrive at the Department of Education not later than 5 pm on 20 July, 1979. 2568(E)

UNIVERSITY OF DUBLIN
Trinity College

POST-DOCTORAL FELLOWSHIP IN BIO-ORGANIC CHEMISTRY

Applications are invited for a post-doctoral fellowship, commencing 1st October 1979, for work with Dr D. H. Grayson of the Department of Chemistry on the mechanism of enzymic conversion of chorismic acid into anthranilic acid.

The appointment, which is supported by the national board for science and technology, would be particularly suitable for an organic chemist with some experience of biochemical systems and/or radio-tracer techniques. This position is up to a total of three years, at a salary of £3,837 — £3,994 — 4,153 pa (currently under review).

Applications, giving details of qualifications and experience to date, and naming two referees, should be submitted to:

The Establishment Officer,
Staff Office,
West Theatre,
Trinity College,
Dublin 2.

Who will receive completed applications up to Friday, 17th August, 1979. 2567(E)

UNIVERSITY OF EXETER
DEPARTMENT OF CHEMISTRY

Studies of Iron-Sulphur Peptide Clusters

Applications are invited for two Science Research Council sponsored

POSTDOCTORAL RESEARCH FELLOWSHIPS

to work in collaboration with Dr Brian Ridge on the synthesis and physico-chemical study of iron-sulphur peptide clusters related to the ferredoxins. (Previous experience in either the synthesis of peptides or the handling of extremely air sensitive materials would be an advantage.)

The appointments are for a maximum of three years, but will be made for one year in the first instance, commencing 1st October 1979. Salary (1A Scale) will be in the range £4,333 to £4,910 pa (under review) plus superannuation.

Applications, with full curriculum vitae and the names and addresses of two referees, should be sent to Dr B Ridge, Department of Chemistry, University of Exeter, Exeter EX4 4QD to reach him by 31st August 1979. 2616(E)

To place your advertisement in these pages

Phone: 01-831-6901

STUDENTSHIPS and FELLOWSHIPS

THE HANNAH RESEARCH INSTITUTE

For Studies Relating to the Production and Utilization of Milk.

RESEARCH STUDENTSHIP

Applications are invited for Graduates holding a first or upper second class honours degree in either Chemistry, Biochemistry, Physiology, Agricultural Sciences or related disciplines, for appointment to an Agricultural Research Council Postgraduate Studentship tenable for 3 years from October 1979 leading to a PhD.

Particulars of the topics offered for research and details of the conditions of the award may be obtained from:

The Secretary
The Hannah Research Institute
Ayr. KA6 5HL
Tel. Prestwick 76013

to whom applications, giving full ciriculum vitae, and the names and addresses of two academic referees, should be sent by: 10th August 1979.

2633(F)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF CHEMISTRY

POST-DOCTORAL RESEARCH FELLOWSHIP IN ORGANIC CHEMISTRY

Applications are invited for a post-doctoral research fellowship, supported by the SRC, to work in collaboration with Dr G.B. Gill. The programme involves an exploratory study of the catalysis of Ene reactions, particularly with a view to developing procedures of preparative value — notably in the area of terpenoid synthesis.

Applicants should have a good background and a general interest in synthetic organic chemistry. The appointment is tenable for 2 years, and is available from 1 October, 1979. Starting salary (1A scale) in the range £3,975 to £4,505 p.a., plus U.S.S.

Applications, including a curriculum vitae and the names of two academic referees should be addressed, as soon as possible, to: The Staff Appointments Officer, The University of Nottingham, University Park, Nottingham NG7 2RD, quoting Ref. No. 705. 2556(E)

UNIVERSITY COLLEGE OF SWANSEA

CASE Studentship

for Medical Physics

Applications are invited from suitably qualified graduates, or students expecting to graduate this year, for an

SRC RESEARCH STUDENTSHIP

associated with a research project to investigate the feasibility of developing an *in-vivo* technique for the determination of Beryllium in human organs. The research is part of a collaborative programme being undertaken by the Department of Physics in the University and the Department of Medical Physics in the adjacent hospital, and will involve work in both establishments. The student will be encouraged to register for the PhD degree of the University of Wales.

Further details and application forms from Professor J Dutton, Department of Physics, University College of Swansea, Singleton Park, Swansea, SA2 8PP, Tel (0792) 25678, Ext 390. 2594(F)

UNIVERSITY OF OXFORD

DYSON PERRINS (ORGANIC CHEMISTRY) LABORATORY

Applications are invited for a postdoctoral fellowship to work on the synthesis of novel chiral phosphines and their application to asymmetric catalysis. The post is in

RESEARCH ASSISTANT GRADE 1A

on the scale £4,232 to £4,776 p.a. according to age and experience, with membership of U.S.S., and is supported by S.R.C. for a period of up to 2 years. The starting date is October 1st, 1979 or as soon as possible thereafter.

Applications with curiculum vitae and the names of two referees should be sent as soon as possible to Dr J M Brown, Dyson Perrins Laboratory, South Park Road, Oxford. OX1 3QY. 2636(E)

STUDENTSHIPS

UNIVERSITY OF BRITISH COLUMBIA

Vancouver, Canada

DEPARTMENT OF PHYSIOLOGY

Applications are invited for a research studentship available from September 1st, 1979. The successful candidate will be expected to work in the field of the reflex control of the cardiovascular and respiratory systems and to register for a higher degree. Applicants should have a First Class or Upper Second Class Honours degree in a biomedical subject.

The studentship will be valued at $6,500 per annum and may be supplemented by additional teaching duties.

Applications including a curriculum vitae and the names of two referees should be sent as soon as possible to Dr J R Ledsome, Department of Physiology, UBC, Vancouver, BC, Canada V6T 1W5. W243(F)

UNIVERSITY OF BRADFORD

SRC CASE RESEARCH STUDENTSHIP IN STRUCTURAL CHEMISTRY

Applications are invited from good honours degree graduates or equivalent to read for a PhD on a joint investigation with B.S.C. (Chemicals) Ltd, into thermally induced structural changes in coal-tar pitch and their components by chromatography, N.M.R. spectroscopy and other techniques. Further details and application forms may be obtained from Dr D W Jones, School of Chemistry, University of Bradford, West Yorkshire BD7 1DP. (0274-33466 ext 288 or 289). Ref RS/DWJ/X. 2561(F)

Southampton THE UNIVERSITY

SOLAR CELL THEORY Post-doctoral SRC Fellowship

Applications are invited for the above post-doctoral post which is available from 1 September 1979, and is suitable for appropriately interested mathematicians, physicists or engineers. Salary up to £4,505.

Applications giving the names of two referees should be sent to Professor P T Landsberg, Department of Mathematics, University of Southampton, Highfield, Southampton SO9 5NH, before 9 August 1979. Please quote ref: N. 2623(E)

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF GENETICS

Applications are invited from men and women for a 3 year Postdoctoral Fellowship tenable 1 October 1979 or as soon as possible thereafter and funded by the SRC for work on the control of cell division in *Saccharomyces cerevisiae*. Applicants should either have experience in recombinant DNA techniques or in microbial genetics. Salary up to £4,910 a year on Range 1A.

Applications including curriculum vitae and names and addresses of two referees to Dr P Sudbery, Department of Genetics, The University, Sheffield S10 2TN as soon as possible. Quote Ref R341/G. 2617(E)

CITY OF BIRMINGHAM POLYTECHNIC

DEPARTMENT OF SCIENCE

SRC STUDENTSHIP IN ENZYMOLOGY

The research programme will involve an investigation of the physico-chemical properties and *in vivo* distribution of therapeutically important, stabilised, enzymes. Candidates should have or expect a good honours degree or equivalent in Biochemistry, Chemistry, Pharmacy or a related science. The appointee will be expected to register for a PhD degree (CNAA). The studentship is tenable for 3 years from 1st October 1979 (subject to confirmation). Informal enquiries may be made to Dr R L Foster (051 207 3581 ext 48).

Further details and application forms are available from Dr J M Hitchen, Head of Department of Science, City of Birmingham Polytechnic, Perry Barr, Birmingham B42 2SU Tel 021 356 6911. 2622(F)

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF CHEMISTRY

SRC-CASE RESEARCH STUDENTSHIP

Applications are invited for a three year SRC (CASE) Research Studentship to work on the effect of impurities on the mechanism of the interaction of nickel with carbon monoxide. The work will be supervised by Professor M.W. Roberts in collaboration with Inco Europe Ltd.

Applicants should hold or expect to hold at least an upper second class honours degree in chemistry.

Applications, with the names of two referees should be sent to Professor M.W. Roberts, Department of Chemistry, University College, P.O. Box 78, Cardiff CF1 1XL. Reference 1850. 2553(F)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF METALLURGY AND MATERIALS SCIENCE

SRC CASE and AERE HARWELL sponsored Research Studentships

Applications are invited from good honours (1st or 2(1)) graduates in Metallurgy/Materials Science or related Physical Sciences for 3 year sponsored research studentships tenable from October 1st 1979. The research projects are in the following areas.

(a) Structure and Embrittlement of Low Alloy Ferritic Steels (2 positions).

Research into the relation between microstructure, segregation of alloy and impurity elements to grain boundaries and susceptibility to intergranular fracture in ferritic steels used for power engineering structures in collaboration with AERE Harwell. Supervisors: Prof B L Eyre and Dr B C Edwards.

(b) Stress Corrosion Cracking in Aluminium Alloys.

Research into the relation between the atomic structure of grain boundaries and observed rates of intergranular stress corrosion in commercial aluminium alloys in collaboration with Alcan Industries. Supervisors: Dr R C Pond and Dr C Tuck.

(c) Light-ion Bombardment of Non-metallic Materials.

To investigate the effects of light, gas ion bombardment on the microstructure and surface of ceramic materials having potential for use in fusion reactors in collaboration with the Culham Laboratory. Supervisors: Dr D J Bacon and Dr K Erents.

(d) Studentships and projects are also available in other areas, viz. heat treatment of steels, computer simulation of crystal defects, development of composite materials, mechanical properties of metals, properties of cellular materials and the properties of bio-materials.

Applications should be received as soon as possible by the Registrar, The University, P O Box 147, Liverpool L69 3BX. Quote ref RV/703/N. 2569(F)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL RESEARCH FELLOWSHIP IN BIOENERGETICS

Applications are invited for a Research Fellowship to investigate the factors controlling the synthesis of ATP after short flash excitation of membranes isolated from the photosynthetic bacteria. The principle aim of this SRC-supported project is to determine the relationship between electron transport reactions through defined segments of the electron transport chain, the development of the electrochemical proton gradient and the extent and rate of ATP synthesis after single and multiple flashes.

The appointment is for three years commencing October 1979 (or as soon as possible thereafter) in the range (under review) £4,333 to £5,488 (from 1 October). Starting salary not expected to exceed £4,910 pa. Candidates should preferably have some previous experience in the field of mitochondrial, chloroplast or bacterial bioenergetics.

Applications, including a short curriculum vitae and the names of three academic referees should be sent by 10 August to Miss L. R. Heyes, Senate Registry, University of Birmingham PO Box 363, Birmingham B15 2TT Further details may be obtained from Dr. J. B. Jackson, Department of Biochemistry. Tel: 021-472 1301 Ext. 3113 2520(E)

THE HARTLEY FELLOWSHIPS of the UNIVERSITY OF SOUTHAMPTON

Applications are invited for three Fellowships normally tenable from 1 October 1980, for up to two years research, in any field for which facilities are available within the Faculties of Arts, Science, Engineering & Applied Science, Social Sciences, Law, Education, Medicine and Mathematical Studies.

Fellows will normally be appointed from persons of high promise whose records show evidence of capacity for original research, and who have up to five years post-doctoral experience or equivalent research record.

Salary will be related to age and experience and be within nationally agreed ranges for research staff in universities. (No provision for travel or removal expenses).

The closing date for applications for the 1980 competition is 1 November 1979.

Details and application forms from Mr E.D. Gordon, The University, Southampton SO9 5NH.

Please quote reference N. 2462(E)

UNIVERSITY OF WARWICK

POSTDOCTORAL RESEARCH FELLOW in Molecular Beam Scattering

Applications are invited for an SRC Postdoctoral Research Fellowship in the Department of Chemistry and Molecular Sciences to work on the investigation of simple ion molecule reactions using a crossed molecular beam apparatus in collaboration with Dr D M Hirst. Applicants should have experience in electronics, high vacuum work and preferably in molecular beams. The post, which is available immediately, will be for one year in the first instance. Salary on the Range 1A Scale £4,333 to £7,521 pa from 1st October, 1979. Application forms and further details from the Academic Registrar, University of Warwick, Coventry CV4 7AL. Ref No 50/A/79.

2614(E)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF MICROBIOLOGY

POST-DOCTORAL RESEARCH FELLOWSHIP

Biochemist or chemical microbiologist needed to purify cell-wall components responsible for the resistance of gonococci to killing by human phagocytes. The project, which relates to a gonococcal vaccine, is supported by the Medical Research Council for three years from 1st October, 1979.

Salary will be in the Research Fellow 1A range £4,333 to £7,521, plus superannuation. Maximum starting salary will be £5,199. For further details apply to Professor H. Smith, Department of Microbiology.

Applications (3 copies) including full curriculum vitae and naming three referees should be sent to the Assistant Registrar, Science and Engineering, P O Box 363, University of Birmingham, Birmingham B15 2TT, by Monday 13th August, 1979. Please quote reference NM7. 2613(E)

UNIVERSITY OF STRATHCLYDE

Applications are invited for a

POSTDOCTORAL RESEARCH FELLOWSHIP IN ANALYTICAL CHEMISTRY in the DEPARTMENT OF PURE AND APPLIED SCIENCE

tenable for one year in the first instance, to work on the design and development of an automatic monitor for the measurement of beryllium metal in atmospheric dusts. Preference will be given to candidates with experience in atomic spectroscopy as the principal technique to be used will be carbon furnace atomic absorption spectrometry. The post is suitable for an experienced analytical chemist and will involve close liaison with colleagues working on methods of dust collection and on instrument design and production. The post is sponsored by the Atomic Weapons Research Establishment, Aldermaston, and will be under the supervision of Dr J M Ottaway.

Appointment will be on Range 1A of the national salary structure for research and analogous staff with a commencing salary from 1 October 1979 up to £6,066 per annum. Placing according to qualifications and experience. Superannuation benefit.

Written applications naming three referees (quoting R26/79) should be forwarded as soon as possible to Dr J M Ottaway, Department of Pure and Applied Chemistry, University of Strathclyde, Cathedral Street, Glasgow G1 1XL, from whom further information may be obtained.

2562(E)

QUEEN MARY COLLEGE University of London

CHEMISTRY DEPARTMENT

Applications are invited for a postdoctoral

RESEARCH FELLOWSHIP

on an SRC-Supported project to study magnetic anisotropy of polynuclear co-ordination compounds with Dr P Thornton. Appointment for 2 years from 1 October 1979, suitable for a chemist or chemical physicist with experience in magnetochemistry or other work with single crystals. Initial salary in range (under review) £4,835 to £5,412 pa (including London Allowance).

Please apply as soon as possible, giving age, qualifications, experience and names and addresses of 2 referees, to The Registrar,(N) Queen Mary College, Mile End Road, London E1 4NS. 2579(E)

QUEEN MARY COLLEGE (University of London)

CHEMISTRY DEPARTMENT

Applications are invited for an SRC-supported

Postdoctoral RESEARCH FELLOWSHIP

to work with Dr D. G. Buckley on the biosynthesis and synthesis of novel pyrrolylpolyene fungal pigments. Experience in the use of ^{13}C- and/or ^{14}C-labelled compounds an advantage. Appointment for two years from 1 October with initial salary in range (under review) £4835 to £5412 p.a. (including London Allowance).

Applications, with curriculum vitae and names and addresses of two referees, as soon as possible to The Registrar, (N) Queen Mary College, Mile End Road, London E1 4NS. 2639(E)

NUFFIELD FOUNDATION

ONE-YEAR SCIENCE RESEARCH FELLOWSHIPS

The Nuffield Foundation is offering to University Lecturers in Science (but not Mathematics) Departments a number of one-year research fellowships to be awarded competitively at the end of 1979 and to be held during the academic year 1980-81. The objective is to enable younger university lecturers to be freed from teaching and administrative duties for one academic year in the first instance; preference will therefore be given to applicants under the age of 40. Awards will cover the cost of replacement teaching and will include an allowance for research expenses.

The closing date is 15 October 1979 and further particulars may be had from the Director, Nuffield Foundation, Nuffield Lodge, Regent's Park, London NW1 4RS 2589(E)

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UNIVERSITY OF DUNDEE

DEPARTMENT OF CHEMISTRY

Applications are invited for a

POSTDOCTORAL FELLOWSHIP

to work with Dr W M Horspool and Dr A W Murray on an investigation of the photochemistry of some benzotriazine-3-N-oxides.

The appointment will be for eighteen months and the salary £4,232 (reviewable from October 1979).

Applications containing a curriculum vitae and stating the names of two referees, should be sent as soon as possible to The Secretary, The University, Dundee DD1 4HN. Please quote reference EST/66/79J.

2564(E)

CONFERENCES

Attachment

NATO ADVANCED RESEARCH INSTITUTE *IN VITRO* TOXICITY TESTING: CURRENT AND FUTURE POSSIBILITIES

Monte Carlo, September 22-28 inclusive, 1979

Organizing Committee: L. Grant, S. Harris, A. Kolber (Chairman), J. McLachlan, T. Wong.

Topics: In vitro Carcinogenesis, Mutagenesis, End-Organ and Behavioral Toxicity Testing. Molecular Mechanisms, Human Criteria and Health Risk Assessment Methodologies. Epidemiology and Development of Environmental/Health Policy.

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Applications should reach *A. Kolber, Chairman,* Organizing Committee, Research Triangle Institute, Research Triangle Park, North Carolina 27709, USA, before August 10, 1979. Please include a curriculum vitae, brief summary of applicant's background, current interests, and reasons for attendance. A small number of fellowships are available.

W233(C)

ASSISTANTSHIPS

UNIVERSITY OF LONDON
ST BARTHOLOMEW'S HOSPITAL MEDICAL COLLEGE
and the
INSTITUTE OF NEUROLOGY

Applications are invited from good honours graduates in Biochemistry or related subjects for a

RESEARCH ASSISTANTSHIP

to undertake investigations into the metabolism of muscle mitochondria in both normal and diseased states. The successful candidate would be expected to register for a PhD degree.

The appointment is for three years from 1st October at an initial salary (under review) of £4,191 per annum including London weighting.

Applications, with c.v. and names of three referees, should be sent by 20th August to Dr J B Clark, Biochemistry Department, St Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ (Tel: 01-253 0661, ext 22). 2628(P)

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF BOTANY

RESEARCH ASSISTANTSHIP

Applications are invited for the post of research assistant to work on an IBM supported-project concerned with computer techniques for the recording and display of quaternary pollen analytical data. A degree in computer science, applied mathematics, or related subject is required; experience in numerical analysis of large data sets and of computer graphics is essential. Starting salary is in the range of £4,232 to £4,776 per annum plus USS. Appointment is for one year in the first instance, starting from October 1979, with the possibility of renewal for a further year.

Applications, with a curriculum vitae and names of two referees, should be sent to Dr H J B Birks, Botany School, Downing Street, Cambridge CB2 3EA by 10 August at the latest, from whom further particulars may be obtained. 2629(P)

UNIVERSITY OF GLASGOW

POSTDOCTORAL RESEARCH ASSISTANTSHIP IN SOLID STATE PHYSICS

Applications are invited for an SRC Postdoctoral Research Assistantship to work on a VG Microscope's HB5 Scanning Transmission Electron Microscope due to be delivered in early 1980. The proposed research covers a wide range of micro-analytical (energy dispersive X-rays spectroscopy, electron energy loss spectroscopy, microdiffraction) and novel imaging techniques. Experience in one or more of the following fields would be an advantage: electron microscopy, electronics, ultra high vacuum techniques, minicomputers.

The post is tenable from 1 October 1979 until 31 July 1982 with an initial salary on Range 1A of the Salary Scales for Research and Analogous Staff (£4,333 to £7,227 per annum). Superannuation benefits will apply.

Applications stating age, academic qualifications, research experience and the names of two referees should be submitted not later than 20th August, 1979 to Professor J. C. Gunn, Department of Natural Philosophy, University of Glasgow, Glasgow G12 8QQ, from whom further details of the research work of the Solid State Physics group may be obtained. In reply please quote Ref. No. 4488M. 2574(P)

UNIVERSITY OF DURHAM
DEPARTMENT OF PHYSICS

Applications are invited for one or more SRC funded

POSTDOCTORAL SENIOR RESEARCH ASSISTANTS

tenable for 2 years from 1 October 1979 to work with the COSMOLOGY group, whose main interest is in the formation and evolution of galaxies. There may also be opportunities to work with large telescopes. Extensive computer experience would be an advantage.

Initial salary in the range £4,333 to £4,910 on Range 1A plus superannuation.

Applications (3 copies) naming three referees should be sent by 31 July 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE from whom further particulars may be obtained. 2554(P)

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ASSOCIATESHIPS

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Research Associateship available for a suitably qualified postdoctoral scientist to work in a research programme on the molecular genetics and application of plasmids that encode pathways for the degradation of organic environmental pollutants. The position, which is funded by the Bundesministerium Für Forschung und Technologie, is available immediately for an initial period of 1½ years, with a possibility for extension. Salary will be about DM3,500 per month (BAT Ib/IIa).

Applications, including a curriculum vitae, bibliography and the names and addresses of two persons who are willing to provide recommendations, should be sent immediately to Dr K Timmis, Max-Planck-Institut für Molekulare Genetik, Ihnestrasse 63, D-1000 Berlin 33, Germany. W231(O)

CONFERENCES

NINTH INTERNATIONAL RESEARCH GROUP IN DEVELOPMENTAL BIOLOGY

to be organised at the HUBRECHT LABORATORY, International Embryological Institute, Utrecht, 12 January-17 July 1981. Open to POST-GRADUATES in natural science, medicine or veterinary science (age limit 35).

TOPIC: The cell surface in morphogenesis: cell contacts, signal transmission, plasma membrane structure and function. MATERIALS: eggs and embryos of several classes, cellular slime moulds, cell lines *in vitro*. METHODS: varied, ranging from simple to advanced (inc. EM, biochemistry, biophysics).

Circular and application form obtainable from the Deputy Director of the Hubrecht Laboratory, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. Closing date for applications: December 1, 1979. W237(M)

COURSES

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"PAIN"

The ETP Winterschool is a one-week lecture course which is being organised and financed entirely through the European Training Programme in Brain and Behaviour Research. The eighth Winterschool will take place in Zuoz (Switzerland) from 5-12 January, 19080. Lectures and round table discussions will be held on the central topic of "Pain".

The following speakers have been invited:

S. Andersson (Goteborg); **M. R. Bond** (Glasgow); **D. Bowsher** (Liverpool); **G. Carli** (Siena); **M. von During** (Bochum); **A. Fanchamps** (Basel); **H. U. Gerbershagen** (Mainz); **H. Handwerker** (Heidelberg); **J. van Hees** (Leuven); **A. Herz** (Munchen); **A. Iggo** (Edinburgh); **W. Janig** (Kiel); **U. Lindblom** (Huddinge); **P. W. Nathan** (London); **P. Procacci** (Florence); **J. Siegfried** (Zurich); **A. Struppler** (Munchen); **L. Terenius** (Uppsala), **L. Vyklicky** (Prague); **P. Wall** (London); **M. Zimmermann** (Heidelberg).

The Scientific Organiser of the 1980 Winterschool is **Professor R. F. Schmidt** (Dept. Physiology, University of Kiel).

The deadline for the submission of completed applications is 15 September and successful applicants will be notified by the end of October. Application forms and further information may be obtained from:

Dr. Stephanie Zobrist
European Science Foundation
European Training Programme in
Brain and Behaviour Research
1, quai Lezay Marnesia
F-67000 STRASBOURG
FRANCE
Tel: (88) 35 30 63
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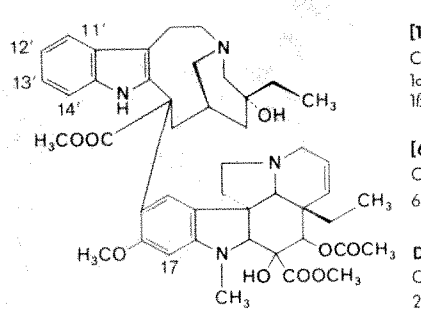
Distribution of labelling in tritium compounds

Modern techniques for the production of tritiated compounds are more sophisticated than those used in the early days of tritium labelling, and produce compounds labelled in specific positions rather than generally labelled. Nevertheless, it is necessary for many tracer applications of tritium compounds to know the *precise* position and configuration of the tritium labels. Traditional chemical methods of doing this are tedious and time consuming and subject to considerable error, and so the routine supply of such information has until recently not been possible.

The Radiochemical Centre, in collaboration with the University of Surrey, has developed over the past eight years the technique of tritium nuclear magnetic resonance (tnmr) spectroscopy for this purpose. This method is much quicker and more accurate than the traditional chemical or biochemical methods for determining distribution of tritium labelling.

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A number of examples are given below:



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1β ..... 46.0% 2β 39.9%

[6-^{3}H]BENZO[a]PYRENE
Code TRK.501
6 ..... >95% 1 ..... <5%

D-[2-^{3}H]GLUCOSE
Code TRK.361
2(α) ..... 40% 2(β) 60%

[G-^{3}H]VINBLASTINE
Code TRK.507
17 ..... 44% 11' 8%
12,13 ..... 46% 14' 2%

L-[3,4(n)-^{3}H]VALINE
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2 ..... 8% 4 ..... 64%
3 ..... 28%

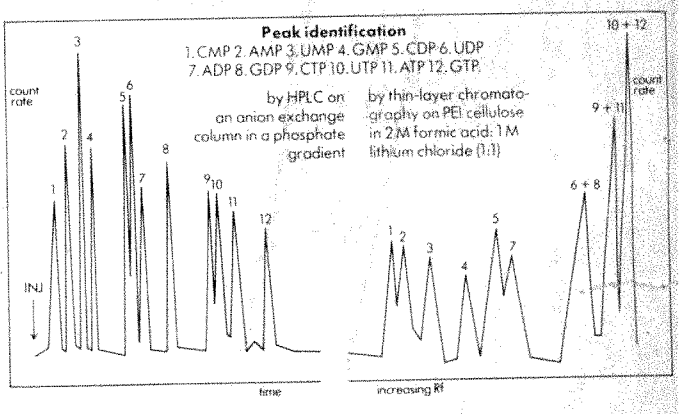
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AL-RAWI, J.M.A., BLOXSIDE, J.P., ELVIDGE, J.A., JONES, J.R., CHAMBERS, V.E.M., CHAMBERS, V.M.A. and EVANS, E.A., *Steroids* vol. 28 (3), p.p. 359-375, 1976.

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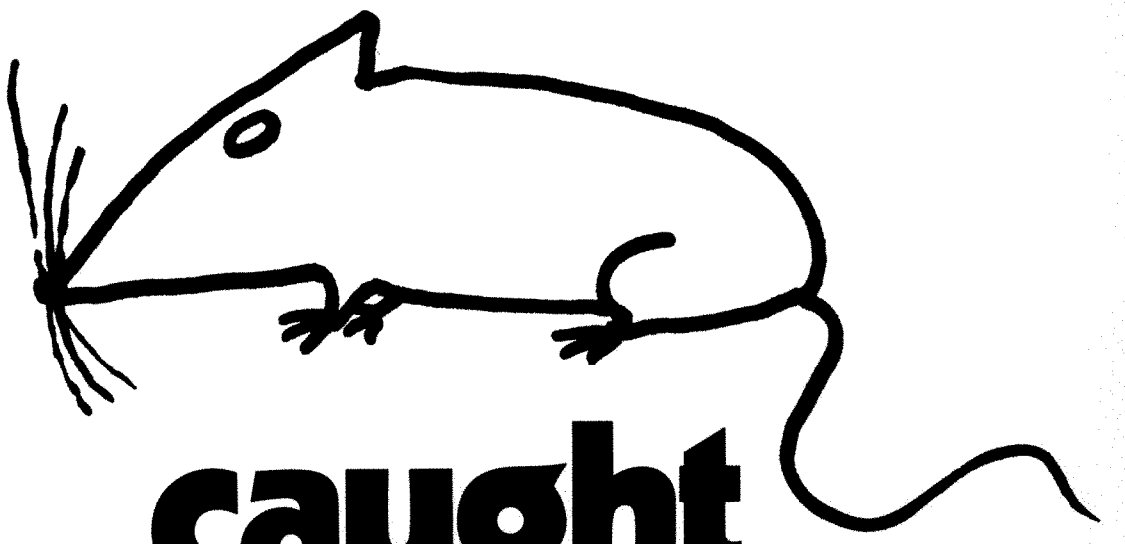
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Cover picture

Demonstration of bacterial chemotaxis showing two mutants plated on agar containing two attractants. Each strain can utilise only one of the attractants and each consumes the attractant it can use, forming a gradient and an expanding ring. The rings expand independently across the agar. Evidence for the involvement of protein methylation in bacterial chemotaxis is reviewed on p. 279.

Vol.280 No.5720 26 July 1979

nature

Vol.280 26 July 1979

How much coal? 261
Developing world 'a permanent desert for research' 262
Call for gene resource conservation programme 263
UN studies new science and technology fund 264
UK energy plans/SRC fellowships/Yugoslavia/China 265
Water quality threatens Polish agriculture 266
In brief 267
Brazil: is it really safe for the scientists to return? 268
Can we afford to make the fast reactor safe? 270

NEWS & VIEWS
Oceanography of fjords/Changing cometary orbits/Progress in thalassemia research/Quark fusion/A new form of electron diffraction by atoms 273

REVIEW ARTICLE
Protein methylation in behavioural control mechanisms and in signal transduction — *M.S. Springer, M.F. Goy and J. Adler* 279

ARTICLES
Riftward younging of volcanic units in the Addis Ababa region, Ethiopian rift valley — *W.H. Morton, D.C. Rex, J.G. Mitchell and P. Mohr* 284
Sequences at the somatic recombination sites of immunoglobulin light-chain genes — *H. Sakano, K. Hüpi, G. Heinrich and S. Tonegawa* 288
Statistical mechanics of supercoils and the torsional stiffness of the DNA double helix — *A.V. Vologodskii, V.V. Anshelevich, A.V. Lukashin and M.D. Frank-Kamenetskii* 294

LETTERS
Weak low-latitude X-ray sources and local galactic structure — *T. Matilsky* 298
Differential solar rotation depends on solar activity — *D.H. Clark, B.D. Yallop, S. Richard, B. Emerson and P.J. Rudd* 299
Origin of the jovian ring and the galilean satellites — *A.J.R. Prentice and D.Ter Haar* 300
Optical absorption coefficients of water — *C.K.N. Patel and A.C. Tam* 302
Two-photon absorption spectra and cross-section measurements in liquids — *A.C. Tam and C.K.N. Patel* 304
Photocatalytically induced fixation of molecular nitrogen by near UV radiation — *R.I. Bickley and V. Vishwanathan* 306
CS_2 and COS in the stratospheric sulphur budget — *N.D. Sze and M.K.W. Ko* 308
Thermohaline intrusions lie across isopycnals — *M.G. Gregg and J.H. McKenzie* 310
Connectance in real biotic communities and critical values for stability of model ecosystems — *M. Rejmáek and P. Starý* 311
Abnormal visual resolution of cats reared in stroboscopic illumination — *T. Pasternak and W.H. Merigan* 313
An intrinsic factor XII–prekallikrein-dependent pathway activates the human plasma renin–angiotensin system — *F.H.M. Derkx, B.N. Bouma, M.P.A. Schalekamp and M.A.D.H. Schalekamp* 315



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Macrophage requirements for mouse B-cell stimulation differ according to the molecular form of anti-IgM antibodies	*M. Hattori, D.J. Scribner and J.W. Moorhead*	316
Increased intracellular cyclic GMP does not correlate with protein discharge from pancreatic acinar cells	*G.R. Gunther and J.D. Jamieson*	318
Rabbit skeletal myosin isoenzymes from fetal, fast-twitch and slow-twitch muscles	*J.F.Y. Hoh and G.P.S. Yeoh*	321
Fast and slow muscles in tissue culture sythesise only fast myosin	*N.A. Rubinstein and H. Holtzer*	323
Filament geometry and the activation of insect flight muscles	*J.S. Wray*	325
Transition temperature of excitation–contraction coupling in frog twitch muscle fibres	*R. Miledi, I. Parker and G. Schalow*	326
Inhomogeneous distrubtion of filipin–sterol complexes in smooth muscle cell plasma membrane	*R. Montesano*	328
Visualisation of ^{11}C-flunitrazepam displacement in the brain of the live baboon	*D. Comar, M. Maziere, J.M. Godot, G. Berger, F. Soussaline, C. H. Menini, G. Arfel and R. Naquet*	329
Partial agonists for the brain GABA/benzodiazepine receptor complex	*C. Braestrup, M. Nielsen, P. Krogsgaard-Larsen and E. Falch*	331
Solvent accessibility properties of complex proteins	*S. Sprang, D. Yang, and R.J. Fletterick*	333
Evidence for non-spliced SV40 RNA in undifferentiated murine teratocarcinoma stem cells	*S. Segal, A.J. Levine and G. Khoury*	335
The relationship of degradative and resistance plamids of *Pseudomonas* belonging to the same incompatibility group	*S.A. Bayley, D.W. Morris and P. Broda*	338
Electron microscopic evidence for the circular form of RNA in the cytoplasm of eukaryotic cells	*M.-T. Hsu and M. Coca-Prados*	339

BOOK REVIEWS

Science and Its Critics (John Passmore)	*J.R. Ravetz*	341
Hückel Theory for Organic Chemists (C.A. Coulson, B. O'Leary and R.B. Mallion); Hückel Molecular Orbital Theory (K. Yates)	*A.J. Stone*	341
Intelligence: Heredity and Environment (P.E. Vernon)	*Paul Light*	342
The Year in Metabolism, 1977 (N. Freinkel, editor)	*R.G.G. Russell*	343
Disorder in Crystals (N.G. Parsonage and L.A.K. Staveley)	*R.W. Cahn*	343
The Principles of Pollination Ecology (K. Faegri and L. van der Pijl)	*Peter D. Moore*	344

ANNOUNCEMENTS xiii

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nature 26 July 1979

How much coal?

There is not the slightest doubt that an immense amount of coal resides under the British Isles. Onshore there are at present many healthy coalfields, and new ones such as Selby, Belvoir and Coventry-Kenilworth could make substantial additions; further down the line the Oxfordshire field could possibly outshine anything that has gone before. Offshore, there is ample evidence of rich fields at great depth below the North Sea. Why then should anyone disagree with the claims of the National Coal Board (NCB), repeated recently in large advertisements, that 'we have proven coal resources to last for at least another three hundred years'? Is it not just an academic quibble to ask for more scrupulous definitions of the term 'proven resources'?

A wide range of geological opinion thinks not. The Flowers' Commission on Energy and the Environment has recently been turning its attention to coal, and has received some tart comments about NCB publicity — this is a dispute which has simmered for many years. Terminology in the assessment of minerals is, if you will pardon the phrase, a minefield. But common practice is to distinguish, first, between reserves and resources. The term 'resource' covers all material about which something is known, although information may be exceedingly thin either about the extent of the resource or about ways in which it could be exploited. And, not surprisingly, a 'resource' includes a large amount of material which will on further research, turn out to be unexploitable because of, for instance, environmental or social unacceptability. So one thing the NCB does not have is a 'proven resource' — a contradiction in terms.

What the board is actually talking about if it uses the word 'proven' is 'reserves': materials that have been mapped out sufficiently well that they can be the subject of mining by known methods. The most important of the several definitions of reserve is the 'workable' or 'operating' reserve — the amount of coal that can be mined economically according to present-day detailed assessment. This figure, like all reserves, may fall if the price of coal were to drop, and may fall just as easily as rise if new equipment were installed that required thicker seams or was sensitive to faulting. In 1973 the NCB's assessment of current workable reserves amounted to 3.9 billion tonnes according to the board's then Chief Geologist (G. Armstrong, *Phil. Trans. roy. Soc.*, **276**, 439-452 (1974)). This is enough for around 30 years at present rates of consumption. Since then a vigorous programme of exploration and assessment has inched the workable reserves up to 6 billion tonnes.

How, then, does the NCB get its figure of 300 years — equivalent to a workable reserve of 45 billion tonnes? Geologists outside the NCB claim that the board tends to be a law unto itself and there is a severe shortage of coal geologists outside the board's embrace able to provide an independent estimate. But one thing does seem to be clear — that the 45 billion tonnes is bound to contain a vast amount of coal that is no more than rough guesswork at present. It seems that the figure arises not from the building up of detailed estimates from individual fields but from an estimate of what could be the total coal (shallower than 4,000 feet and in seams greater than 2 feet thick) under Britain; the total is then cut down to reflect the fraction of this coal which, on past experience, has actually been brought to the surface. The picture of a coal industry progressively and steadily working its way through the production of 45 billion tonnes over the next 300 years is thus, at best, no more than a giant extrapolation; at worst it could be totally deceptive.

But does it matter? Shouldn't the NCB be allowed a certain amount of exuberance concerning its future? In private, yes; there is little harm in a bit of optimism about an energy source that Britain has somewhat neglected in the recent past. But continued public statements on the subject could have a very harmful effect, as investment in energy technology and the development of other types of energy sources is bound to be distorted once the NCB's point of view gains general currency, particularly amongst politicians.

Most prudent energy industries like to look ahead at least fifteen years, and whilst NCB is talking about figures such as 6 billion tonnes it is being equally prudent. In making guesses at much larger figures and calling them 'proven resources' it is surely taking quite unnecessary liberties. □

Developing world 'a permanent desert for research'

Six countries account for 85% of the world's total spending on research and development. This monopoly is a major feature of global inequality, writes **Anil Agarwal**

MOST countries in the world today form a permanent research desert, the remaining few forming a small number of R&D oases. This is the conclusion of Dr Jan Annerstedt of Roskilde University in Denmark who has conducted a detailed survey of R&D activities across the world. Six nations (USA, Soviet Union, Japan, West Germany, France and the United Kingdom) employ nearly 70% of the world's R&D manpower and spend nearly 85% of R&D funds. The USA and Soviet Union account for more than half of the world's total R&D expenditure. Together, these six countries spend a quarter of every R&D dollar in the world for military purposes.

This "concentration of research and experimental development to a small number of highly industrialised countries is one of the major features of global inequality", says Dr Annerstedt in his paper on the present global distribution of R&D resources, recently released by the Vienna Institute for Development. Developing countries account for nearly 70% of the world's population but they contribute less than 3% of the world's R&D expenditures, and about 13% of the world's R&D scientists and engineers.

As far as the developing countries are concerned, this distribution of world R&D activity has remained almost unchanged. Based on a sample of countries for which R&D statistics existed in 1963 — 1964 (excluding most socialist and developing countries), the United Nations had estimated that developing countries accounted for only 2% of the world's R&D expenditure. The US was responsible for 70% and other market economies for the remaining 28%. Using this same sample of countries, Dr Annerstedt finds that their research expenditures have increased from less than $30 billion in 1963-64 to over $60 billion in 1973. But the share of developing countries has only increased from 2% to 2.8%. The share of the US has dropped from 70% to 50.7%, while that of the other developed market economies has increased dramatically from 28% to 46.5%.

Annerstedt's own survey of world R&D expenditures in 1973, however, also covers socialist countries in Europe and Asia and several more developing countries. He estimates that the world spent a total of about $96.4 billion on R&D in 1973. Developing countries accounted for 2.9% of this sum. The rest was spent by rich countries, primarily in Europe and North America.

Annerstedt's data shows that within the Third World, South and Central American countries were spending most money on R&D in comparison to their populations — about 33% of the Third World's R&D expenditure on 11% of its population. Asian countries (excluding Japan, Israel and Turkey) spent over 50% of Third World R&D expenditure but they contain over 75% of the Third World's population. African countries contain 14% of the population and spend about 10% of the R&D resources.

The distribution of R&D manpower is more in favour of the Third World than the distribution of R&D expenditures. Annerstedt's study shows that developing countries had more than 12% of the world's scientists and engineers engaged in R&D, or 290,000 of the world's 2.3 million researchers. Three quarters of the Third World's R&D workers were active in Asia, about 10% in Africa (excluding South Africa) and the rest in Latin American countries. The latter, however, had the highest number of researchers per million economically active persons (EAP) that is, 460 R&D workers per million EAP.

But if all R&D personnel are included (that is, technicians and other supporting staff), then the Third World fares even better. The Third World, and in particular Asian organisations, have a higher number of technicians and supporting staff per researcher than the developed countries have.

Annerstedt believes that the distribution of overall scientific and technical manpower may be changing fast in the favour of the Third World. Educational expansion is faster in the developing than in the developed countries. In 1973, there were 4.6 million students graduating from universities and other institutions of higher learning all over the world. 23% of the third level graduates were from the developing countries (not considering those who were foreign students in developed countries and excluding China because of lack of reliable statistics). Among the twenty countries in the world with the highest total number of university graduates were seven developing countries: India, Brazil, the Philippines, Bangladesh, Democratic Republic of Korea, Egypt and Pakistan. The Philippines, Lebanon, Argentina, Venezuela and Chile had 54,500 to 48,050 third level students per million economically active persons. When compared in this way, they were only

Table 1: Distribution of world R&D expenditures among major regions and by average share of gross national product and per economic active person, 1973

	R&D expenditures: in mn US dollars	— in % of world total	per EAP in US dollars	in % of GNP at market prices
WORLD Total	96,418	100.0	66.4	1.97
DEVELOPING COUNTRIES	2,770	2.9	3.0	0.35
Africa (excl. South Africa)	298	0.31	2.8	0.34
South and Middle America	902	0.94	9.0	0.37
Asia (excl. Japan)	1,571	1.63	2.1	0.34
DEVELOPED COUNTRIES	93,648	97.1	182.1	2.29
Eastern Europe (incl. USSR)	29,509	30.6	160.0	3.82
Western Europe (incl. Israel and Turkey)	21,418	22.2	135.1	1.55
North America	33,716	35.0	331.1	2.35
Other (incl. Japan, Australia)	9,005	9.3	129.8	1.76

Source: Jan Annerstedt, On the present global distribution of R&D Resources, Vienna Institute for Development, Occasional Paper No. 79/1

Table 2: Distribution of researchers (R&D scientists and engineers) among major regions and per million economic active population, 1973

	Researchers total (000)	(R&D scientists and engineers): in % of World total	per mn EAP
WORLD Total	2,279	100.0	1,570
DEVELOPING COUNTRIES	288	12.6	307
Africa (excl. South Africa)	28	1.2	271
South and Middle America	46	2.0	461
Asia (excl. Japan)	214	9.4	292
DEVELOPED COUNTRIES	1,990	87.4	3,871
Eastern Europe (incl USSR)	730	32.0	3,958
Western Europe (incl Israel & Turkey)	387	17.0	2,441
North America	548	24.1	5,386
Other (incl Japan, Australia)	325	14.3	4,687

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behind the US, Canada, Israel and New Zealand.

In terms of sheer numbers, India claims to possess the world's third largest scientific and technical manpower, only behind the US and USSR. India had at the end of 1977, 2.3 million people with qualifications in science, engineering, medicine and agriculture. In April 1976, 54,000 professional scientists were engaged in R&D work supported by some 40,000 technicians and another 50,000 people engaged in administrative and non-technical jobs.

China too has a vast stock of scientific and technical manpower. Aqueil Ahmed of the Administrative Staff College of India believes that India and China should be alternatively either third or fourth in international comparisons of scientific manpower. The eight-year science and technology plan (1978-85) recently announced by the Chinese government aims to increase the number of professional R&D scientists to 800,000 (compared to the total Third World complement of 288,000 in 1973).

But the fact that developing countries spend so little (2.9%) on R&D even though they have a much larger proportion of scientists (12.6%), means that each R&D worker in the developing countries gets few resources to work with. Many scientists have, in fact, emigrated from the Third World to the developed countries complaining that they do not enjoy adequate support. □

Military research the big spender

MORE than a third of the global research and development budget is spent on military and space research, and less than a tenth on health and agricultural research, according to a report published this week by the Worldwatch Institute in Washington. In addition, over 95% of R&D funds are spent in the industrialised countries; yet these contain less than 20% of the global population.

The report, written by Colin Norman, a senior research fellow at the institute, covers ground similar to the Annerstedt report (see left). Norman says that imbalance in research priorities — both between subject areas, and in meeting the needs of the rich rather than the poor — urgently need to be reordered. It points out, for example, that the US Government is expected to spend about $670 million in 1979 on research and development aimed at improving the productivity of American agriculture; and that this sum far exceeds the agricultural R&D expenditures of all the developing countries put together.

"The worldwide distribution of R&D capacity closely matches the global distribution of economic power," says the report. The disparity applied both to financial and manpower resources. It had been calculated, for example, that during the early 1970s developing countries had about 300 scientists working on R&D for every million workers, compared to the developed countries, which had 4,000 scientists for every million workers.

The report says that the world's research and development priorities need to be reordered by, for example, channelling more money into neglected research areas, new research organisation and Third World laboratories, as well as encouraging the increasing expenditure by industrialised countries on research appropriate to developing country needs, and stimulating co-operative research efforts between the developing countries themselves.

However, the report warns that such efforts will not, by themselves, be sufficient to solve the world's major problems. "Many tasks are too urgent to wait for R&D to provide solutions and many cannot be solved by science and technology alone. Indeed when new knowledge is used to bolster and extend the power of governments, corporations and ruling elites, it can aggravate the social injustices that lie at the root of many of the world's most urgent problems," says the report. □

Knowledge and Power: The Global Research and Development Budget, by Colin Norman. Worldwatch Paper 31. Available from the Worldwatch Institute, 1776 Massachusetts Avenue NW, Washington DC 20036.

New US group calls for gene resource conservation programme

A DIVERSE group of scientists, concerned over the lack of support for efforts to protect genetic resources met recently in Berkeley, California, to form the National Gene Resource Program Co-ordinating Committee. The committee will initiate a train of events which, it hopes, will culminate in a national conference on gene resource conservation in 1981.

The committee's first action was to circulate a letter requesting other scientists and people concerned with bioresources to bring the problem to the attention of the federal government.

In an interview with *Nature*, the co-ordinator of the committee, geneticist David Kafton, elaborated on the goals of the group. "What we envision is making gene resources a high-priority national issue," he says. The task of the new committee will be to "provide a vehicle for effective action — not just talk."

Critics of the so-called Green Revolution have long pointed out the double-edged danger of over-reliance on just a few strains of essential crops: the chance that disease could wipe out whole harvests very suddenly and that further breeding potential could be lost forever as wild strains disappear. The devastation left by flare-ups of wheat rust, southern corn leaf blight and Dutch elm disease in the United States in recent years has illustrated this point.

Some action has been taken. The National Seed Storage Laboratory in Fort Collins, Colorado, has pioneered practical genetic preservation by storing and protecting seeds of more than 100,000 kinds of food and fibre plants. Bernard Finkle, one of the founding members of the new committee, and his colleagues at the Western Regional Research Laboratory of the US Department of Agriculture in Albany, California, have been experimenting with methods of storing in liquid nitrogen the individual cells of plants usually propagated by cuttings rather than seeds.

Such efforts, however, are too poorly funded to assure the preservation of genetic variability for even essential crops, Kafton says. He cites a memorandum sent last March to Secretary of Agriculture, Robert Bergland, by the National Plant Genetic Resources Board — an advisory body to USDA — outlining the need for greater effort in gene conservation. The National Academy of Sciences has also warned that the "genetic diversity for many species is severely threatened" and has recommended the setting up of a new agency for preserving germ plasms.

Meanwhile, an estimated 200 plant species disappear every year, particularly in areas of developing countries where land is cleared to raise fast-growing new hybrids only. Current levels of sample collection preserve only a small fraction of the existing natural diversity of crops and forests, Kafton and his colleagues insist, and too little research is directed towards finding new methods of conservation.

The letter they are now circulating — really a petition for action — calls for "the establishment of an effective and comprehensive conservation program to ensure the protection of this nation's irreplaceable and invaluable gene resources." Specifically they call for a national conference "whose primary task will be to initiate and design such a comprehensive program."

Technical support for the committee, and other action groups like it, is being supplied by a small, new, non-profit firm called Inquiring Systems Inc., of Berkeley. Loren Cole, an ecosystemologist, is executive director of the company, and David Kafton is one of the staff scientists. Kafton says that the new company is designed to work with groups like the new gene resource committee to stimulate action on social issues through such activities as organizing meetings, training personnel and publishing information.

John Douglas

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UN studies new science and technology fund

Keen to avoid an impasse at the United Nations Conference on Science and Technology for Development when it meets in Vienna next month, UN officials are studying the idea of setting up a new mechanism to support activities meeting the goals established by the conference in a way that is acceptable to both developed and developing nations.

The Group of 77 which is currently negotiating such issues on behalf of over 120 developing countries, has already proposed that an international 'financing system' for science and technology for development be established by the UN, largely financed by the developed nations and aimed to grow to $2,000 million by 1985, and $4,000 million by 1990.

Many developed countries, however, have pointed out the political difficulties that they would face in committing themselves to such a fund, both in terms of the size of the contributions involved, and the form in which it is proposed that they be made — namely as 'automatic' contributions assessed, for example, as a percentage of the balance of trade in manufactured goods with the developing countries.

What is now being sought is a formula that might eventually lead to a transfer of funds for scientific and technological activities of the same order of magnitude as the Group of 77 have proposed, but in a form which would be politically acceptable to the countries expected to be the major donors.

A possible way in which this could be done, according to one line of argument now being actively pursued in the UN and elsewhere, is through an initially relatively modest financial allocation — perhaps in the order of $250 million to $300 million — which could both provide some immediate additional support for science and technology activities and establish the basis for exploring the desirability and feasibility of scaling up to the higher budget levels.

The need to increase the finance for science and technology in development has been emphasized in a paper presented to the UNCSTD secretariat by the United Nations Development Programme. One of the suggestions made in this paper is that the conference should examine and, if appropriate, establish "international arrangements to generate and channel additional funds to developing countries . . . . to stimulate research and development activities in priority areas."

The UNDP suggests, for example, setting up an international financing facility which, at the request of a particular country, would support selected research projects in key areas, as well as the development, field testing, pilot production and dissemination "which are so essential in the conversion of research into widespread practice"

Such a facility, it says, would aim to ensure that adequate resources were channelled into key areas, and would arrange to recover its costs in whole or in part in sectors such as industry, where appropriate. It would also attract funds from non official sources, combining an intergovernmental mechanism with a mixture of public and private funds through co-financing.

"If the facility were both effectively managed and secure, it could attract, through co-financing, substantially greater levels of funding than are likely to become available from official sources alone. It would thus provide an important new source of support for a variety of critical links in the development process in many developing countries", says the UNDP.

Addressing a meeting of the development assistance committee of the Organization for Economic Co-operation and Development in Paris two weeks ago, Mr Bradford Morse, UNDP administrator, stressed the importance to developed and developing nations alike of a successful outcome to the UNCSTD conference, for substantive economic and political reasons.

He stated that the UNDP was prepared to upgrade its own scientific and technological activities in line with the general goals agreed by the conference. And he also suggested a possible scenario for agreement between UNCSTD delegates that would include

- The immediate provision of additional funds to support scientific and technological activities in developing countries;
- A committment by the developing countries to work out their most pressing needs in science and technology;
- A careful review of alternative financing mechanisms for meeting these needs, and how these could be built up; and
- Investigation of ways to ensure the adequate involvement of the scientific community in development efforts.

Speaking at a joint Senate and House hearing in Washington last week, Mrs Lucy Benson, US Undersecretary of State for Science and Technology, said that US negotiators at the UNCSTD preparatory committee meeting in New York last month had considered the Group of 77's proposal of a financing system was "somewhat unrealistic".

"We all feel that we will not be able to come up with what is considered an adequate sum of money" Mrs Benson told the congressional hearing. However, she said that members of the US UNCSTD delegation were waiting to hear more about the plans being discussed in the UN for a funding facility along the lines suggested by UNDP. and in particular were waiting for decisions to come out of the executive branch about the type of commitments that the US would have to make in Vienna.

Ambassador Jean Wilkowskizn, US co-ordinator for UNCSTD, told the meeting that there was a suggestion within the UN that a new science and technology facility, if established, might "very much involve the Arab nations".

Officials in Washington last week confirmed that, in principle, those responsible for allocating aid funds within the US are sympathetic to some new type of facility being established for science and technology, particularly since it might parallel on an international basis the type of bilateral activities proposed for the Institute for Scientific and Technological Co-operation (whose fate, following its rejection by the Senate two weeks ago, still hangs in the balance).

The rationale for the ISTC has already been successfully argued for within the administration. On an international level, many may feel that a logical place for such a facility would be the UNDP. Much will depend, however on what type of financial commitment the administration feels that it is able to make in both the short and the long term.

Meanwhile, some speakers at the congressional hearing used the opportunity to express their frustration at the apparent lack of initiatives which the US seemed to be proposing to take at Vienna. "Our objectives for Vienna are fine, but the effort we proposed is scarcely commensurate, unless things change in the next few weeks", said William Carey, executive officer of the American Association for the Advancement of Science.

Mr Thomas Pickering of the State Department, however, told the hearing that the administration had been studying a wide range of possible initiatives, and was making a final selection of those it considered most feasible. Prime candidates are thought to be plans for resources monitoring satellite systems for developing countries (using technology developed by the National Aeronautics and Space Administration), a proposal to assist in setting up a global information network, and plans to link ISTC — if approved — to similar institutions in other developed countries.

Finally, asked whether the developed and developing nations were on the same wavelength as far as negotiations for UNCSTD were concerned, ambassador Wilkowski gave a frank appraisal of the situation. "It came as a shock to the National Academy of Sciences when it realised that this conference is not about science and technology but is political, because it deals with developing country concerns about inequality and dependence. Those are the catalytic forces which have to rise to this conference. It is the desire to overcome them which led to UNCSTD." **David Dickson**

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UK energy minister backs conservation and nuclear power

"We must have a balanced energy policy, drawing on every one of the resources available to us", David Howell, Britain's Secretary of State for Energy, told a gathering to celebrate 25 years of the UK Atomic Energy Authority last week.

Mr Howell (above) backed nuclear power — without it, he said, the UK "would not have a realistic policy for meeting this country's long-term energy requirements". But he also stressed the need to tap coal, and conservation, "where savings of 30% or more are available". Mr Howell announced that he was reviewing the UK conservation programme "to see what reinforcement may be necessary in this key part of our energy strategy" □

China eases ideological grip

"CLIMATE" science in the last sixty years" is the title of an article appearing in a recent issue of *Hong qi* (*Red Flag*) written by Chou Pei-yuan — who is in charge of the Chinese Scientific and Technological Association.

Chou expresses his opinion on the further progress of Chinese science, and refers to the role of marxism. While no substitute for science proper, materialism and the dialectics of nature nevertheless constitute a powerful methodological tool in scientific research. He quotes the 'straton' model of hadrons in particle physics and the chemical synthesis of biologically active insulin as examples of advances made by Chinese scientists under the guidance of marxian ontology.

On the other hand, real scientific discoveries may also be made by experimentalists who are not consciously materialists and dialecticians. In the development of any discipline, the general policy should be "let a hundred schools contend", he says. Academic freedom includes the understanding that no scientific model or theory will be rejected, or supported, purely on the basis of politico-philosophical arguments. Lysenko-type mistakes, such as the uncritical refutation in the late 1950s of western psychology, must be avoided.

Chou also reviews the slow and uncertain development of science in China before 1949, when research was hampered by the lack of experimental facilities, and when significant original work was confirmed to geology, biology, palaeontology, archaeology and mathematics. He describes how Chinese science developed in the quarter century after 1949 with the socialist transformation of scientifgic and educational institutions. However, in the last few years, research of a fundamental nature has been largely neglected, and, he says this needs to be remedied quickly.

Chou's final point about the position of scientists is perhaps the most problematical. He mentions the phenomenon of being "hot at both ends but cold in the middle": that the enthusiasm for scientific modernisation among the top leadership and the rank-and-file scientists does not find its way to the mid-level cadres in some parts of the country. He argues that the majority of intellectuals in China are now part of the working class, and that more say in the administration and funding of science should be given to the scientists themselves. They should have the right working conditions so they can concentrate on research. Other problems which need attending to are the rational allocation of research duties to specialists and the implementation of arrangements to reduce the amount of time they spend on household labour. **T.B. Tang**

●China is to spend £3,500 million on cultural, educational, scientific research and health undertakings this year. Nearly half of it will be devoted to the running of institutes under the Academia Sinica and to maintaining scientific research various other departments, according to the latest state budget announcement. □

UK fellowship scheme approved — in principle

BRITAIN'S Science Research Council has given general approval to the establishment of a programme aimed at easing the critical staff stagnation problem which now affects the country's universities. However, its central council last week decided to call for more specific details before giving the final go-ahead to the scheme which would provide special release fellowships to help senior research workers vacate tenured posts that could then be filled by young scientists.

There are a number of drawbacks associated with the scheme but council members were particularly concerned that the programme, which could cost up to £450,000, could give universities an excuse to cut back on their own financing of science departments and transfer funds to other faculties. To prevent this, more specific regulations are now being prepared and the plan will be discussed by the SRC again in the autumn. □

Yugoslavia: no more oil burning

YUGOSLAVIA'S Federal Chamber last week passed an emergency law, banning for the time being any further investment in new power stations and heating plants based on liquid fuel. This move follows a report from the Federal Executive Council which noted that, as a result of increased OPEC prices, Yugoslavia's oil import bill for 1979 will be 37,650 dinars, some 72% higher than the original estimate. Furthermore, increased domestic consumption is unable to keep up with existing supplies, so that last month, for example, certain Yugoslav airports were closed for several days, due to a total lack of aviation fuel.

The emergency bill, however, did not have an easy passage. Originally scheduled for 27 June, it was held up for three weeks by the delegates from Vojevodina province, who demanded a special procedure and moratorium, claiming that the law would endanger the vital interests of the province, and that the situation did not justify a state of emergency. Vojevodina has small but promising oil resources of its own, and recent prospecting operations by the Naftagas enterprise suggest that in the future, Vojevodina may be able to supply up to 5% of the crude oil required by the Belgrade, Novi Sad and Pancevo refineries.

In spite of these discoveries, Yugoslavia's energy planners are rapidly rethinking their strategies to include new developments of the abundant lignite reserves and a re-emphasis on the country's coal mines, many of which have been closed in recent years. Nuclear power, too, receives a new emphasis, and it is hoped to commission one new nuclear plant a year, from 1990 onwards. However, additional funding for the first such plant, at Krsko, is still on the "pending" list of investment, while the proposed site of the second such station on Vir island has had to be abandoned, following protests by local residents and tourist enterprises. As an extraordinary measure, the station will now be built at Prevlaka, a village on the Sava near Zagreb, although Rade Pavlovic, head of the Croation Electricity Board, stresses that all such stations in the future will have to be built on the coast.

Vera Rich

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Water quality threatens Polish agriculture

Following an unusually severe winter and dry spring, Poland faces a short-fall in the harvest, a situation in which Party Leader Edward Gierek, meeting agricultural 'activists' earlier this month, could only advise them to "take a close look at every field and harvest as much grain as possible. We must not run round looking for possible places where grain can be bought. Firstly, the money is not there, and secondly, we cannot buy grain anywhere".

Although attributable in the short term to exceptional climatic conditions, this short-fall is a symptom of one of the major problems bedevilling the Polish economy, a chronic lack of water. Not only do many parts of the country have an overall deficit of water, even where there is abundent rainfall, there is unsufficient reservoir capacity to store it. In all, Poland's existing 140 reservoirs can hold only 2,800 million m^3 water, some 5% of the mean annual flow of the rivers (for comparison, Bulgaria stores 15%, Czechoslovakia 12% and the Soviet Union more than 14%).

Although the new Vistula Programme, formally inaugurated on 18 June 1978, is designed to remedy this situation to give a storage capacity for the Vistula of approximately 6,000 million m^3, in addition to modernizing the river's transport facilities and building hydroelectric stations. However, provision of water is not sufficient. The Vistula is now so polluted that for much of its length, the water is not even up to the 'second class' of cleanness required for agriculture. A spokesman for the Programme, told *Nature* that "the main purpose of the Programme is to provide the nation with pure water. All the other aspects, transport, hydroelectricity etc, could be replaced by some other means. But without pure water there is nothing we can do."

Like all major technological undertakings in Poland, the Vistula Programme draws heavily on scientific potential throughout the country. Virtually all major Universities and Polytechnics are involved, especially Warsaw, Krakow and Wroclaw. Coordination is the responsibility of the Academy of Sciences.

Although work on the Vistula Programme is not due to start until 1981, the extreme urgency of the situation has led those involved to anticipate the official inauguration. A few administrators have already moved into the half-built office block in the Warsaw suburbs assigned to the Programme, while on the river itself preliminary dredging operations are already in operation. Yet even at this early stage, the engineering operations seem likely to be held up by environmental problems.

Plans for the river include floodwalls which will enclose the present channel and the water-meadows susceptible to flooding; dams will then be built between these floodwalls to create reservoirs with a minimum depth of 2m. The Institute of Ecology of the Academy of Sciences has decided, however, that no dams should be built until the water in that area is up to second class standard, a demand of unparalleled severity.

Cleaning up the Vistula to an extent where largescale engineering operations can commence will involve a considerable number of interests. In addition to the routine problems of industrial outfall and domestic sewage, there is heavy mineral pollution from the mining and industrial belt of Silesia, to say nothing of the Nowa Huta Steelworks near Krakow, where the ecological price of the annual steel output of 7 million tonnes is an emission of 100,000 tonnes of dust and some 70,000 tonnes of SO_2.

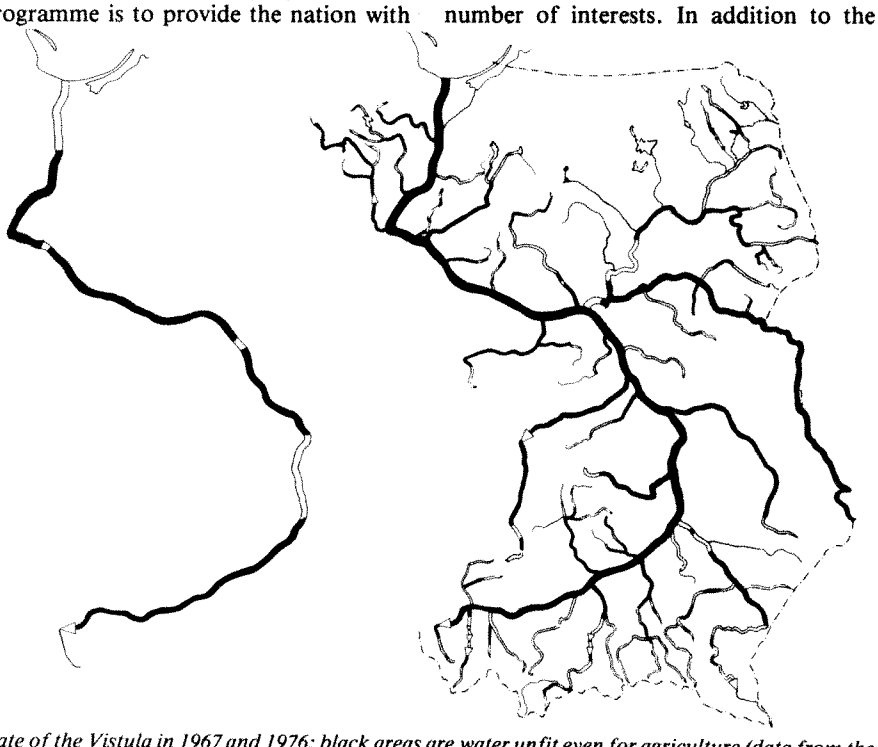
Many ecologists concerned with the Vistula Programme see this indirect pollution *via* soil and air as a greater problem than that of wastes discharged into the water, which can, at least in principle, be dealt with by improved sewage, recycling, oil-trap and purification systems. The Academy's Environmental Engineering Institute (situated appropriately, at Zabrze in Silesia), is working on a new electric filter, now at the pilot model stage. Filters and precipitators have in fact installed on all new furnaces at Nowa Huta since the mid-1960s; of them, Professor Roman Andrzeiewski, of the Environmental Engineering Institutes Krakow office simply observed "The filters would be fine if they had an efficiency of 90%". What is really needed, he added is "a technology without dust and without gas!"

Until that utopian situation is achieved, the Institute of Ecology is trying to work out some ecological solution to the pollution problem. In Silesia, valuable data have already been collected on the uptake of minerals. Although it is far too early to propose a general strategy, some partial solutions have been suggested. Zinc pollution, for example, can be remedied by planting birch trees, which can apparently accumulate zinc without harm. Similar methods, say the researchers, can also be applied to agricultural run-offs.

To avoid eutrophication of the future reservoirs, they propose filter zones of live ecosystems on either side of the river. Alder woods are particularly valuable, as too is grass-land, which can profitably be used for grazing. Preliminary studies on the disposal of agricultural chemicals by such filters is going forward in the Mazurian lake disrict. Not only the absorption by flora is being investigated, but also the uptake and "export" of the chemicals *via* the small fauna.

But, faced with a pressing lack of water, as highlighted by the current harvest estimates, will the planners be prepared to wait? It is not, of course, the specific task of the Institute of Ecology to hold them off — but the consensus of opinion seemed optimistic. "We asked the engineers about this last year", said one ecologist, and they said that they would never put a dam where it would make the water quite dead!" "We fell sure that the planners will do what we suggest," said another, "they are actually trying to improve their contacts with us".

Vera Rich



State of the Vistula in 1967 and 1976: black areas are water unfit even for agriculture (data from the Instytut Ksztaltowania Srodowiska, Wroclaw)

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news in brief

Army worms on the march: Reports from a number of sources indicate that army worms, second only to locusts as a threat to agriculture over much of Africa, are occurring in very large numbers this year in West and East Africa and in the southern Arabian peninsula. Army worms (pictured right), larvae of the Lepidoptera genus *Spodoptera*, attack food, especially cereals, fodder as well as cash crops such as cotton. But in East Africa the most serious damage may be to grazing. In earlier outbreaks, the pest have tended to move northwards in a series of waves and may cause a cattle famine anywhere from Transvaal to southern Ethiopia.



CO1: Crown copyright

The current situation adds urgency to research on the possibility of using the *S exempta* virus as a pesticide. This research was held up last year because no one could locate any army worm outbreaks. Use of *S. Exempta* is the second stage of the virus-and-pheromone concept, the first stage of which, directed against the Mediterranean army worm, (*Nature*, 7 December 1978) has now reached the stage of extended field trials on 5,000 acres in the Egyptian Fayoum — *from Peter Collins in Geneva.*

Soviet call for co-operation on global problems: Academician Dzherman Gvishiani, deputy head of the Soviet Union's State Committee for Science and Technology, has called for a "concerted approach" to the solution in Europe, and ultimately the world, of such major problems as environment, population, water resources, health care, and desertification. Writing in *Pravda*, Gvishiani stressed that such problems can no longer be solved by the efforts of one country alone. In this he differs markedly from Academician Anatolii Aleksandrov, chairman of the Soviet Academy of Sciences, who averred last April that "there is no scientific-technical problem that would not be within our power." Gvishiani, urges the setting up of all-European or inter-state forums to help turn the Helsinki accords into specific action for environmental protection, the development of transport, and energy.

Fire at Windscale causes radiation exposure: Eight workers suffered radiation exposure when a fire broke out on 17 July at a decanning cave at British Nuclear Fuel's Windscale reprocessing plant. The most serious case was 0.3 rem exposure, the equivalent of 10 chest X-rays. This is below the unit of 3 rem in any 13 weeks set for radiation workers by the International Committee for Radiation Protection. The fire occurred when a piece of irradiated fuel rod became jammed in a fuel assembly. Dismounting machinery failed to remove the jammed rod and the fire started, presumably due to friction between the assembly cover and the uranium rods. The fire was extinguished after 45 minutes by flooding the area with water manually through access hole in the top of the cave. Numerous questions remain to be answered about the incident, including the reason for the escape of material and the failure of safety provisions to prevent the event. Community representatives on a BNFL community liaison committee have called for a full investigation "on behalf of the local people and not as a public relations exercise for BNFL".

Czech scientists await trial: Five scientists — mathematician Vaclav Benda, psychologists Jiri Nemec and Dana Nemcova, and (engineers) Ladislav Lis and Petr Uhl — are among ten Czech human rights activists now awaiting trial for "subversion on a large scale". In plain terms, their offence is their membership of VONS, the "Committee for the Defence of the Unjustly Prosecuted", a subgroup of the Charter 77 movement. Since its inauguration, the committee has issued 113 statements on alleged breaches of justice and legality. The ten detainees were arrested on 29 May, and their trial is expected in the next few days. If convicted, they face prison sentences of up to 10 years.

No future for nuclear power plants in Iran: "Only a miracle can rescue both nuclear power stations" an official of the West German engineering company Kraftwerkunion GmbH said last week. He told *Nature* that work permits for the last 50 members of Kraftwerkunion staff will expire by the end of this month. Earlier in the year, there were still hopes that the 6.9bn works would be finished by 1981 the contract was 80% completed. But influential officials are taking a strong line against any nuclear power stations in Iran, and Kraftwerkunion now sees little hope of a resumption of work. The prospect of the plant's cooling towers ending up as grain silos now appears far more likely than before. — *Klaus Höpfner.*

ILL ready for its "deuxième souffle": The renewal programme for one of the world's leading neutron research facilities, the Institut Laue-Langevin in Grenoble, is close to final approval. Funds for ILL's "deuxième souffle" — amounting to 104 million French francs to update instruments and techniques — were approved by the member countries, Britain, France and Germany on 25 May. But because of prior French budget commitments, the detailed spending for the five year programme has been postponed by the French until October. ILL Director John White calls the 25 May approval "extremely good news" and is optimistic that the French difficulty will be resolved.

Jupiter space shot survives budget threat: Project Galileo — NASA's plan to send a joint probe and orbiter mission to Jupiter in 1982 — has survived a threat from a key Congressional subcommittee, some members of which had been threatening to cut off funds to help pay for overspending on the space shuttle. The threat to kill the project came primarily from Senator William Proxmire, chairman of the Senate appropriations Subcommittee responsible for the budgets of both NASA and the National Science Foundation. Senator Proxmire, no great friend of the basic science community, had also suggested that about $20 million be taken out of the budget of the proposed space telescope. However, at a meeting last week the subcommittee refused to go along with either of these proposals, and agreed to recommend to the full appropriations committee that NASA be granted the full $3,822 million in the President's budget request for the fiscal year 1980. Within this total, however, the subcommittee recommended that budget cuts totalling $17 million be allocated to five new projects, including preliminary design studies for a national oceanographic satellite system (a proposed successor to SEASAT), and a multispectral resource satellite.

Islamic technical training centre opens in Bangladesh: An Islamic Centre for Technical and Vocational Training and Research (ICTVTR) began work at Dacca last month, with a board of directors including representatives of Saudi Arabia, Libya, Iran, Iraq, United Arab Emirates, Turkey, Indonesia, Senegal, Gambia, and Bangladesh. The Centre will be built on a 30 acre site provided by the government of Bangladesh at Tongi, 10 miles north of the capital. It will cost $9.6m and is expected to be completed by April 1983. Saudi Arabia has already announced a grant of $2m for the centre. ICTVTR has drawn up a programme for a ten month interim training course in machine shop technology for 50 instructors; and to meet the technical manpower needs of Islamic countries it will undertake training programmes in chemical and mechanical technology, design and drafting, computer programming and technical innovations in oil and gas. — *from M. Kabir in Dacca.*

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Brazil: is it really safe for the scientists to return?

Brazil's stern leader, President Ernesto Geisel (right), has relaxed some of the restrictions on academic freedom that forced many scientists to leave the country. **Maurice Bazin** reports on the sceptical reaction among the exiles.

THE Brazilian government has recently proclaimed that it will create a gradual democracy and re-establish the right of *habeas corpus*. With this emerges the possibility that some exiled Brazilian scientists may return permanently. Although many exiled scientists have returned on visits in recent months, it is not clear whether the military government really wants them or if they are being tolerated only temporarily. These scientists had been engaged in creating respected research institutions in the early 1960s, but their work was nullified by arbitrary firings and forced retirements imposed by a government afraid of 'intellectual subversion'. As a result, many have been forced into exile, contributing involuntarily to the very 'brain drain' which their earlier efforts in Brazil aimed at eliminating.

A point of convergence for all scientists, both in exile and in Brazil, is the annual meeting of the SBPC, the Brazilian Society for the Progress of Science, now taking place in Fortaleza, a coastal city in the north-east of Brazil. The motto of this year's meeting "Sing there while I sing here" shows how prominent the theme of reunification has become within Brazilian scientific circles.

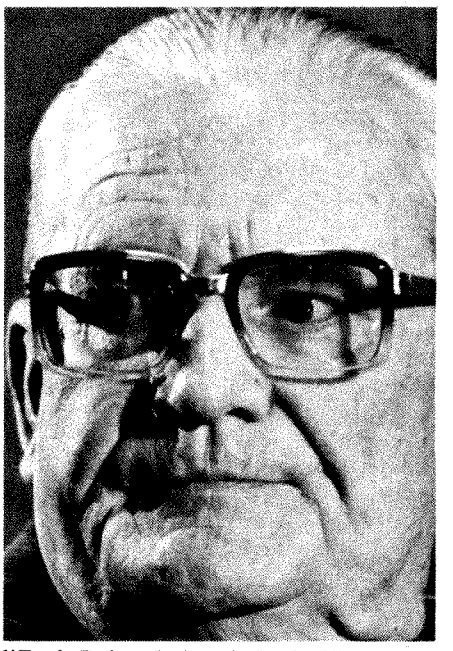
Meetings of the SBPC in past years have been marred by conflicts with the military authorities who wanted to restrict their impact. Two years ago the government forbade all scientists working in federal institutions or using government funds from participating. The meeting's venue should have been the Federal University in Fortaleza, but it was forced to move to a private institution, the Catholic University in São Paulo.

In a well-publicised 'Declaration of Scientists to the Brazilian People', approved by acclamation at the 1977 meeting, the SBPC asked that "scientists fired through dictatorial decrees be reinstated." The declaration also stated that the SBPC "repudiates all censorship of artistic, literary, journalistic and scientific activity. In particular, it repudiates the recent decision of the government to impose censorship upon foreign publications. We see this as a retrograde action, negating the universal character of knowledge and forcing the cultural isolation of Brazilian intellectuals." On its 30th birthday, last year, the SBPC ironically asserted: "the revolution of 1964 was a great stimulus to our economy; the firings and deportations of 1968 and 1969 were also. Each exiled professor costs the country 50 thousand dollars and another 50 thousand to import another who is not *non grata*."

The fact that this year's meeting will finally take place in Fortaleza is a positive step in the process of liberalisation but how many exiled scientists will attend and how many would resettle in Brazil are very much open questions. According to Professor Rocha e Silva, a founder of the SBPC, "some scientists are now living much better in foreign countries. The problem is not of an amnesty, but to convince those who receive the amnesty to come back." For others, however, it is not a question of their standard of living, but doubt about the liberalisation process itself.

"It is ironical that I should return to my country on the occasion of the SBPC meeting; it was during the Society's meeting in 1964 that I was arrested," says Professor Luis Pereira da Silva who now directs the Department of Parasitology of the Institut Pasteur in Paris. "I am going only to renew contacts with the Brazilian scientific community and meet old friends again." In 1964 Pereira da Silva was Assistant Professor in the Medical School of the University of São Paulo; his work on Chagas disease and the chemotaxis of tripanosomes had already received international recognition. He and eight colleagues were fired, with no justification given, under the First Institutional Act, a dictatorial decree of the military regime. At the forthcoming SBPC meeting in Fortaleza he will chair a discussion session entitled 'Who should be responsible for the orientation of scientific research'.

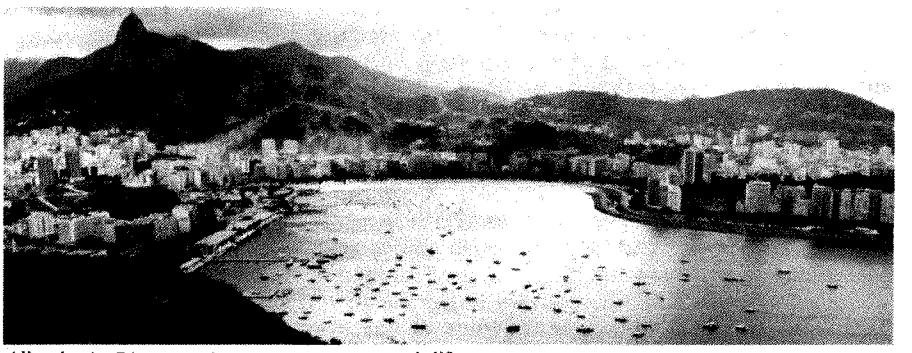
How did Brazil suffer such a massive loss of scientists in the mid-1960s? "We were all free intellectuals, independent thinkers. We wanted to set up modern institutions on a standard academic basis", says Roberto Salmeron, now Directeur de Recherche in high energy physics at the Laboratoire de



l'Ecole Polytechnique in Paris. He was one of the group who conceived the idea of the University of Brasilia, and in 1965 he was coordinator of its Science Division. "We wanted a democratic way of running a university. We used the word 'coordinator' to avoid the authoritarian 'director'. The university was to be a true community and teachers and students were to reside on campus — a very new situation for Latin America."

While Salmeron was trying to build up the science departments, the Rector of the university was changed twice. "The authorities named persons who had the confidence of the military and who were totally subservient. One day the Rector told me that I could not hire a certain professor; he had been called in by unspecified superiors and warned that my candidate was an 'agitator'. Our lectures were taped secretly and we could be asked to explain what we had said in class. Even the names of the composers whose magic was to be played at free public concerts on Saturdays had to be cleared in advance; a conductor was called in for having scheduled a Prokofief work.

"Although the government started dismissing professors during the very first month of the military regime (13 social scientists were dismissed in March 1964) we succeeded in building up a Division of Science with 150 personnel by 1965." The



All calm in Rio — or is it just a temporary lull?

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process of confrontation escalated when a new Rector announced that he would dismiss six other professors. "In spite of our efforts to explain directly to the highest military authorities the value to our country of a modern university, and despite the fact that their sons and daughters were attending it, those in power remained completely insensitive." The result was a series of assemblies of teachers and students, with talks of strikes; finally the army took over and sealed off the campus.

"Nobody could enter the campus, and all our research animals and the flies of the geneticists were going to perish. I alone obtained an authorisation, which read: 'Professor Salmeron is authorised to enter the grounds of the University of Brasilia'. I have kept this piece of official democracy to this day." Finally, towards the end of 1965, as the Rector demanded that Salmeron countersign the firing of not six but 16 scientists, he resigned instead and made public 250 other individual letters of resignation that his colleagues from other departments had prepared. "For five months, we tried to find work as a group of physicists in other Brazilian universities, away from the centre of power that Brasilia represents. We did not succeed and I finally came to Europe, accepting an invitation of Victor Weisskopf, then General Director of CERN."

'I have no confidence in this political overture'

As to whether he will return to Brazil now, Professor Salmeron says "I do not understand what is going on in Brazil. I have no confidence in this political overture — people only do what they are allowed to do. There is some influence coming from the intellectuals, but in the case of the nuclear agreement with West Germany, the influence of the scientists was zero. They were told only after things happened. One should not have illusions. I can only imagine going back in the distant future, and it would depend on the existence of a real stable change in the Brazilian situation which would allow me to undertake projects with a perpsective. I shall not drop everything in Paris to take the risk of losing everything again."

Most other senior scientists who suffered the persecutions and destructions of the past hold the same cautious opinion. Professor Pereira da Silva explains that he "met twice with the fate of losing everything after building a research group; it's already too much." After his first exile in 1964 he agreed to return to a medical school in São Paulo, leaving behind the work on lambda phage on which he was working with Nobel prize winner François Jacob. "This was in 1968 when there appeared to be a political lull. But after nine months spent organising a new laboratory in microbial genetics, I and my colleagues were fired once more, and I went back to the Institut Pasteur."

Another decree of exception, the Fifth Institutional Act, was used in 1969 to fire 25 professors from the University of São Paulo and 44 from the Federal University of Rio de Janeiro. Among the latter was physics professor Jose Leite Lopes, who had also gone back to Brazil in 1968, to the Federal University in Rio. He was also reappointed director of the Theoretical Division of the CBPF, the Brazilian Centre for Physics Research, to which he had previously attracted Richard Feynman and C.N. Yang on sabbatical. Federal funding for physics research had improved substantially in the meantime. But in April 1969, Professor Leite Lopes heard on the radio that he had been dismissed from the University of Rio. "The 44 names were read in alphabetical order; you only waited for yours to come up. That was the end."

A few months later, while lecturing in Pittsburgh, he learned of his further dismissal from the CBPF. "Four colleagues resigned in protest; I got telegrams of support; the SBPC and international societies protested. It was all very comforting at the time. Then there was a lull, to be followed by 12 more firings in 1972 . . . .". He now heads the Theoretical Division of the Nuclear Physics Laboratory in Strasbourg, and says "I shall be going back this summer for the first time in ten years, at the invitation of the physics department of my former university in Rio. Beside this I want to see what it is possible to do, and to discuss ideas. It looks from here as if some progress is being made and I am eager to see how far it goes."

Dr Julio Pudles in Paris takes a still more hesitant attitude. "I was among the nine doctors fired from the Faculty of Medicine in São Paulo in 1964; I was also part of the group which prepared a model for the University of Brasilia in 1963. I was one of those found innocent in our trial in

How the military banned 'exotic ideas'

The life and death of the Oswaldo Cruz Institute in Manguinhos is typical of the long-lasting unstable interaction between scientific institutions and government in Brazil. The Institute was created at the turn of the century, and its first director, Oswaldo Cruz, received his initial training at the Institut Pasteur in Paris. At that time Rio de Janeiro was a swampy, dirty place, harbouring all kinds of epidemics and chronically affected by yellow fever.

In 1902, Oswaldo Cruz publicized Finlay's discovery that the mosquito *Aedes aegypti* was a transmitter of yellow fever, and coordinated a public sanitation plan to eliminate it. Although this plan succeeded in eradicating the disease within a few years, official resistance was great and Cruz was often ridiculed in the press, and even in Congress, for bringing in "exotic theories".

Two years later, Cruz proposed to institute compulsory vaccination against smallpox. A virulent Antivaccination League immediately emerged, bringing together military men, workers' organisations and old-fashioned medical surgeons — whom the young scientists at Manguinhos described as "always wearing dark suits and using their medical authority to declare quarantines and sign autopsy reports in times of epidemics." Their opposition to compulsory vaccination was in the name of individual freedom of conscience: "vaccination was forced upon us and we may even use force against it. This government must be rejected by the people." Partly as a result of the League's campaign, military cadets rebelled in 1904, in an attempt to bring down the first republican government of Brazil. The government, however, won the day and the combined medical and military victories led to dramatic increases in funding for medical research at Manguinhos. In the following years malaria was intensely studied; Carlos Chagas identified the *Trypanosoma crusis* and left his name attached to Chagas disease. Serums and vaccines of all kinds were developed.

"Today," says Professor Ubatuba, "all these achievements seem to be forgotten. That house of science at Manguinhos was destroyed in 1970 when ten of us were forcibly retired. Furthermore, eight of us had our political rights taken away for ten years as stipulated in the Fifth Institutional Act, the act which closed down Congress." The tone had been set by the Health Minister of the military government who had declared, when a new director of the institute took office: "if it is true that there are no frontiers for science, it is also certain that there are frontiers for scientists. The exotic ideas that have infiltrated Manguinhos will be definitely banned . . . Manguinhos of the future will be a busy hive and not what a few wanted, a centre of subversive ideas. The institute will have all the support it needs for its research."

Professor Herman Lent, one of the eight, and former member of the Department of Zoology, recalls in his book *The Massacre of Manguinhos* that the director of the institute had accused its researchers of "conspiring in their laboratories".

A commission of inquiry, headed by a military man, did not find any evidence but it praised the director who "although a poor professional and a poor administrator is a reliable anticommunist". **M.B.**

1966, and I fought unsuccessfully for six months to recover my university post. Dr Pudles would like to go to the SBPC meeting in Fortaleza but hesitates because of the cost. "I paid my own way in 1966, all for nothing."

He sees the SBPC as a remarkable society which has consistently combated arbitrariness and shown tremendous dignity in its support of scientists. But he expresses doubts about returning for good. "My bitterness from the past is very deep. The university may let us return, but with the same positions we had in 1964. We'd find there those same people who wrote anonymous letters denouncing us to the military. When you are over fifty, you cannot run the risk of having to look for a job in France again. France accepted me three times in the past, but the new legislation concerning foreigners working in France is very tough and makes it almost impossible for anyone coming anew to stay permanently."

Passport problems stop some from returning

Some Brazilian research institutions have been modified so much over the past fifteen years that exiled researchers cannot imagine going back. Professor Fernando Braga Ubatuba, a pharmacologist working at the Wellcome Foundation near London finds himself in this situation. "The research institute of Manguinhos in Rio de Janeiro where I worked did fundamental research in medical science with departments of microbiology and parasitology of great renown, but today it has been transformed into a mere generator of vaccines. (see box)

"We all rejoiced on New Year's day this year when President General Geisel revoked the Fifth Institutional Act, and I have recently been back for the Congress of the Latin American Society of Pharmacology. But the case of my colleague Professor Haity Moussatche, now teaching in Venezuela, is more difficult, because our government refuses to renew his passport."

The problem of passports always comes up in the interviews. Those unable to obtain one had to take refugee status in their country of residence abroad. "Now the Brazilian government demands that we first renounce our refugee status before it considers whether it will give us passports," says Alice Rivera of the Centre for the Study of Weak Radioactivity in Orsay near Paris. "I would like to go back but I do not believe I could find work in Brazil now, although my only crime is to be married to an ex-deputy whose mandate was annulled when the military took over in 1964." □

Maurice Bazin is a physicist and science journalist specializing in Latin American affairs.

Can we afford to make the fast reactor safe?

The safety of fast reactors is more difficult to guarantee than that of thermal reactors, so the cost of making them safe is likely to be high. Norman Dombey, of Sussex University, argues that international collaboration is the only way to proceed.

FAST reactors, in particular the liquid metal fast breeder reactor (LMFBR), differ substantially from thermal reactors; indeed as a physicist I take the view, based on the intrinsic physics of the systems, that fast reactors should be considered as a different genus from thermal reactors.

A discussion from this viewpoint illustrates some of the problems of fast reactor development. In this article I should also like to introduce to non-specialists some of the features characteristic of fast reactors, as this information is not readily available outside the technical literature, and finally, I shall draw some conclusions for fast reactor development generally and for the British programme in particular.

The main physical differences between thermal and fast reactors are given in Table 1, the most important point being the last: thermal reactors are designed to be in their most reactive nuclear configuration. This follows from the presence of a moderator without which the natural or slightly enriched uranium fuel of the reactor would not go critical at all. The corollary of this for fast reactors is that as there is more than one critical mass of fissile material in the core of a fast reactor and no moderator, it is not difficult to envisage a rearrangement of fuel to provide a more reactive configuration. As the reaction goes by fast neutrons, it is possible to attain a prompt critical reaction with a large energy release over a very short time. (About 0.2% of the neutrons in a fast reactor are delayed, i.e. come from radioactive daughter nuclei with various half-lives of the order of one second; it is these delayed neutrons which allow a reasonable control of the fast reactor because the prompt neutrons are captured in about one microsecond. A fast reactor is therefore designed to be just critical including the delayed neutrons, but not critical without them; a prompt critical reaction is one which is critical by prompt neutrons alone.) A large energy release over a short time is called a nuclear excursion and cannot occur in normal types of power-generating thermal reactors.

Another important point in this comparison follows from the absence of a moderator in the fast reactor, which allows the fast reactor core to be much smaller than a thermal reactor of the same power. The power density of a fast reactor is about five times that of a light water reactor (LWR). (We should remember that LWRs themselves have a power density about ten times higher than British gas-cooled reactors.) So the coolant used in a fast reactor must have very high thermal conductivity and all the present prototypes use liquid sodium.

It is a job for the licensing authorities in each country to consider hypothetical accidents and to insist on measures to minimise the risk of accidents, and to minimise and contain their effects if they do occur. The discussion of hypothetical accidents begins with an analysis of the reactivity coefficients of the reactor. These coefficients measure the change in reactivity of a reactor as the physical state of the reactor changes. The important coefficients for safety purposes are those related to an increase in power and temperature of the reactor: if the power or temperature increases, a negative coefficient implies that the reactivity will decrease. As commercial thermal reactors operate in the configuration of maximal reactivity, all nuclear reactivity coefficients in these thermal reactors will be negative. For fast reactors, the reactivity coefficient which allows these reactors to be considered as potentially suitable for commercial use is the Doppler coefficient, which measures the change of neutron reaction rates with temperature in the reactor materials themselves. For uranium 238, neutron absorption increases strongly with temperature and hence, there will be fewer neutrons available for fission, so the Doppler coefficient is large and negative.

The fissile material in the core will be plutonium or ^{235}U; for the latter the Doppler coefficient is positive in the same temperature range whereas for plutonium it is small. So in a fast reactor of the design envisaged in CFR 1 or Superphenix with about 20-25% plutonium and 75-80% natural uranium (99.3% ^{238}U) in the fuel rods, there will be no overall negative Doppler coefficient.

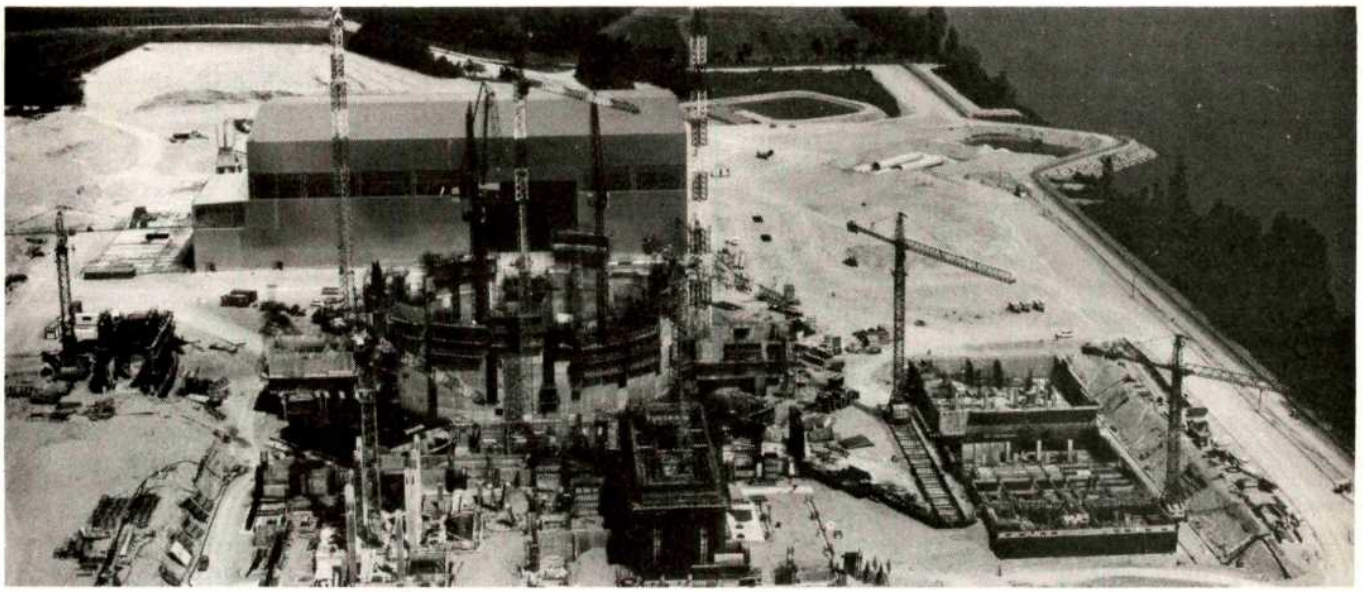
It is important to emphasise that the Doppler coefficient, although dependent on core geometry, is primarily an intrinsic nuclear quantity. It depends on the nuclear composition of the fuel used and its

Table 1 Primary differences between thermal and fast reactors

Thermal	Fast
natural or slightly enriched (2-3%) uranium fuel	20-30% enriched uranium or plutonium-uranium fuel
less than 1 critical mass	more than 1 critical mass
moderator	no moderator
thermal neutrons, $\frac{1}{40}$ eV	fast neutrons, keV-MeV
most reactive configuration for nuclear reaction	not most reactive configuration for nuclear reaction

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Superphenix, Europe's largest (1200 MWe) fast breeder reactor, under construction at Creys-Malville, France.

arrangement in the core; once an acceptable configuration is found, there is no reason why this configuration should not be scaled up to a larger reactor design.

The second most important reactivity coefficient in fast reactors is the sodium void coefficient. Although fast reactors do not have a moderator, the sodium coolant flows in the core and scatters the neutrons, so to some extent it acts like a moderator in slowing down the neutrons. It also absorbs some neutrons. If the core were to overheat, the sodium could begin to boil; the void coefficient then measures the change in reactivity of the core caused by bubbles of sodium gas forming in the liquid sodium flowing through the core.

Here there are two competing effects. First, sodium gas would absorb and scatter fewer neutrons than liquid sodium, so there would be more neutrons of higher energy and the reactivity would tend to increase. Second, the presence of sodium bubbles would increase the probability of neutrons escaping from the core through the bubbles, so this tends to decrease reactivity.

The first effect leads to a positive void coefficient and is proportional to the volume of sodium in the core, whereas the second effect is an edge effect which is proportional to the effective surface area of the sodium. So for small reactors, where all the sodium can be taken to be near the surface, the edge effect wins and the void coefficient is negative. This was the case for the old 15 MWe Dounreay Fast Reactor. As the reactor gets larger, the volume effect will begin to dominate and the present prototypes such as the Prototype Fast Reactor (PFR) or Phenix have a positive void coefficient, albeit small. The planned CFR 1 or Superphenix, however, will have a large positive void coefficient, about five times larger than that of the present prototypes. So scaling up from a 250 MWe prototype to a 1300 MWe 'commercial' reactor is not simple, because the problems connected with voiding get much worse.

In addition to a comparison of reactivity coefficients, three other factors need to be considered in any comparison of fast reactors with thermal reactors: the fuel-coolant interaction, other non-nuclear problems arising from the use of liquid sodium as coolant, and the general problem of recriticality.

The fuel-coolant interaction is not a nuclear effect: it is the potentially explosive interaction arising when a molten metal comes into contact with a liquid at a temperature much above the boiling point of the liquid. It is well known in the steel industry from incidents where molten steel has come into contact with water, and it is also a problem inherent in water-cooled thermal reactors, as it has been known for a piece of hot fuel rod to melt and interact explosively with the surrounding water. For LWRs, however, the fuel-coolant interaction is less critical than in large LMFBRs because there is an overall negative coolant void coefficient.

The best known difference between thermal reactors and the LMFBR stems from the difficulties of using liquid sodium as coolant in the LMFBR. Sodium is, of course, highly corrosive and interacts vigorously with both air and water on contact. All those large prototypes at present operating (PFR, Phenix and the Soviet BN-350) have been plagued by sodium leaks.

In fast reactors there are also recriticality effects to consider. As the core is not in its most reactive configuration and there may be hundreds of critical masses of plutonium in it, a disruption of the core may lead to a much more reactive configuration; for example, a coolant channel gets blocked, the sodium boils, the reactivity increases because the void coefficient is positive, the cladding of some fuel rods melts and pieces of fuel containing plutonium fall and form a molten mass of plutonium which if large enough can then become prompt critical leading to a rapid release of energy.

The analysis of the energy release and the pressures created by such hypothetical core-disruptive accidents is being pursued in all the countries engaged in fast reactor technology. Enormously complicated computer programs are being prepared to simulate these conditions. Whether or not the present programs are reasonably accurate in describing fast reactor accidents, it is clear that the role of negative reactivity coefficients will be to limit the energy release whereas positive coefficients will enhance it. Thus for a reactor of the size of PFR or Phenix with uranium-plutonium fuel, the energy release for a core-disruptive accident of the type described is not expected to be larger than 400 megajoules (equivalent to about 200 lb of TNT). But in a reactor the size of CFR 1 or Superphenix, the sodium void coefficient is much larger and positive so the energy release for a similar accident could be 2000 megajoules (1000 lb of TNT).

Now the point is not that fast reactors, or large fast reactors, will blow up — it is the job of the licensing authorities after all to ensure the safety of such systems. But the licensing authorities will insist

- on minimising the risk of an accident
- on limiting the damage to the reactor core in the event of an accident
- on maintaining the integrity of the primary reactor system, even in the event of a core-disruptive accident leading to a large energy release
- even in the worst case of an accident breaking the primary system, that radioactive materials are contained.

These are the four lines of assurance of the US authorities, but the practice of the British Nuclear Installations Inspectorate should be similar. The point that I want to make is that measures to deal with the safety and other engineering problems specific to fast reactors will add appreciably to the capital costs of fast reactors compared with thermal reactors of a'

similar size. Yet the goal of a commercial fast reactor system is to produce cheaper electricity than other systems such as thermal reactors. Fuel costs play a minor role in the total cost of nuclear electricity, so that fast reactors will only be competitive with thermal reactors if their capital costs are comparable (unless the price of uranium were to increase dramatically). The special safety requirements of fast reactors and the difficulties of using sodium in LMFBRs both suggest that the capital cost of a LMFBR will be much higher than a comparable thermal reactor. Furthermore, as has been indicated, the safety problems get worse for large fast reactors. It may be thought that my comments are overly pessimistic or overly theoretical — I am after all a theoretical physicist. But the one case study available on the capital costs of fast reactors does indeed bear out my conclusions.

The prototype German fast reactor SNR-300 has increased in cost (at constant 1972 prices) from the initial estimate of DM300 million at the time the reactor was authorised to the most recent estimated cost DM1223 million in October 1975 (O.Keck, University of Sussex Doctoral Thesis, 1977). These are not the final figures and the cost escalation is therefore by at least a factor of four in real terms. Keck also demonstrates that the initial cost estimate of the SNR-300 was in no way based on any evidence about the likely capital costs of fast reactor systems. The estimate was simply a number which the fast reactor proponents thought to be reasonable in view of the likely cost of a thermal reactor system of comparable size — I suspect that the figures we have heard from the AEA about the likely cost of CFR 1 have been arrived at in a similar way. Keck now estimates that the capital cost of the SNR-300 is five times that of a comparable LWR system ordered at the same time.

The importance of this case study is that the SNR 300 is the first and so far the only prototype fast reactor which has been built as if it were a commercial reactor; that is to say it has been built specifically with the goal of satisfying both the utilities and the licensing authorities. Therefore the utilities and the licensing authorities have been consulted from the early design stage in 1969. Keck says "After the designs were submitted drastic alterations became necessary as a result of the safety and environmental provisions demanded by the licensing authorities and substantially increased the costs of the plant... The most severe licensing requirement related to hypothetical accidents. The safety philosophy of the original design placed the emphasis on measures to prevent a core meltdown and a subsequent hypothetical nuclear excursion, and it was thought that this accident could be made sufficiently unlikely to avoid measures accommodating its consequences. The licensing authorities, however, not only required more stringent measures for prevention of this accident, but also stipulated that the impact of a nuclear excursion should be safely accommodated by the reactor vessel and the containment. . . .

"The demands of the licensing authorities not only increased the costs of the prototype plant, but also necessitated a lot of additional research and development in industry and government laboratories, which added considerably to the costs of the fast breeder project. For some items, the licensing requirements even went to the limits of technical feasibility, and imposed heavy tasks on scientists and engineers in industry and government laboratories."

So on the basis of this case study, there is every reason to believe that the licensing requirements for the fast reactor, arising specifically from the physics problems I have outlined, will cause an appreciable increase in capital costs relative to thermal reactor systems. Furthermore, the situation will get worse for larger fast reactors such as CFR 1 and Superphenix.

The relative higher capital costs of fast reactors compared with thermal reactors must be weighed against the prospective future price of uranium. In the words of The Times Mining Correspondent (*The Times*, 30 October 1978) "colossal new discoveries of uranium" have been made in the past three years in Australia and Canada. Therefore "prices are unlikely to go up faster from their current $42 a pound than inflation warrants". So there can be no urgency about developing a commercial fast reactor for economic reasons; it would be more cost effective to spend money on improving the present designs of thermal reactors. Although the AEA talk about a commercial fast reactor, there is no reason at present to use the term commercial. There still remains an enormous amount of basic work to be done on these systems before they can be regarded as a safe, sure and reasonably-priced source of electricity.

My final point is that fast reactors are too hard for us in Britain working alone, since we have neither the resources in money nor in people to do the job by ourselves. The greatest part of the UKAEA's budget and most of its qualified scientists and engineers since the mid-1960s have been committed to the fast reactor but, nevertheless, we cannot match the spending and manpower committed in the United States on fast reactor systems.

The UK is favourably placed for energy until the end of the century, while fast reactors cannot hope to be commercial until next century. A modest programme of research and development is indicated which does not strain the UK's resources, so that it is in a position to benefit from any future exploitation of fast reactors next century. The problem is similar to that in the field of fusion power and the solution is similar: there should be a common European programme of research and development, similar to JET and to the programme of research in elementary particle physics based at CERN in Geneva. If it is too late for the UK to join in the Superphenix project, it should indicate its willingness to join the next reactor after that. With its continuing experience of PFR at Dounreay and its large and assured stock of plutonium it can expect to be welcomed as a partner. I agree with Lord Flowers (*Nature* **264**, 496; 1976) that 'fast reactor developments is likely to be beyond our resources. It seems to me essential that if we propose it at all we should do so on a European basis'.

So it does not make sense for the UK to build CFR 1. On the other hand there is scope for being involved in planning the reactor to be built as a European collaboration (perhaps even the US and Japan would also be interested in participating) after Superphenix. The considerations I have outlined here would suggest that a sensible size for a LMFBR which avoids the problems connected with a large positive sodium void coefficient and which may have commercial potential next century would be about 500 or 600 MWe. PFR, Phenix and SNR-300 provide a sensible basis for the design of a reactor of this size.

France minimises risk of 'worst accident'

WHEN construction was authorised in May 1977 of Superphenix — the fast breeder reactor now being built in south-east France — the licensing authority stipulated that it should be built to meet certain safety specifications. One was that the double-walled reactor vessel, the lid and the dome housing the reactor, should be able to withstand the accidental release of 800 megajoules of mechanical energy. This was the energy which way believed might be released in the 'worse possible accident', that of criticality followed by a sodium explosion.

More recent studies by the French atomic energy authority and others (reported in *Le Monde*, 5 July) however, have concluded that the likelihood of such an accident happening is much lower than had previously been thought: 1 in 10^8 instead of 1 in 10^6. Three independent rod systems, the studies conclude, lower the probability of criticality. Even if the reactor did go critical, then it is highly unlikely that the energy released would be 800 MJ.

The recent studies have also shown, however, that the reactor buildings would be unlikely to withstand 800 megajoule explosions. (It is not ever clear precisely what maximum energy they could withstand.) The question therefore remains whether, given the reduced probability of an 800 megajoule explosion, the licensing authority will still insist that the reactor housing be built to withstand such energies — which would mean a substantial increase in cost. □

news and views

The oceanography of fjords

from David M. Farmer and Herbert E. Huppert.

FJORDS, inlets or sea lochs are deep, twisting arms of the world's oceans, generally formed by glacial erosion. In sharp contrast to the depth of the main basin — up to 1700 m — most fjords have a shallow sill at their mouth, of between 1 and 10^2 m, and sometimes with second, third and further sills. All fjords are influenced by the tides, the prevailing winds and fresh water input from rivers. Some, particularly those in the Artic, are covered with ice for part of the year. Many people live along their shores, especially in Scandinavia, and have come to rely on the fjords for water, food, the disposal of waste and recreation. All these demands are interconnected, so that the ill-considered construction of a hydroelectric scheme, for example, will modify the water circulation, which in turn can decrease the production of herring in the fjord and of codling on the continental shelf. Man's intervention can also seriously disturb the millions of juvenile salmon which use fjords as highways on their seaward migration and depend throughout their journey on food produced in the fjords.

Scientifically, fjords provide a relatively simple system for investigations which can also contribute to knowledge of the open ocean. All these aspects were borne in mind at a recent NATO Advanced Research Institute on the physical, biological and chemical oceanography of fjords.*

G.L. Pickard (University of British Columbia) and B.R. Stanton (New Zealand Oceanographic Institute) have compiled a detailed description of the water characteristics in Pacific fjords. In contrast to the open ocean, their density structure is controlled almost entirely by salinity (rather than temperature). In Pacific fjords the salinity almost always increases with depth, whereas the temperature and dissolved oxygen generally decrease slightly. The river run-off near the head of each fjord, although very variable, can make an important contribution to the circulation. Fjords with high river run-off have well-mixed layers up to 10 m in depth, and there tends to be some movement in the deep water. The Alaskan and Chilean fjords with glaciers flowing into them, in contrast to those without, tend to have a positive dissolved oxygen anomaly at depth and a stepped salinity structure. This can be explained in terms of the predominantly horizontal flow, in a series of layers, of the glacier melt water and the extra oxygen contained in the ice as suggested recently (Huppert & Turner *Nature*, **271**, 46; 1978). Although morphologically similar to Pacific fjords, Scandinavian fjords typically experience smaller tides and this may have important consequences for the types of mixing process which dominate. A further distinction is that New Zealand fjords are short, have relatively deep sills and their circulation, in contrast to other fjords, tends to be set by the oceanographic conditions along the coast.

R.R. Long (Johns Hopkins University, Baltimore) felt that rare but severe disturbances, such as bad storms, could be responsible for a considerable amount of the vertical mixing and affect the circulation in fjords and in the deep ocean. The concept that rare, large, inputs might be considerably more influential than the inputs usually observed, was much discussed, but the final consensus was that the available data from all sources were not sufficient to make a definitive statement.

The renewal of deep water, due to the occasional flow of relatively heavy water over the sill into a fjord may occur over a range of time-scales — as infrequently as only once every 7 years in one case — in response to a combination of tidal range, strength of the off-shore winds, amount of river run-off and interaction of the flow produced by these effects with the major sills (A. Edwards, Dunstaffnage Marine Research Laboratory, Scotland). In between renewal, diffusion and mixing slowly decrease the salinity at depth, thus helping to promote the next renewal. Mixing may be the result of the breaking of internal waves and of turbulence generated by the tidal flow past the boundaries, although the magnitude of both these mechanisms is uncertain. D. Dyrssen (Göteborg University, Sweden) drew attention to the practical importance of deep-water mixing in connection with pollution. Deep water exchange is also crucial in determining the amount of available oxygen, which in turn produces changes in the distribution of species and alterations in feeding behaviour.

From the results of the JASIN (Joint Air-Sea Interaction) experiment R. Pollard (Institute of Oceanographic Sciences, UK) pointed to three mixing processes believed to be relevant to the oceanic thermocline and possibly to the halocline in a fjord. First, there is the instability, wave breaking and mixing due to shear, which Pollard believed to be infrequent and unimportant in the oceanic thermocline. Second, there is the motion along isopycnals, as for example, across fronts. Third, there is the motion across isopycnals due to double-diffusive effects. The importance of the last effect is still controversial, but data from the open ocean continue to affirm its influence and Pollard discussed additional confirmation in data taken by M. Gregg (University of Washington, Seattle). Laboratory experiments by B.R. Ruddick and J.S. Turner (*Deep-Sea Res.*, in press) indicate that double-diffusive effects may also play a part in mixing across fronts. Pollard then pointed to processes believed to be relevant in the mixed layer: the generation of Kelvin-Helmholtz billows, with subsequent mixing, surface wave breaking and Langmuir circulation due to interactions between surface waves and shear in the water. Various theoretical models have been proposed to predict features of the upper mixed layer, but it seems that the models are more sophisticated, though less realistic, than warranted by the data.

One of the most comprehensively observed North American fjords is Knight Inlet in British Columbia and an impressive array of phenomena have been documented there. Acoustic soundings indicate that there is a large amplitude internal response to the tidally forced flow

* The Fjord Oceanographic Workshop was held at the Institute of Ocean Sciences Patricia Bay, British Columbia from 4 - 8 June, 1979. The proceedings are to be published by Plenum in their Marine Science Series, edited by D.M. Farmer and H.J. Freeland.

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over the sill. At the beginning of the ebb tide, the flow separates behind the inner sill and there is a large amplitude periodic disturbance on the shear layer emanating at the sill crest, at a depth of approximately 60 m. As the tidal flow increases, a large-amplitude, stationary lee-wave train, or more usually an internal hydraulic jump, is set up in the stratified layer in the lee of the sill. Such an event is shown in the illustration along with the laboratory simulation. The strong flow associated with the accelerated flow down the lee face of the sill suppresses the separation, in agreement with recent theoretical and laboratory experiments of P. W. M. Brighton (Ph.D. Thesis, University of Cambridge). As the tide slackens, waves propagate upstream, since their phase velocity is now larger than that of the opposing tidal flow. This is the same phenomenon as documented by acoustic measurements in the vicinity of Stellwagen Bank in Massachusets Bay (Haury, Brisco & Orr *Nature* **278**, 312; 1979), a model of which was investigated in the laboratory (Maxworthy *J. geophys. Res.* **84**, 338; 1979). From measurements taken by the small manned submersible Pisces IV, A. Gargett (IOS, British Columbia) found that the wave train in Knight Inlet has an amplitude of 10 m, is highly turbulent and has a very strong down-ward velocity at its front. The flood tide may set up a similar train of waves, which propagate downstream as the tide slackens, but they are of smaller amplitude and possibly of different character because of the asymmetric geometry of the sill.

Long discussed refinements of his two-layer frictional model for the circulation in a fjord, presenting an updated parameterisation of the friction to allow for a better comparison with data from Knight Inlet. Nevertheless, a consequence of the model, that the Froude number at the mouth be 1.0 (the critical value) is not reflected in recent data taken in Knight Inlet, which indicates that the value is close to 0.3.

Some of the many practical problems in fjords were skillfully described by Scandinavian workers. Careful consideration of the energy exchange between the barotropic and baroclinic tidal response over a sill indicates that some fjords have constrictions which make them close to resonant. This suggests that relatively small modifications of the constriction, such as altering the depth of the sill or placing a floating bridge or barrier over the sill, may bring the fjord closer to resonance. This would increase the tidally-generated internal response and lead to enhanced mixing.

The siting of hydropower installations and the depth of the fresh water discharge into a fjord can have enormous influence on the water characteristics; in particular, a fresh water layer, sufficiently deep and cold, on the top of a fjord can promote undesirable ice cover throughout the winter. T. Carstens (Norwegian Institute of Technology, Trondheim) explained that submerging the output and introducing compressed air into it enhances the mixing in the upper layers and increases the salinity at the surface, thereby inhibiting ice formation. T.A. McClimans (Norwegian Institute of Technology, Trondheim), described models for the point release of a fresh water supply into a fjord, incorporating tidal effects, which have been successfully used in a number of situations.

A novel technique for studying the circulation in the Clyde Sea Area fjord involving the use of radioactive pollutants from Windscale was presented by I.G. McKinley (University of Glasgow, Scotland). Caesium-134 and 137, which have different half lives (2 and 30 years), provide an ideal tracer pair for studying vertical diffusion, sedimentation rates and other exchange processes. The caesium was detectable as far away as off Northeast Scotland, suggesting that this procedure may be ideally suited for ocean circulation studies elsewhere. □

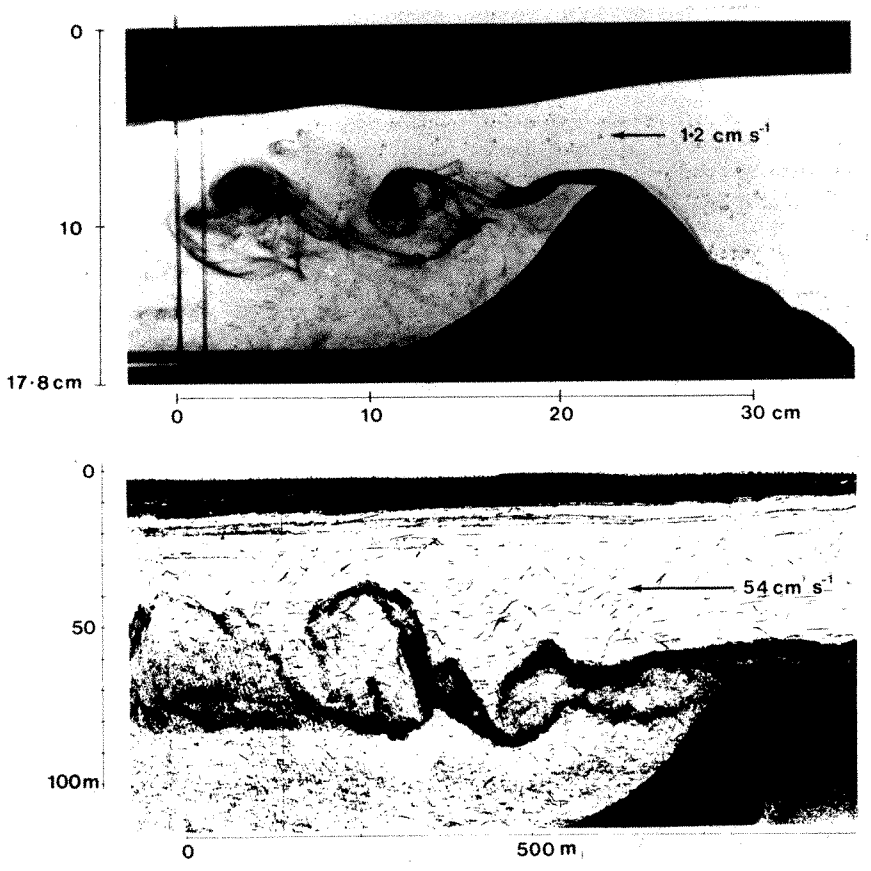


Fig. 1. Tidally-generated flow in the laboratory (bottom) and Knight Inlet, British Columbia (top), showing the effects of flow separation and large-amplitude billows.

Changing cometary orbits

from David W. Hughes

Two principal mechanisms are available for changing the orbit of a comet. The first is gravitational and relies on the proximity of a planet to perturb the comet's path. The change in the reciprocal of the semi-major axis of the orbit then suffers a random walk which is approximately proportional to the square root of the number of returns the comet makes to the inner Solar System. The second is non-gravitational and is due to the fact that gases given off by the cometary nucleus are not emitted symetrically in all directions. The importance of this second force was first realised in the early 1820s when Comet Encke consistently passed perihelion 2.5 h before the predicted time.

Imagine an icy conglomerate cometary nucleus which is not rotating. The maximum emission of the sublimating volatiles will occur on the sunward side, around the subsolar point. This jet of gas will effectively exert a force on the nucleus in the direction away from the Sun. Now if the nucleus rotates and if there is a time lag between the absorption of the solar radiation by the dusty surface of the nucleus and the sublimation of the cometary ices, then the effective force is not radial; this force will have a transverse component along the orbit of the comet and also a normal component perpendicular to this orbit. Marsden, Sekanina and Yeomans (*Astron. J.* **78**,

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211; 1973) found that the radial force was typically 10 times the transverse force and that the normal component was either zero or undetectable. This implies that the lag angle (the angle between the solar direction and the line from the centre of the nucleus to the maximum emission point) is on average about 6°. Such a low value indicates that the nucleus is either spinning slowly, with a period of 24 h or more, or that the spin axis is nearly perpendicular to the orbital plane, or that both these conditions are operative.

Interestingly there seem to be as many comets with positive transverse forces as with negative ones. So direct rotations (like the Earth's) and retrograde rotations are equally probable.

The effects of these transverse forces are also rather interesting and they have been investigated by Paul R. Weissman (Jet Propulsion Laboratory, California) in a recent edition of *The Astronomical Journal* (**84**, 580; 1979). If the cometary nucleus has a direct rotation, the transverse force increases the energy and the angular momentum of the orbit and both the perihelion distance and the aphelion distance get larger. The comet moves away from the Sun. The opposite occurs for retrograde rotation. Now planetary perturbations produce a random walk variation in orbital parameters whereas non-gravitational forces are much more effective. Assuming that the spin axis direction does not vary, the value of the reciprocal of the semi-major axis undergoes regular, unidirectional steps and the total change produced in this quantity is simply a function of the number of times the comet passes close to the Sun.

Non-gravitational forces are responsible for changing the orbits of many of the short-period comets. Weissman suggests for example that they have decreased the semi-major axis of the periodic comet Encke and have pulled it away from the influence of Jupiter. This effect can thus greatly increase the inner Solar System lifetime of these short-period comets and there is the possibility that they could lose all their ices without violent disruption and so evolve into Apollo or Amor-type asteroids. The action of non-gravitational forces on long-period comets is much more difficult to assess simply because these are only seen at one apparition.

Weissman also considers the Kreutz family of Sun-grazing comets. The change in the reciprocal of the semi-major axis, a, that these non-gravitational forces can produce, increases strongly as the perihelion distance gets smaller. The Kreutz family have perihelia in the region between 0.005 and 0.009 AU, well within the solar Roche limit. Typical values of $\Delta(1/a)$ lie in the range 10^{-3} to 10^{-1} AU^{-1} and can easily change the aphelion distance from around 4×10^4 AU (well within the Oort cloud) to their present value of about 100 AU, in two or three returns.

Of course there are still many problems requiring solutions. Do the spin periods and spin axis directions of cometary nuclei vary with time? What made them spin in the first place? Are the spin axes directions randomly orientated in space and if so why? F.L. Whipple (*IAU Colloquium* **39**, 25; 1977) reminds us that the cometary nuclei are irregularly shaped objects that can lose up to 1% of their mass at each perihelion passage. Also the mass loss is irregular and often occurs disruptively. These factors can all lead to a change in the spin axis direction and the spin rate.

Another mechanism that can change the spin rate has been discussed recently by R.L. Hawkes and J. Jones in *The Monthly Notices of the Royal Astronomical Society* (**185**, 727; 1978). They consider the effect of micrometeoroid ablation on the spin rate of the meteoroids responsible for the radio and visual meteors seen in the Earth's atmosphere. Particle impacts on an irregular body will produce a change in the angular momentum and the total angular momentum will increase in a random walk fashion. Even though on average there will be as many collisions causing increases in the spin rate as there are causing decreases, there will be an incomplete cancellation due to the statistical fluctuations of the opposing torques. According to Hawkes and Jones the meteoroids spin up until rotational energy becomes comparable to the translation energy. The meteoroids would then be spinning at about 1000 revolutions per second indicating that comets with their spin periods of many hours have a long way to go. □

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Progress in thalassemia research

from David G. Nathan

INVESTIGATIONS of normal haemoglobin have paved the way for understanding the anatomy and physiology of the human genome and research into Cooley's anaemia has been well in the vanguard. At the fourth symposium on Cooley's anaemia,* an international group of clinicians and basic scientists met to review the remarkable progress made in understanding, treatment and prevention of this inherited disease.

Cooley's anaemia, or the β-thalassemia syndrome, together with the various α-thalassemia syndromes are the result of unbalanced synthesis of globin chains secondary to decreased production of functioning mRNA. This mRNA deficit is sometimes due to specific gene deletions. More commonly, the structural gene is present but ineffectively contributes to globin synthesis. Application of restriction endonuclease gene mapping techniques to normal DNA and to DNA bearing globin gene deletions by R. Bernard, R. Flavell and R. Williamson (University of Amsterdam and St. Mary's Hospital Medical School London) and J. Mears and A. Bank (Columbia Univesity, New York), has provided a physical map of the $G\gamma$ - $A\gamma$ - δ - β globin regions in chromosome 11, one that spans more than 40 kb (kilobases). The intergenic distances are now established. Maps of several different deletions in this region, all of which cause complete absence of β globin synthesis (β^0 thalassemia) have been provided by B. Forget (Yale University), T. Maniatis (California Institute of Technology; also *Nature*, **279**, 598; 1979), Mears and S. Orkin (Children's Hospital Medical Center, Boston). They range from a 0.6 kb deletion at the 3′ end of the β gene to much larger deletions that cause either $\delta\beta$ thalassemia or hereditary persistence of fetal haemoglobin. In the latter diseases the known deletions include one of at least 30 kb encompassing all of the DNA from the area immediately 3′ to the G γ gene through to the 3′ end of the β gene and another of at least 13 kb encompassing the 3′ half of the δ gene through to the 3′ end of the β gene. Whether a firm relationship exists between the extent of gene deletion 5′ to the β, and γ chain synthesis is uncertain from the few cases studied to date.

Two laboratories have reported successful cloning of the β^+ thalassemia genes. So far molecular lesions clearly responsible for disturbances in transcription or mRNA processing have not emerged. Forget reports no abnormality in the 5′ end of the larger β intron (intervening sequence). Unambiguous interpretations of these clones may be difficult because the data already suggest substantial normal molecular heterogeneity in and around the 5′ regions of the β gene. The search for a molecular basis of non-deletion forms of thalassemia will require painstaking sequencing of long stretches of DNA and use of functional systems for detecting gene expression.

Chang and Y.W. Kan (University of California, San Francisco) have clearly ascribed one form of β^0 thalassemia to a nonsense mutation at nucleotide codon 17. The nonsense-containing mRNA can be translated in a cell-free system if a suppressor $tRNA^{Ser}$ is added. This is the first description of a nonsense mutation in man.

The α-thalassemias have been investigated by S.E. Embury (University of California, San Francisco) and Orkin. 'Non-deletion' forms of α-thalassemia genes are commoner than formerly appreciated and single deletions of one of

*The Fourth Symposium on Cooley's anaemia, co-sponsored by the New York Academy of Sciences and the Cooley's Anaemia Foundation Incorporated, was held in New York on 21-23 May.

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the pair of duplicated alpha genes is very common in blacks accounting for the rarity of α-thalassemia-induced hydrops fetalis in that race.

Kan, B. Alter (Children's Hospital Medical Center, Boston), A. Cao (University di Cagliari, Sardinia) and D. Loukopoulos (University of Athens) all reported that prenatal diagnosis of β-thalassemia by fetal blood sampling is now an established procedure. Endonuclease mapping of fetal fibroblast DNA can also be used in the deletion forms of β-thalassemia, in certain West Africans with sickle cell anaemia and α-thalassemia.

R. Propper (Boston), A. Nienhuis (National Institutes of Health, Bethesda) and S. Piomelli (New York University Medical Center) reviewed therapeutic advances in which new approaches to transfusion with young red cells and continuous subcutaneous infusion of deferoxamine can achieve negative iron balance in these repeatedly transfused patients. New approaches to computerised radionuclide cardiac scanning will probably permit better evaluation of the effects of this treatment on cardiac function.

Whether the long-sought goal of increased fetal haemoglobin synthesis can be achieved in patients with β-gene defects was debated somewhat inconclusively by G. Stamatoyannopoulos (University of Washington, Seattle), Nienhuis and Nathan. Normal erythroid stem cells do form differentiated colonies that vary in their capacity to express γ genes. One human erythropoietic cell line (K562) derived from a patient with chronic myelogenous leukaemia was reported by T. Rutherford and D. Weatherall (The Radcliffe Infirmary, Oxford). When stimulated with haemin, this cell line exhibits α, ζ, ε, and γ gene expression (*Nature* **280**, 164; 1979). W. French Anderson (National Institutes of Health) reported progress in cell fusion studies inthat hybrids formed by human fibroblasts and 2-S mouse erythroleukaemia cells produced human β, but not γ mRNA. A specific positive regulator is apparently present in these interesting hybrids.

It seemed clear to all participants that advances in haematopoietic stem cell culture and in globin gene cloning may lead to marrow autotransplantation experiments in animal models in which new functioning globin genes may be incorporated into replicating stem cells and globin chimaeras created in the laboratory. Whether these techniques will be applied in man will await much more refined methodology. In the meanwhile, prevention by prenatal diagnosis and improved therapy are available. Progress at the bench and bedside is indeed exciting in this fascinating field. □

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Quark fusion

from Peter Landshoff

EXPERIMENTS in which beams of high-energy electrons, muons or neutrinos are scattered on a hydrogen target give good, though indirect, evidence that the proton is made of quarks. The evidence is indirect because no free quarks have yet been seen, and indeed most high-energy physicists have come to believe that quarks can only occur in bound states such as protons or π-mesons. For this reason, it is valuable to have other types of experiment that confirm the quark structure and give more information about it. In the experiments that are most straightforward to interpret, a lepton pair is produced in the collision of a proton or pion beam with a hydrogen target, or with some heavier nucleus. The lepton pair may be either an electron and a positron, e^+e^-, or a muon pair $\mu^+\mu^-$.

It was experiments of this type that made the important discoveries of the J/ψ and Υ resonances. The Υ are responsible for the structure near 10 GeV in the data shown in Fig. 1 from the Columbia-Fermilab - Stony Brook collaboration, where the production cross-section is plotted against the invariant mass, m, of the lepton pair. Attention is now turning to a more accurate exploration of the continuum, that is, to values of m not near a resonance. In 1970, S.D. Drell and T.-M. Yan predicted that, in experiments with high-energy beams, dilepton production in the high-mass continuum would take place through quark fusion. The Drell–Yan mechanism is best visualised in the centre-of-mass frame, in which the beam particle and the target both move towards each other with large momentum and collide. At that time, it was already known, from experiments in which high-energy electrons scattered on protons, that a substantial part of the momentum of a fast-moving

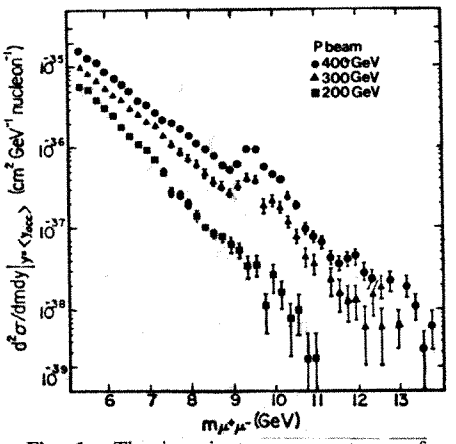


Fig. 1. The invariant-mass spectrum of $\mu^+\mu^-$ pairs produced by high-energy proton beams colliding with a heavy target. The structure around 10 GeV is associated with the Υ resonances (Columbia-Fermilab-Stony Brook). From a talk given by John Dowell at an Institute of Physics meeting in April, 1979).

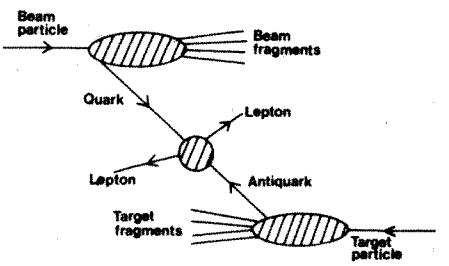


Fig. 2. The Drell–Yan mechanism, in which a quark and an anti-quark fuse to form a lepton pair.

proton is carried by three quarks, known as its valence quarks, but that in addition the proton contains a large number of slow-moving pairs of quarks and antiquarks. In the Drell–Yan mechanism, a quark from one of the two initial colliding particles fuses with an antiquark from the other, and the energy of the quark-antiquark pair then materialises again at the lepton pair (Fig. 2).

The experimental results agree well with this picture. In particular, the proton-beam data verify that the production cross-section $d\sigma/dm$ obeys a scaling law. This is predicted from the fact that quarks and antiquarks have very small radius, or maybe are even pointlike. This implies that the production must be essentially independent of any fixed parameter that carries dimensions, and, since $d\sigma/dm$ has dimension (mass)^3 in units where $\hbar = c = 1$,

$$\frac{d\sigma}{dm} = \frac{1}{m^3} F(\tau).$$

Here F is a dimensionless function of the only dimensionless variable associated with the production, $\tau = m^2/s$, where $\sqrt{s}$ is the total energy measured in the centre-of-mass frame. The test of scaling is shown in Fig. 3, where it is seen that $m^3 d\sigma/dm$ plotted against τ is independent of beam energy, or equivalently of the centre-of-mass energy $\sqrt{s}$. Data at rather higher centre-of-mass energies are now beginning to come from the CERN Intersecting Storage Rings, and they also seem to be in agreement with scaling. In the case of π-meson beams, scaling sets in only at rather larger values of m; why this should be is not understood.

The proton-beam data agree well with quantitative calculations of $F(\tau)$. These calculations use as input the momentum distributions of the quarks and antiquarks within the proton, as measured in the electron, muon and neutrino scattering experiments. However, these experiments determine well the momentum distributions of the valence quarks, but much less well those of the antiquarks, and the dilepton production experiments actually give the best determination of the antiquark distributions. Because of its very short lifetime, the π-meson cannot be used as a target, and so there is no information

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about its structure from electron, muon or neutrino scattering. But muon-pair production from pion beams, when interpreted in the Drell-Yan picture, agrees well with theoretical prejudices about the momentum distributions of quarks within the pion. Quarks are known to have spin ½ and at values of the invariant mass m high enough for scaling to have set in, the angular distribution of the produced muons is found by the Chicago-Illinois-Fermilab collaboration to correspond with that predicted from the fusion of a pair of particles of spin ½.

One surprising property of the Drell-Yan continuum was discovered by the Columbia-Fermilab-Stony Brook collaboration. It was expected that the dilepton system would emerge from the reaction with its total momentum in a direction closely parallel to the incident beam momentum, but it is found that rather often this is not so. Theorists would like to interpret this as a natural consequence of quantum chromodynamics. This is believed to be the quantum field theory associated with the strong interactions that hold quarks and antiquarks together within protons and pions. Just as in quantum electrodynamics the electromagnetic force is transmitted by a particle, the photon, so in quantum chromodynamics the strong force is

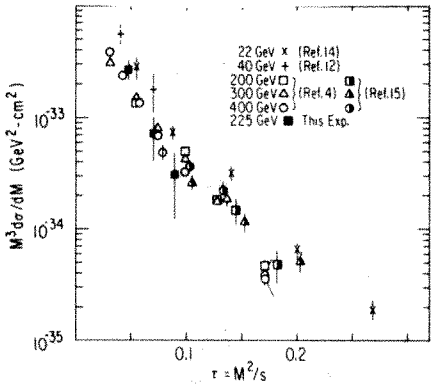


Fig. 3. Test of scaling: plots of $m^3 d\sigma/dm$ for various energies of the incident proton beam (Chicago-Illinois-Princeton). From Anderson *et al. Phys. Rev. Lett.* **42**, 944; 1979).

thought to be transmitted by a particle called the gluon. The bremsstrahlung of gluons by the quarks and antiquarks before they fuse can appreciably change their directions of motion, and so may account for the large transverse momentum component of the dileptons. If this is the correct explantion, the gluon bremsstrahlung will have other consequences that can be checked in accurate experiments, including small but well-defined deviations from scaling and from the simple expectations for the angular distributions. The strong interest of both experimentalists and theorists in the Drell–Yan mechanism seems likely to continue. □

A new form of electron diffraction by atoms

from C. B. Lucas

STUDIES of electron scattering by free atoms have now over 50 years of history, so it is surprising that a new diffraction phenomen has just been discovered by Geiger and Morón-León (*Phys. Rev. Lett.* **42**, 1336; 1979).

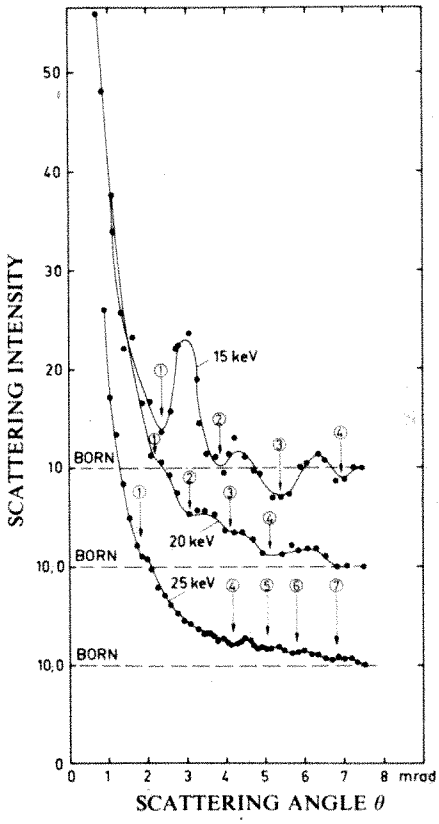
The first experiments which showed that electrons have a wave nature and so undergo diffraction effects were those of Davisson and Germer in 1927, using a crystalline taget. These experiments overshadowed the studies of electron scattering from free atoms which started at about the same time. As Bullard and Massey stated in 1931: "It will be seen that the . . . curves exhibit general similarity to those representing the intensity of light scattering from small spheres . . . However the analogy must not be pushed too far as the electron wavelength varies very rapidly in the region of the atom."

In so well-established a field, little general interest is aroused by the majority of current publications on elastic electron scattering. Recent progress has mainly been directed towards increased accuracy of experimental data and increasingly sophisticated numerical calculations. Though great interest was aroused by the discovery of resonance phenomena, which caused narrow structure to be seen in the scattered intensities, certainly the most outstanding discovery of recent times was also made in the 1960s — that electrons scattered from heavy atoms are spin-polarised. This confirmed predictions made by Mott in 1929, using the relativistic form of the Schrödinger equation, which had been introduced by Dirac only a year earlier. Even this exciting discovery did little to raise the prestige of elastic electron scattering studies as very few surprises emerged. Also, the unfortunate fact was soon discovered that large values of polarisation were only obtained in the scattering minima. This is because the polarisation is caused by an angular displacement of the scattered intensity for electrons of different spin directions, but the maxima are broad and thus insensitive to the displacement. In other words, electron scattering has only yielded very small currents of polarised electrons, so interest in ways of producing polarised electrons rapidly switched to other fields.

The electron scattering maxima and minima are found at electron energies ranging from a few electron volts up to many hundred, especially in the heavier atoms. Quite deep minima in the scattering intensities are observed at scattering angles ranging from beyond the forward peak to well into the backward scattering region.

The new electron diffraction was discovered by Geiger and Morón-León at higher electron energies and at scattering angles of less than 0.5°. It occurs not by diffraction on the atom itself but from a kind of shadow around the atom produced by other scattered electrons. This diffraction had not been predicted theoretically, although the reason for its occurrence is easily understood.

In general, the intensity of electrons which are scattered elastically at any scattering angle is larger than the intensity of electrons which have lost some of their energy, for example by exciting or ionising the atom. These inelastically scattered electrons, however, dominate the forward scattering, especially at high energies, where their intensity is typically 1000 times greater than the elastic. So if only the



Shadow diffraction in neon at electron energies of 15, 20 and 25 keV. For clarity the three curves have been displaced and the orders of the diffraction minima have been numbered.

THE statement on page 4 of the *News and Views* article prepared by Leffert and Koch, entitled 'Ionic Landmarks Along the Mitogenic Route' (*Nature*, **279**, 104; 1979) is incorrect. Proteolysis does not convert myelin basic protein into a mitogenic peptide whose sequence is Thr-Pro-Pro-Pro-Ser-Gln-Gly-Lys. What I stated at the conference was that from our data the brain FGF activity resides within region 91-117 of the basic protein. However because of the effects of other portions of the molecule on mitogenicity one would not expect that this region itself would be mitogenic.

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elastically scattered electrons are detected, it appears as if the atoms were surrounded by large opaque fuzzy-edged disks, or shadows, since almost no elastically scattered electrons can appear at small scattering angles. It is these disks which are responsible for the diffraction effects.

It is clear by comparison with light diffraction that diffraction effects become easier to observe when the diffracting object is not very large compared with the wavelength of the diffracted beam. So it is not surprising that shadow diffraction has remained undetected for so long. The experimental techniques necessary for its observation are more difficult than for large angle scattering, and there was no theoretical prediction.

The apparatus used by Geiger and Morón-León is similar to an electron microscope in which the specimen is replaced by the target gas at a pressure of about 0.1 Torr. A diminished electron optical image, 8 μm in diameter, of the cathode is formed in a filter lens. This is so arranged that all electrons which have lost energy by any inelastic collision with the target atoms see a potential hill which they cannot surmount, and so fall back; that is, they are reflected by the filter lens. The remaining elastically scattered electrons are imaged by the filter lens onto a photographic plate.

Measurements were made in helium, neon and argon. Typical results obtained with neon are shown in the figure, from which it is clear that the diffraction effects are more pronounced at lower energies. The well-known Born approximation, which is flat over this narrow angular range, is used for normalisation purposes. The orders of the diffraction minima are numbered. Geiger and Morón-León assume that the radius R of the disk is equivalent to a 0th order diffraction maximum of angular full width at half height $\theta = \Delta E/E$ where ΔE is the energy lost by the inelastically scattered electrons of primary energy E. This gives an energy dependent radius R of the disk given by $R = 2/k\theta$, where k is the wavenumber of the incident electrons. In the case of the 15 keV electrons scattered from neon, if it is assumed that the main energy loss, ΔE, is due to excitation of the resonance line at 16.9 eV, the radius of the scattering disk is 30 Å, or some 23 times larger than the kinetic theory atomic radius. This agrees exactly with the value of the radius which can be obtained by substituting the measured minima shown in the figure in the Fraunhofer diffraction equation. Good agreement is also obtained in all other cases, where the diffraction angles are not so accurately measureable. So the remarkably simple model of an opaque shadow disk without a fuzzy edge gives an excellent explanation of the observations.

Clearly shadow diffraction now awaits a more detailed theoretical treatment, preferably including electron spin effects. Rapidly changing intensities are an indication that spin polarisation effects are to be expected. For example, in addition to the spin polarisation measurements discussed above, electrons elastically scattered in the neon resonances were shown to be polarised by Franzen and Gupta in 1965. Since spin-up and spin-down electrons could see a different-sized shadow, shadow diffraction may well produce polarised electrons where the intensities are by no means small. □

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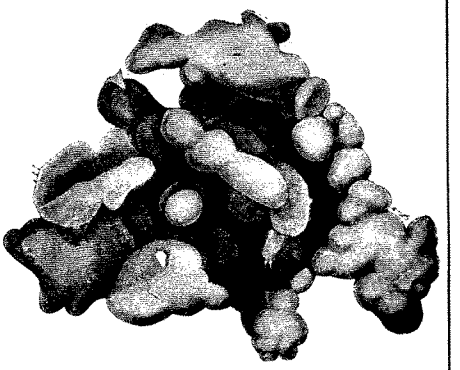


100 years ago

THE eminent zoologist, Dr. Karl Moebius, of Kiel, has recently published a treatise, "Der Bau des *Eozöon canadense* nach eigenen Untersuchungen verglichen mit der Bau der Foraminiferen" ("The Structure of *Eozöon canadense*, according to my own Investigations, compared with the Structure of Foraminifera"), which first appeared in the "Palaeontographica" (vol. xxv.), and was afterwards republished separately. Prof. Moebius inclines entirely towards the view of King and Rowney (*Proc. Roy. Irish Acad.* ser. 1, x. and ser. 2, i.) disputing the organic character of Eozöon. . .

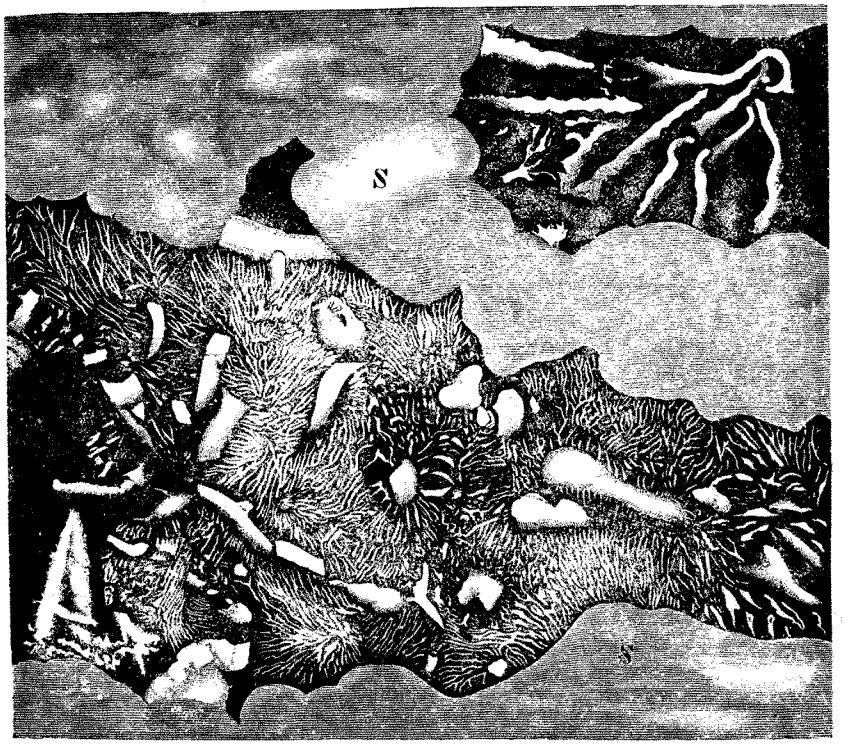
According to Dr. Moebius *Eozöon canadense* consists principally of alternate layers of yellowish green serpentine and whitish limestone. . .

"If the Eozöon pieces from the Laurentian or 'Urgneiss' formation



were really remains of an undoubted foraminifera species, then we should possess in them *certain* proofs that even during the formation of the most ancient strata of the earth's crust living beings occurred, and that the first organisms belonged to the lowest animals, by which biology and geology would have gained two highly important facts. Yet by the scientifically justified elimination of Eozöon from the domain of organic beings it is not proved that during the Laurentian period no living beings existed. Perhaps the graphite of the Urgneiss formation has its origin in organic beings."

from *Nature* **20**, 17 July, 272, 24 July, 300; 1879.



review article

Protein methylation in behavioural control mechanisms and in signal transduction

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In both prokaryotes and eukaryotes methyl groups can be added to and removed from the carboxyl groups of proteins. Recent work has revealed that these reactions have a role in several behavioural phenomena. The nature of this role has been uncovered in one case—that of bacterial chemotaxis.

AMONG the problems addressed by biologists, one of the most intriguing is that of defining the mechanisms that underlie behaviour at the cellular level. This includes the transduction of one signal into another. For example, sensory stimuli interact with sensory receptors to bring about an alteration in cellular output, neurotransmitters combine with synaptic receptors to change ion fluxes, and hormones affect cell surface receptors to produce responses in target cells. In the past few years it has become apparent that reversible covalent modifications of macromolecules play an important role in some of these mechanisms. The phosphorylation and dephosphorylation of proteins are perhaps the best characterised examples[1-4]. Recently, another class of covalent modification, methylation of protein-carboxyl groups, has been linked to the control of behaviour and to signal transduction in both prokaryotes and eukaryotes. In this article we will summarise the evidence for the involvement of protein methylation in bacterial chemotaxis, a simple behavioural response in which the role of this modification has been characterised in some detail. We will also provide a brief discussion of the evidence linking protein methylation to behaviour in eukaryotes.

The response of bacteria to chemical stimuli

Motile bacteria such as *Escherichia coli* and *Salmonella typhimurium* are attracted by certain chemicals and repelled by others, a phenomenon known as chemotaxis[5-15]. In nature the cells respond to spatial gradients of these chemicals by monitoring concentration as a function of time and reacting to the changes experienced as they swim from one place to another. The bacteria, then, are actually sensitive to temporal changes in chemical concentration[16-18]. In the laboratory it is convenient to provide temporal stimuli directly, by the sudden addition or removal of a chemical[16,17].

Before stimulation, the cells show a pattern of motility characterised by periods of smooth swimming interrupted at random intervals by brief periods of chaotic tumbling[16,19]. Smooth swimming is produced by anticlockwise rotation of the flagella[20], which act as propellers to drive the cells through the surrounding medium[21-23]. Tumbling results when the direction of rotation is reversed[20,24].

When an attractant is added (Fig. 1*a*), the cells respond with a suppression of tumbling[16]. After a lag period, however, the frequency of tumbling returns to the pre-stimulus level[16,25,26]; the cells have in effect become insensitive to the continued presence of the attractant. The initiation of the behavioural response is referred to as sensory excitation and the subsequent loss of sensitivity as sensory adaptation. The adaptation process begins as soon as the attractant is added and proceeds until it reaches completion and terminates the response[26]. The time required for this is a function of the strength of the stimulus (defined by the magnitude of the increase in concentration) and can be many minutes following a large concentration change[16,25,26].

When the attractant is removed, sensitivity is rapidly regained[26], that is, the bacteria can respond again if the attractant is added back. The restoration of sensitivity can be thought of as the inverse of adaptation and is referred to as de-adaptation. A curious asymmetry should be pointed out: the de-adaptation process is very much faster than the corresponding

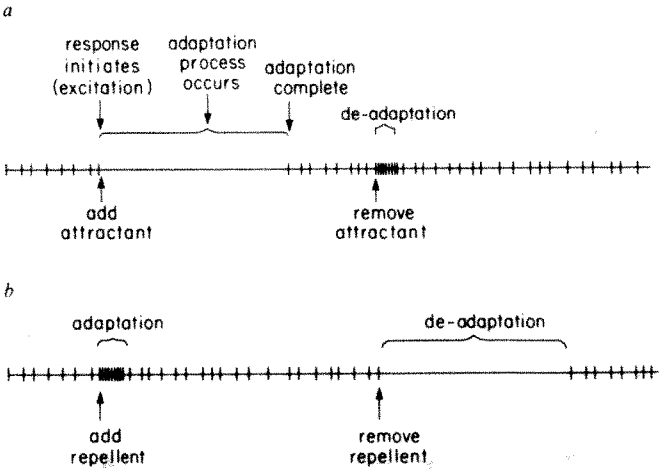


Fig. 1 Schematic representation of temporal responses. Each vertical line represents a tumble. The absence of vertical lines that follows addition of attractant or removal of repellent represents suppression of tumbling, while the increased frequency of lines that follows removal of attractant or addition of repellent represents continuous tumbling. *a*, Effect of attractants; *b*, effect of repellents.

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adaptation process, requiring no more than a few seconds even for a large decrease in attractant concentration[16,26]. This asymmetry is indicated in Fig. 1*a*. Note that de-adaptation is accompanied by a brief period of enhanced tumbling[16,26].

Repellents have behavioural effects which by several criteria are opposite to those described for attractants. First, addition of a repellent (like removal of an attractant) results in a short period of increased tumbling, whereas removal of repellent (like addition of an attractant) causes a longer period of suppressed tumbling[17] (Fig. 1*b*). Second, if an attractant and a repellent are added simultaneously the two tend to cancel each other's effects[17,27]. These results suggest that, in terms of the underlying biochemical mechanisms, there is an analogy between the process which follows addition of repellent and that which follows removal of attractant and similarly between removal of repellent and addition of attractant.

The mechanisms that underly these behavioural responses have been analysed by using mutants that are defective in various aspects of chemotaxis. The mutants fall into several classes which can be used to define a pathway of information flow, as shown in Fig. 2. The first class, called specifically non-chemotactic, suffer from lesions in one or another of the different kinds of chemoreceptors[28] that detect specific compounds. These mutants respond normally to most chemicals and lack responses only to those chemicals detected by the receptor that has been affected[28–34]. The second class contains mutants that have lost responsiveness to the chemicals detected by several different kinds of receptor, but that retain responsiveness to compounds detected by the remaining receptors. This class of mutants, known as multiply nonchemotactic mutants, contains three complementation groups, called *tsr*, *tar* and *trg*, which define three major branches of the pathway[35–39] (see Fig. 2). The receptors that feed information through the branch defined by the *tsr* gene are called type I receptors, and those feeding through *tar* and *trg* are called type II and type III, respectively. Ultimately, information from all the receptors is integrated and used to control the direction of rotation of the flagella and consequently the frequency of tumbling, as described above. It is in this final, integrated pathway that a third class of mutants is found. These are the generally non-chemotactic (*che*) mutants; they fall into eight complementation groups[15,28,40,41]. A last class, the non-motile mutants, has lost the ability to synthesise or rotate the flagella[15,42] (Fig. 2).

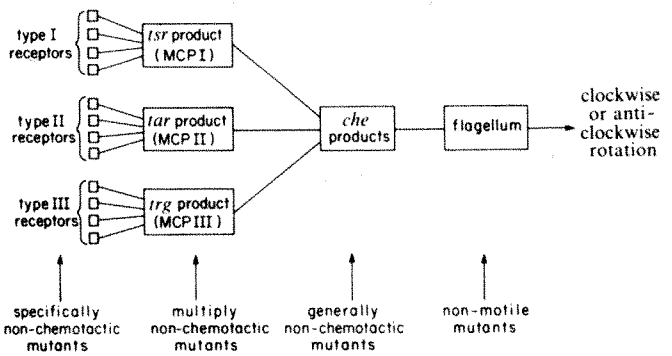


Fig. 2 Information flow during bacterial chemotaxis. The lines represent the pathways of information flow; each of the empty boxes represents a different kind of chemoreceptor. The arrows at the bottom indicate where this flow of information has been interrupted in the various mutants.

Information flow through methylated proteins

Several years ago it was discovered that a protein methylation reaction has an essential role in bacterial chemotaxis[43]. Two criteria were used to demonstrate this. First, the extent of methylation of several cytoplasmic membrane proteins[35,36,39] was shown to be altered by chemicals that elicit a chemotactic response[43]. Second, the methylation reaction failed to occur in some generally non-chemotactic mutants and, when such a mutant was reverted for chemotaxis, methylation simultaneously reappeared[43]. The proteins were therefore called the methyl-accepting chemotaxis proteins (MCPs).

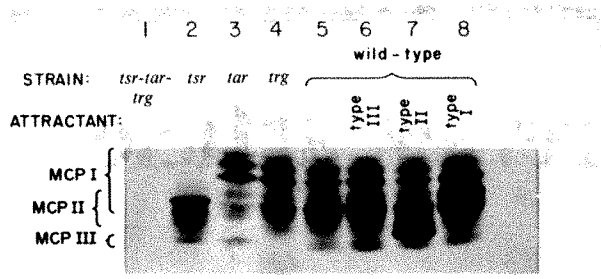


Fig. 3 Methylation patterns from a chemotactically wild-type strain and from *tsr* (lacking MCP I), *tar* (lacking MCP II) and *trg* (lacking MCP III) mutants. Bacteria were incubated with [*Me*-^{3}H]methionine in the presence of a protein synthesis inhibitor, as described previously[53]. The attractants used, D-ribose (5 mM), DL-α-methylaspartate (5 mM) and α-aminoisobutyrate (50 mM), are type III, typeII and type I stimuli, respecnvEqual amounts of protein were layered on a 7% SDS polyacrylamide gel. The fluorographic image shows the region of the gel where proteins of molecular weights 56,000–65,000 are found. The brackets indicate the bands which compose MCP I, MCP II and MCP III. The lower two bands of MCP I and the upper two bands of MCP II overlap[35]. Strains used were AW405, AW518, AW539, AW701 and AW660 for the wild type, *tsr*, *tar*, *trg* and *trs-tar-trg* mutants, respectively.

Where do the MCPs function in the pathway of information flow described above? Three different MCPs have been defined by the three complementation groups of the multiply nonchemotactic mutants[35,36,39]. This is documented in Fig. 3, which shows the methylation patterns obtained by incubating bacteria with [*Me*-^{3}H]methionine (the source of the methyl group). As shown in Fig. 3 (lanes 1–5), the *tsr* mutant lacks a set of methylated bands collectively called MCP I (refs 35, 36), the *tar* mutant a set of bands called MCP II (refs 35, 36) (note that there is some overlap of MCP I and MCP II), and the *trg* mutant a set of bands called MCP III (ref. 39); the triple mutant lacks all these methylated bands[39]. Evidence suggests that *tsr* and *tar* actually code for the MCP I and II polypeptides, respectively[36,44], and by analogy it is thought that *trg* may code for the MCP III polypeptides[39]. Why each of these genes gives rise to more than one polypeptide is not clear, although there is some indication that this results from post-translational modification of the gene products[45].

Information from each of the three different types of receptor (as defined in Fig. 2) causes a change in the level of methylation of the appropriate MCP. Apparently, the cells maintain a basal level of methylation in the absence of external stimuli. If a type I, II or III attractant is added there is a large increase in the level of methylation of the appropriate MCP with little or no increase in the methylation of the other two MCPs[35,36,39,46]. This is shown in Fig. 3, lanes 5–8. As illustrated in Fig. 4*a*, the basal level of methylation of an MCP (defined by the plateau of radioactivity incorporated by cells from labelled methionine in the absence of added stimuli[43,46]) is rapidly increased in response to an attractant until a new plateau is reached. The methylation will remain at this new level as long as the attractant is present, and returns to the basal level only when the attractant is removed[46]. Addition of a repellent, on the other hand, causes a decrease in methylation[46] (Fig. 4*b*), and removal of repellent causes re-methylation (M.S.S. and M.F.G., unpublished); these results might be expected as repellents and attractants have opposite effects on behaviour.

The chemical reactions which are used to regulate the level of methylation of the MCPs are shown in Fig. 5. Methyl groups are transferred from *S*-adenosylmethionine to the MCPs[47] by esterification of the γ-carboxyl group of one or more glutamic acid residues[48,49]. Methyl groups are removed by a reaction in which the final product is methanol[50,51]. Both reactions have been demonstrated in *in vitro* systems[47,50]. A methyl transferase has been partially purified and found to be a soluble protein with

a molecular weight of about 41,000 (ref. 47). An esterase has also been identified; it is a soluble protein and has a molecular weight of about 100,000 (ref. 50).

Involvement of methylation in sensory adaptation

What role do these changes in methylation play in the chemotactic response? This question can be investigated by observing the behaviour of cells in which the methylation reaction has been blocked. The discovery that *cheX* mutants of *E. coli* have the acceptor proteins present in the membrane but lack the ability to methylate them[35,36,43,47] provided an opportunity for this type of experiment. A number of independent *cheX* mutants have been examined: each initiated the appropriate behavioural responses when subjected to attractant and repellent stimuli, but in each the responses were abnormally prolonged[52,53]. For example, wild-type cells typically exhibit a 5-min period of smooth swimming following the addition of the attractant α-aminoisobutyrate (50 mM final concentration) but a *cheX* mutant failed to adapt to this stimulus even after 36 h of exposure[53]. It seems, then, that the process of sensory adaptation is defective in these mutants.

Methylation has also been blocked by starving methionine auxotrophs of methionine and consequently depleting them of

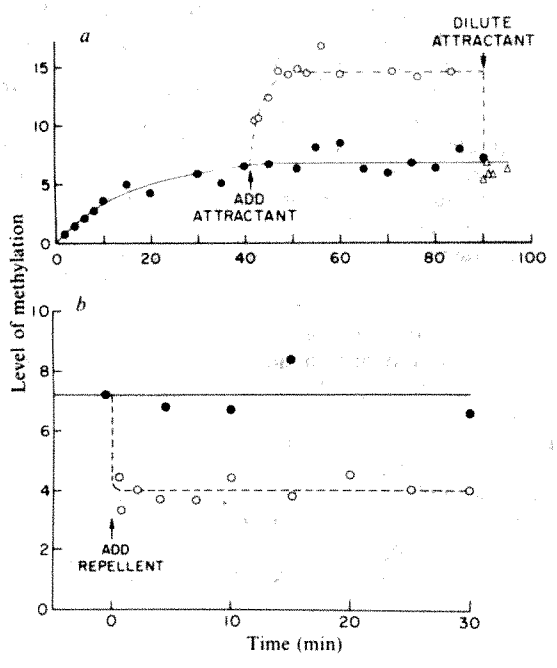


Fig. 4 Effect of chemical stimuli on the methylation of MCP (from ref. 46). Samples were electrophoresed on a 12% SDS polyacrylamide gel. A fluorographic image of the gel was obtained and the MCP region in the developed film scanned with a densitometer. The 'level of methylation' of each sample is the area under the densitometric curve divided by the amount of protein applied to the gel, and is expressed in arbitrary units. The data were obtained by scanning the entire MCP region and thus represent the behaviour of aggregate MCP and not that of any one band. *a*, [*Me*^{3}H]methionine was added at time zero in the presence of a protein synthesis inhibitor. After 40 min incubation the culture was divided in two. One group of cells (○) was stimulated by the addition of the type I attractant α-aminoisobutyrate (50 mM final concentration), the other group of cells (●) received no addition. The concentration of α-aminoisobutyrate was subsequently lowered by diluting the stimulated cells 100-fold into medium containing no α-aminoisobutyrate (△); the first point was obtained 15 s after this dilution. (A 100-fold dilution of stimulated cells into medium containing α-aminoisobutyrate had no effect on the level of methylation.) Similar data have been obtained for type II and type III attractants. *b*, Cells were incubated with [*Me*^{3}H]methionine for 30 min and the culture divided in half. One part received no addition (●) and a mixture of type I repellents was added to the second (○). Zero time refers to the time at which the stimulus was added. The first sample was obtained 20 s after stimulation. When the repellent was removed (at 2 min) by filtration and the cells resuspended in medium containing [*Me*^{3}H]methionine, the level of methylation returned to its initial value 20 min after filtration, the earliest time studied (data not shown, M.S.S. and M.F.G., unpublished).

the methyl donor for the reaction. Cells partially starved of methionine respond to the addition of attractants but do so for abnormally long periods[54,55]. The severity of this adaptation defect increases as the bacteria become further depleted of methionine[55] until finally adaptation fails entirely[56]. Thus, two independent methods of blocking methylation lead to the same conclusion: methylation is involved in the process of sensory adaptation.

The changes in methylation level following addition or removal of stimuli (see Fig. 4) could serve as the biochemical mechanisms which regulate the processes of adaptation and de-adaptation[46,56]. This hypothesis may be summarised as follows: (1) the level of methylation of the MCPs observed before a stimulus is added corresponds to a state in which the cell is fully sensitive to the stimulus; (2) the increase in methylation level that follows the addition of an attractant constitutes the process of adaptation to the attractant; (3) the final level of methylation corresponds to the state of complete adaptation to the attractant, and maintenance of the level corresponds to the maintenance of that state; (4) demethylation, which results from removal of the attractant, constitutes the process of de-adaptation whereby sensitivity is re-established; (5) for repellents, as suggested by the opposite nature of the behavioural responses, the adaptation and de-adaption processes are reversed relative to attractants, so that the decrease in methylation that follows addition of repellent represents the process of adaptation, whereas the increase in methylation that follows its removal corresponds to the process of de-adaptation.

There is considerable evidence to support this proposal. First, the methylation and demethylation reactions show the same asymmetry as the adaptation and de-adaptation processes[16,17,26,46]. Specifically, methylation, like the process of adaptation which follows addition of an attractant, is slow, while demethylation, like the process of de-adaptation which follows removal of an attractant, is fast (Figs 1*a*, 4*a*). This analogy holds true in reverse for repellents. Second, if the methylation reaction actually controls the adaptation process, the cells should respond to an attractant only while the level of methylation is increasing. Data indicate that the time required for the increase in methylation is very similar to that required for adaption[46]. In fact, when two stimuli are compared it is found that the ratio of the length of the responses to these two stimuli is equal to the ratio of the half-times of the changes in methylation induced by the stimuli[46]. Third, there is a striking correlation between the methionine dependency of the behavioural response and the methionine dependency of methylation[46,56]. Adaptation to attractants is methionine dependent, but once a cell has adapted to an attractant it will remain adapted even if methionine is subsequently removed; furthermore, de-adaptation can occur in the absence of methionine[56]. Correspondingly, it has been demonstrated that methylation requires the presence of methionine, but that both maintenance of an attractant-induced level of methylation and demethylation when the attractant is removed do not[46]. Taken together, these observations support the hypothesis that the state of methylation of the MCPs regulates the state of adaptation of the cell.

The control of the level of methylation

As seen in the preceding sections, the methylation and demethylation of the MCPs are carefully regulated. A study of the generally non-chemotactic mutants suggests that the products of some of the *che* genes play a part in the control of these processes. The *che* mutants can be divided into two groups based on their methylation phenotypes.

cheX and *cheB* mutants of *E. coli* (*cheR* and *cheX* of *S. typhimurium*) incorporate virtually no radioactive methyl groups into the MCPs[35,36,43,47]. Present evidence indicates that the product of the *cheX* gene is the methyl transferase[47], and the product of the *cheB* gene is the methyl esterase[50,57]. (Apparently, *cheB* mutants fail to incorporate label into the MCP polypeptides because they are always fully methylated[57]

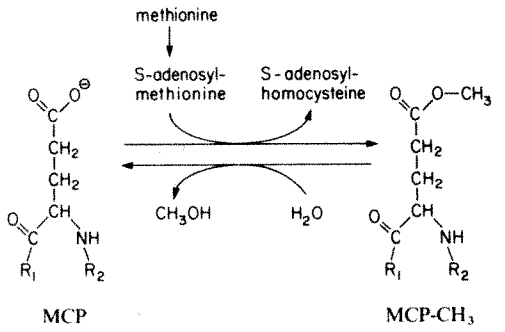


Fig. 5 Protein modification reactions discussed in this article.

and cannot demethylate to provide sites for the incorporation of labelled methyl groups.) Interestingly, lesions in either of these genes affect the methylation of all three MCPs[35,36,43,47,50,57], so the methylating and demethylating enzymes are probably not specific for any single MCP. From biochemical studies[84] it seems that the specificity required to permit a particular stimulus to affect the methylation of only MCP I or MCP II or MCP III results from physical contact between a chemoreceptor and one of the three MCPs, an interaction that alters the ability of that MCP to be methylated or demethylated.

Mutations in the other *che* genes produce strains that do incorporate radioactive methyl groups, although in some cases the amount and/or the distribution of label incorporated into the various MCPs is altered (refs 35, 36, 43, 47 and M.S.S. and M.F.G., unpublished). However, no interpretation of these defects has been formulated, and the functions of the products of these genes remain unknown.

Sensory excitation in bacterial chemotaxis

In the above discussion the chemotactic response has been separated into two processes, called excitation and adaptation. The validity of this separation is demonstrated best by the *cheX* mutants, which become excited in response to stimuli but fail to adapt. Excitation, like adaptation, seems to involve the MCPs[35,36], as mutations in *tsr*, *tar* and *trg* (the genes that apparently code for MCP I, II, and III) produce strains that are defective in excitation of type I, II and III stimuli, respectively[35,36,39,58], and a triple mutant carrying all three lesions is unable to produce an excitatory response to any stimulus tested (C. B. Ball and H. Kondoh, unpublished). However, unlike adaptation, excitation does not involve methylation of the MCPs, as *cheX* mutants, which have the MCPs but cannot methylate them, can still give an excitatory response[52,53]. The molecular details of the excitatory process remain to be elucidated.

A working model for the role of methylation in bacterial chemotaxis

Regulation of the chemotactic response is ultimately manifested as control over whether the flagella rotate in the anticlockwise or clockwise direction and hence whether the cell swims smoothly or tumbles. Some parameter, which we call the rotation regulator, must determine the sense of this rotation. (In previous work this parameter has been called the response regulator or the tumble regulator[9,12,14].)

In our model there are two processes that involve the MCPs: an excitatory process, whose activity changes rapidly when a stimulus is presented, and an adaptational process, whose activity changes slowly (Fig. 6*a*). It is the ratio of these two activities that determines the level of rotation regulator. When the system is at rest (that is, when the cell is not responding to any stimulus) the two processes are balanced so that the rotation regulator is present at a level that allows both smooth swimming and tumbling (see Fig. 6). If an attractant is added, the receptors immediately produce a signal that results in a rapid increase in the activity of the excitatory process, and the level of the rotation regulator rises. The flagella rotate exclusively anticlockwise and tumbling is suppressed (that is sensory excitation occurs). At the same time, the receptor signal also causes a slow increase in the activity of the adaptation process, namely, an increase in the level of methylation of the appropriate MCP. This tends to counteract the effect of the excitational process, and the level of the rotation regulator begins to return to the resting level. Ultimately, when the new plateau level of methylation is reached, the two processes are again in balance, and the level of the rotation regulator has returned to a point that allows both smooth swimming and tumbling (Fig. 6*b*, *c*). The cell has adapted, and the response is terminated. When the attractant is removed, both the excitatory process and the level of methylation fall to their original value. However, the removal of methyl groups lags slightly behind, and there is a brief decrease in the level of the rotation regulator, resulting in a brief tumbling response. Once this is completed the cell has de-adapted and is ready to respond again. (According to this model the response to addition of a repellent would be exactly analogous to the response to removal of an attractant, and removal of repellent would be like addition of attractant.) A molecular interpretation is presented in Fig. 7.

Our model is representative of a class of models which use two competing processes to initiate and terminate behavioural responses[59–61]. Such two-process models have been successfully applied previously to explain the properties of the chemotactic response in bacteria[16,26]. Although our version accounts for most of the phenomena of chemotaxis, including the relationship of methylation to the response, there are several observations which do not fit well into the framework as presented.

The model predicts that *cheX* strains, which do not have the complete apparatus with which to methylate their MCPs[47], should not tumble at all (since the activity of the excitational process should always exceed that of the adaptation process) whereas *cheB* strains, whose MCPs are permanently methylated[50,57], should tumble continuously (since the activity of the adaptation process should always exceed that of the excitational process). Indeed, this is true in most conditions[14,15]. However, *cheX* mutants can be made to tumble by the addition of a repellent[47,52,53] and *cheB* mutants can be made to swim smoothly by addition of an attractant[14]; thus, additional studies of these mutants and/or refinements of the model are required.

Several important questions pertaining to the methylation and demethylation reactions remain unanswered. First, the identity of the rotation regulator has not been established, although evidence has been presented that in *Bacillus subtilis* it may be intracellular calcium ions[62]. Second it remains to be seen how the MCPs function to control the level of the rotation regulator. For example, they could be enzymes that synthesise the rotation regulator, or they could be ion gates that control the

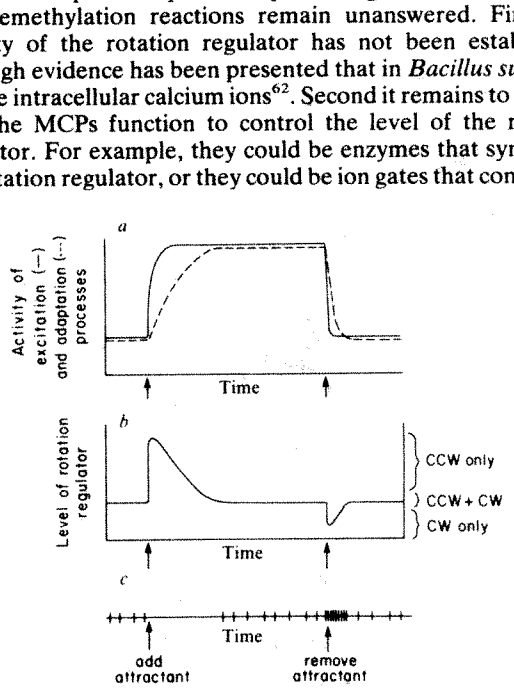


Fig. 6 Model for the role of methylation in bacterial chemotaxis. CCW stands for anticlockwise rotation of the flagella, which corresponds to smooth swimming, and CW stands for clockwise rotation, which corresponds to tumbling. Figure 6*c* is repeated from Fig. 1*a*. See text for explanation. (Increase in level of rotation regulator producing anticlockwise rotation is an arbitrary choice; it could just as well be a decrease.)

level of an ion which serves as the regulator. Third, we do not know the details of how the binding of stimulatory chemicals to chemoreceptors influences the methylation of the MCPs.

Despite the work that remains to be done, the identification and characterisation of the methylation and demethylation reactions represent a significant step forward in the effort to understand how bacteria transduce sensory signals into a behavioural response.

Carboxyl methylation in eukaryotic systems

The existence of protein-carboxyl methylation in eukaryotes was known before the discovery of the reaction in bacteria[63-71]. However, its function in eukaryotic cells is only now beginning to be understood. This reaction seems to regulate several processes, some behavioural in nature. The one most closely related to the bacterial system described above is leukocyte chemotaxis.

A number of different types of white blood cells use chemotaxis in the initial phase of their response to infectious or inflammatory agents[72]. Many natural and synthetic substances act as attractants for these cells[73]. During the past two years several groups have investigated the possibility that a carboxyl methylation reaction might be involved in this phenomenon[74,75]. Measurement of the methanol released when carboxyl-methyl esters are hydrolysed at high pH[74] has shown that the addition of an attractant to leukocytes causes a perturbation of the level of carboxyl methylation[74], and that both the behavioural response and the change in carboxyl methylation can be prevented by the prior addition of an attractant antagonist[76]. Furthermore, it has been found that incubation of leukocytes with compounds that inhibit methylation reactions in general[75,77,78] blocks the behavioural response[75,79]. Thus methylation seems to be involved in the sensory response of leukocytes.

It is interesting that methyl-acceptor proteins and a methylating activity are also found in high concentrations in mammalian testis, spermatazoa and spermatids[66,71,80]. It is possible that here, too, there is involvement of protein methylation in a chemotactic response. Although there is at present no evidence for or against the idea, it may be that mammalian spermatazoa use chemotaxis as an aid to fertilisation; certainly, this occurs in simpler animals[81].

Protein-carboxyl methylation also plays a part in neural and endocrine functions, as indicated by the following results. (1) Protein-carboxyl methylase is found in most mammalian organs but most abundantly in nervous tissue and endocrine glands, for example brain and pituitary[66,70,71]. Methyl-acceptor proteins are also widely distributed, the pituitary gland being the richest source[70,71]. (2) The methylating enzyme is apparently transported down axons and occurs in synaptosomal preparations[71]. A function at the synapse is indicated by the fact that synaptosomes from the hypothalamus undergo changes in the level of methylation following depolarisation[82]. (3) Stimulation of the adrenal medulla, which results in the secretion of catecholamines, produces an increase in methylating activity[76,83]. When methylation is blocked by an inhibitor, secretion does not occur[76]. (4) In the parotid gland β-adrenergic stimulation, which releases amylase, is accompanied by an increase in the level of methylation of methyl acceptor proteins[80].

Taken together, these results suggest that some of the functions of carboxyl methylation in eukaryotes, as in prokaryotes, concern signal transduction and the control of behaviour at the cellular level.

Conclusion

The methylation and demethylation of protein-carboxyl groups are reactions which may play important parts in the regulation of cellular activities. Protein-carboxyl methylation has already been implicated in several behavioural control mechanisms in a variety of prokaryotes and eukaryotes. That the widespread occurrence of this reaction has only recently been recognised is due at least in part to the lability of carboxyl esters which hinders their identification by usual analytical techniques. With improved methodology protein-carboxyl methylation may be found as ubiquitously as the better known protein phosphorylation reactions.

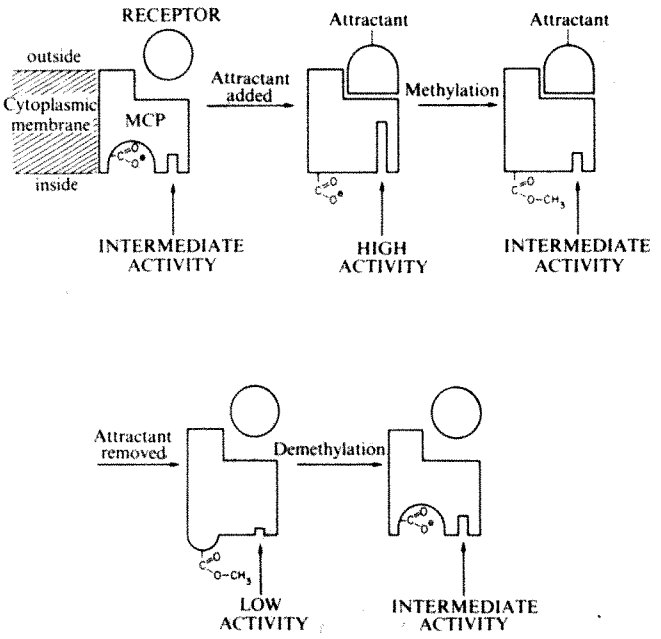


Fig. 7 Speculative molecular model for effect of attractant on the activity of MCP. This activity (ability to generate rotation regulator) is indicated in the figure at various points in the response. In the unstimulated state (top left) MCP has intermediate activity leading to an intermediate level of rotation regulator, and thus both anticlockwise and clockwise rotation can occur (see Fig. 6). When the attractant is added (top centre), it binds to the chemoreceptor and brings about a physical interaction between the receptor and the appropriate MCP. This has two effects: (1) via the excitation process, there is a rapid increase in activity and thus a rise in the level of rotation regulator, so that only anticlockwise rotation occurs; and (2) carboxyl groups become exposed and available for methylation. Slowly methylation then takes place (top right) and causes the activity to return to the intermediate level; thus adaptation occurs. Removal of attractant (bottom left) decreases the activity of MCP; this leads to a decrease in the level of rotation regulator and thereby produces clockwise rotation. Demethylation (bottom right) restores the activity to the unstimulated level. As discussed in the text, neither the nature of the rotation regulator nor the means of its generation by MCP is known. The model shows several features which have been experimentally determined. First, MCP is believed to span the cytoplasmic membrane: interaction of chemical stimuli with chemoreceptors must take place on the outside of the cytoplasmic membrane, and at least some of the receptors are found in the periplasm (the space between the cytoplasmic membrane and the cell wall)[8]; methylation and demethylation must take place on the inner surface of the cytoplasmic membrane, since the enzymes that catalyse these reactions are cytoplasmic[47,50]. Second, upon interaction with ligands, conformational changes in chemoreceptors have been described[85,86]; these observations have been used previously to postulate a ligand-induced conformational change as an obligatory first step prior to the interaction of receptor with subsequent components of the chemotaxis machinery[87]. Third, chemoreceptors appear to interact with MCP directly[84].

Bacteria such as *E. coli* and *S. typhimurium* offer a favourable system for the study of the relationship of protein methylation to behaviour, because these organisms are relatively simple and well characterised both biochemically and genetically. From studies of sensory transduction in bacteria a set of facts and concepts may emerge that is generally applicable to our understanding of the phenomena that underlie behaviour and signal transduction in higher organisms, as evolution seems to have conserved at least some of the biochemical mechanisms involved.

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articles

Riftward younging of volcanic units in the Addis Ababa region, Ethiopian rift valley

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A general riftward younging of surficial volcanic units is a feature of the Addis Ababa sector of the Ethiopian rift valley. The narrowing of the volcanic zone with time is due to crustal necking and topographical control.

THE development of continental rift valleys can be accompanied by a progressive narrowing of the zone of tectonism and volcanism as crustal thinning proceeds[1]. In the Ethiopian rift valley, this phenomenon is difficult to discern owing to voluminous cover from Quaternary rhyolitic ash-flow and air-fall tuff eruptions. However, the complex and perhaps atypical Addis Ababa embayment[2] reveals disconnected exposures of older volcanic units with an overall gentle southeastwards dip towards the rift axis[3–12]. This embayed sector of the western margin of the rift has developed as a broad downwarp lacking any major escarpment; although faulting is common, it is limited to block tilting with associated downthrows to the south-east of tens of metres[2,8].

We review here radiometric ages of volcanic rocks along a 100-km-wide swathe, south-east from the Abbay (Blue Nile) basin via Addis Ababa on the rift margin to the rift floor axis at

* Deceased

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Table 1 K–Ar age determinations on volcanic samples from the northwestern sector of the Addis Ababa–rift floor traverse

Sample	Locality	Lat (grad N)	Long (grad E)	Analysed portion	% K_2O	Vol ^{40}Ar ($\times 10^{-4}$ mm^3 g^{-1})	% atmospheric ^{40}Ar	Age (Myr)
Aphanitic basalt (2211)	19 road Km N. of Addis Ababa	10.17	43.06	Whole-rock	1.47	11.17 10.65	56.6 13.3	22.8 ± 1.5
Porphyritic trachyte (ENT)	North side Entoto, 5 km north of Addis Ababa	10.13	43.07	Felspar	5.31	38.01 37.86	44.6 30.9	22.0 ± 0.8
Olivine basalt (2213)	North–Central Addis Ababa	10.07	43.07	Whole-rock	0.72	1.69 1.83	65.5 64.6	7.5 ± 0.6
Basalt (2214)	Northeastern Addis Ababa	10.06	43.09	Whole-rock	0.94	1.95 2.09	64.8 68.2	6.6 ± 0.5
Basalt (2215)	Southwestern Addis Ababa	9.995	43.010	Whole-rock	3.07	3.71	37.5	3.7 ± 0.4
Fiammé-rich welded tuff (A1)	Eastern Addis Ababa	10.020	43.080	Feldspar	1.56	2.61 2.56	80.4 92.9	5.11 ± 0.4
Welded tuff (A2)	Eastern Addis Ababa	10.020	43.085	Felspar	6.28	6.30 6.92	54.7 70.3	3.26 ± 0.1
Felsphyric basalt (2207)	South side Mr Yerer	9.87	43.30	Whole-rock	2.28	2.60	31.3	3.5 ± 0.4
Felsphyric basalt (2207)	South side Mt Yerer	9.87	43.30	Feldspar	0.79	1.00	78.7	3.9 ± 0.4
Trachyte (2208)	South side Mt Yerer	9.87	43.30	Feldspar	3.67	4.34	41.6	3.7 ± 0.4
Rhyolite (2209)	South side Mt Yerer	9.87	43.30	Feldspar	5.08	5.35	69.2	3.3 ± 0.4
Porphyritic rhyolite (MEN)	Mt Menegasha tholoid	10.04	42.87	Feldspar	5.75	5.76 5.70	55.6 44.2	3.09 ± 0.12
Porphyritic trachyte (Z1)	Northeastern base of Mt Zukwala	9.51	43.195	Feldspar	4.14	1.64 1.79	80.4 96.1	1.28 ± 0.15
Porphyritic trachyte (Z2)	Half-way up northeastern side Mt Zukwala	9.50	43.185	Feldspar	5.52	1.57 1.71	80.9 79.8	0.92 ± 0.04
Porphyritic trachyte (Z3)	North rim of Zukwala crater	9.493	43.170	Feldspar	5.40	1.41 1.54	88.1 90.6	0.85 ± 0.05

Samples collected by W.H.M., analysed by D.C.R.
$\lambda_e = 0.581 \times 10^{-10}$ yr^{-1}; $\lambda_\beta = 4.962 \times 10^{-10}$ yr^{-1}; $^{40}K/K = 1.167 \times 10^{-2}$ atom %.

Wolenchiti (Fig. 1), and include 33 new K–Ar age determinations on samples critically selected for their position along the rift transect.

Plateau sequence

The plateau basalt sequence exposed along the Abbay gorge road section, 150 km north-west of Addis Ababa, is observed in tributary canyons to thicken southeastwards. The Abbay basalts have yielded K–Ar ages ranging from 27.9 Myr (basal flow) to 24.0 Myr (top flow)[13]. These data are consistent with values obtained by Zanettin and coworkers[9] on equivalent basalts and a rhyolitic ignimbrite from the upper reaches of the Mugher tributary, but are appreciably older than the 22.1–20.2 Myr age-range obtained by Jones[14] on the same Mugher basalt flows. Maximum ages of 29 Myr have been obtained for basalts from the opposing, eastern shoulder of the rift valley[11,12].

On the 2,600 m plateau immediately north of Addis Ababa, the top flood basalt at Sululta yields an apparent age of 22.8 Myr (Table 1), conforming with the Abbay basalt ages and the observed addition of intervening, less far-travelled flows derived from the rift-margin sources[15]. Basalts (~13 Myr old) of the Gorfu Mts volcanic centre[9] are built on the plateau.

Embracing Addis Ababa, the arc of the Entotto hills is formed of gently north-dipping comenditic lavas and obsidian-rich tuffs overlying related olivine trachytes[16,17]. Our date of 22.0 Myr for a northern Entotto lava is concordant with a previous 22.6 Myr age for a southwestern Entotto comendite[9]. These early Miocene silicic volcanics may represent a localised terminal episode to massive Oligocene fissure–basalt activity in the Addis Ababa region, but their stratigraphic relations to the Sululta basalts immediately to the north are not exposed because of a faulted contact. The only other silicic rocks of known similar age occur near the bottom of the Kassam gorge within the rift valley, 40 km east of Entotto, where separate investigations have produced radiometric ages in moderate agreement. Justin-Visentin *et al.*[9] obtained an age of 23.9 Myr, and Rex *et al.*[18] an age of 21.4 Myr for the lowermost silicic ignimbrite within the exposed 1,000 m thick Kassam basalt section. (The base of the basalt pile farther down the Kassam gorge has not been sought since Francaviglia's provocative identification there of a restricted window of unfossiliferous, supposedly Mesozoic sediments[19].) The available evidence for early Miocene silicic volcanism in the Addis Ababa region suggests an isolated centre at Entotto, with erupta thinning both riftwards and plateauwards: this centre was later strongly upfaulted as a NE–SW trending horst at the margin of the developing rift.

Above the lower basalts of the Kassam gorge, a 100 m thick trachytic bed at 2,100–2,200 m elevation in the northwestern wall has yielded an age of 16.0 Myr, suggesting a downwarped equivalent to plateau-surface (2,800 m) outcrops in the Debra Berhan region, 60 km farther north-east[9]. Thin rhyolitic ignimbrites of the upper Kassam and Shano area (Fig. 1), with ages in the 13–11 Myr bracket, interdigitate with basalts of the Gorfu and Meghezez piles[9], and on the opposing, eastern side of the rift valley, ignimbrites of the same age-range form the 'main silicic formation', up to 300 m in thickness[20].

In the environs of Addis Ababa, the oldest visible rocks post-dating the Entotto rhyolites are alkali olivine basalts lapping the southern flanks of the Entotto horst[4,21]. These very localised basalts yield ages clustering around 7 Myr (Table 1 and ref. 22), and seem to have no time/composition equivalents except on the opposing, eastern shoulder of the rift valley[11]. However, thin but widespread rhyolitic ignimbrites that occur along the plateau margin north-east of Addis Ababa yield ages in a broad 9.2–7.9 Myr range[9].

In Addis Ababa town itself, the north-east-trending Filwoha fault separates, to the north, the 7 Myr-old basalts here overlain by pantelleritic welded tuffs from strongly welded trachytic tuffs to the south. The latter yield apparent ages of 5.1 and 3.3 Myr (Table 1). These ages overlap with the periods of activity of two important trachyte volcanoes: Wachacha, 15 km west of Addis Ababa, and Yerer, 25 km south-east of Addis Ababa (Fig. 1). Wachacha erupted a terminal lava phase (thick biotite–anorthoclase trachyte flows) dated at 4.6 Myr[9,23]. Isolated patches of welded tuff in the Sululta region, dated at 5.4 Myr[9], as well as the

5.1 Myr welded tuff in Addis Ababa, were probably derived from the Wachacha centre before the Entotto and Filwoha faulting. Likewise, welded tuff exposed in the Akaki river canyon, 25 km south of Addis Ababa and dated at 5.3 Myr, was probably derived from Furi, a satellite of Wachacha.

Yerer volcano lacks the lava cap featuring Wachacha, and its more bare aspect belies the clustering of K–Ar ages at 3.6 Myr (ref. 24 and Table 1). The succession at Yerer is[25]:

(4) NW–SE trending basaltic and subordinate trachytic dykes
(3) Vesicular porphyritic basalts (<20 m), flanking 2
(2) Thick trachytic agglomeratic tuffs and subordinate lavas
(1) Massive welded tuffs

In Table 1, the rhyolite and trachyte ages of 3.3 and 3.7 Myr belong to unit (2), and the basalt ages of 3.5 (whole-rock) and 3.9 (plagioclase) Myr to unit (3). A tendency for ^{40}Ar migration into plagioclase phenocrysts of Ethiopian rift basalts has been detected by J.G.M. (unpublished data). Contemporaneous activity formed the isolated Managasha tholoid on the northern flanks of Wachacha volcano, but extensive volcanism was now migrating south-east towards the axis of the proto-rift.

For the sector between Bishoftu and Mojjo (respectively 45 and 70 km south-east of Addis Ababa) no radiometric ages are available. In contrast to the Addis Ababa–Bishoftu sector, where NE–SW faulting upthrown north-west exposes units derived from Yerer, Wachacha and superimposed end Pliocene–Pleistocene basalt cones[22,25], exposures on the plains south-east of Bishoftu are restricted to small silicic centres and young Quaternary basalt cones associated with the Bishoftu maars[25,26]. At the Mojjo River canyon, Pleistocene volcaniclastic and diatomaceous sediments a few metres thick lie on 20 m+ of ignimbrite, but thicken riftwards to 15 m+ (base not seen) in the Melka Lemi canyon 3 km east of Mojjo[27,28].

At Tedi village, 5 km east of Mojjo, the Gadamsa segment of the Wonji fault belt (WFB) is encountered, a zone of closely-spaced, anastomosing normal faulting[29,30]. The WBF here is 10 km wide and comprises some 12 strip-like blocks, each 700–1,000 m wide, that are tilted down to the ESE at 2–3 grads dips. The separating faults trend NNE and have upthrows to the east averaging about 15 m. However, throws of up to 100 m are responsible for narrow horsts at the margins of the fault belt. The summary stratigraphy within the WFB here is:

(3) Weakly-welded tuff, thickening and more crystal-rich eastwards (2–4 m)
(2) Massive pumiceous sediments with occasional well-bedded sub-units and palaeosols (10–20 m)
(1) Strongly welded tuff, base not exposed (55 m+)

Unit (1), exposed also in the Mojjo River canyon, is presumed to have been derived from the Mukie volcanic centre 15 km north of Adama (Fig. 1). Unit (2) thickens both east and west, away from the higher topography of the WFB in the Mojjo–Adama sector. On the eastern side of the WFB, feldspar-phyric basalts are interbedded near the top of unit (2). They were derived from sources aligned along the subsequently faulted western margin of the Adama graben[30], although basaltic eruptions continued within the Adama graben after the deposition of unit (3). Unit (3) resembles unit (2) in being thinner or absent from the higher blocks and horsts of the WFB, but additionally thins out eastwards to zero.

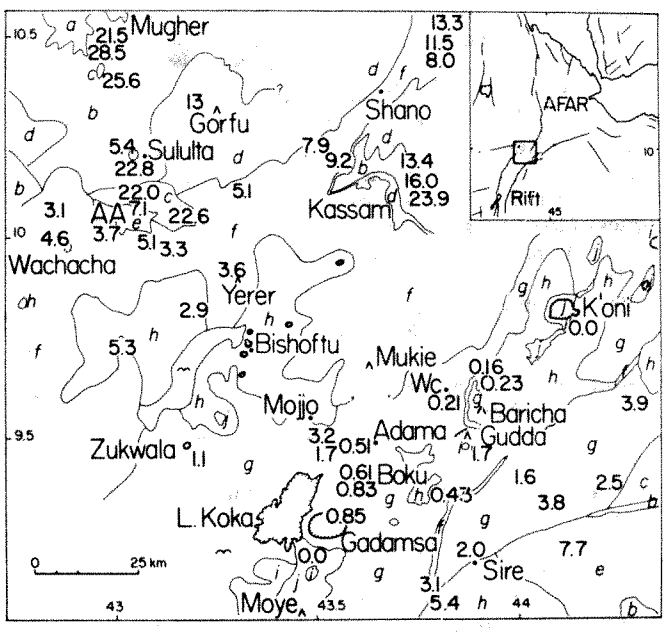


Fig. 1 Chronostratigraphic map of the Addis Ababa region and northern sector of the Ethiopian rift. Numbers are K–Ar ages in Myr. The lettered formations are: *a*, Mesozoic sediments; *b*, plateau basalts (older than 22 Myr); *c*, silicic lavas and tuffs (23–21 Myr); *d*, shield basalts and subordinate rhyolites (16–13 Myr); *e*, olivine basalts (8–6 Myr); *f*, ignimbrites and lavas (11–3 Myr); *g*, ignimbrites (<3 Myr); *h*, olivine basalts (<3 Myr); *i*, Holocene obsidian flows; *j*, Holocene mafic lavas. AA, Addis Ababa; Wc, Wolenchiti. The detailed fault pattern is omitted. Latitude and longitude in grads provide scale; 1 grad latitude equals 100 km.

New K–Ar dates for the WFB transect

We present four new K–Ar dates for this WFB transect (Table 2). Unit (3), sampled from the eastern foot of the Kimbibit horst[22,29], yielded an apparent age of 0.51 Myr. The basalt flow interbedded in unit (2) was sampled from the 'Stegosaur' horst overlooking the Adama graben, and yielded an apparent age of 0.61 Myr identical with that of a small olivine basalt flow lapping Gara Boku, 7 km farther south-east. Unit (1) was sampled 4 km east of Tedi and yielded an apparent age of 1.7 Myr, coeval with major silicic volcanism at Boseti Gudda volcano (on the Boseti segment of the WFB, east of Adama) and shield volcanism on the future eastern shoulder of the rift valley[11,12,31]. However, older rocks floor the rift under the Gadamsa WFB segment, according to the age of porphyritic comenditic obsidian forming the Gara Mariam horst at Tedi (Table 2). Our new date of 3.2 Myr revises previous values for this tholoid[22], and proves activity contemporaneous with Yerer and at the eastern, Siri margin of the rift[11,12]. Gara Mariam, like Entotto, is a discrete silicic igneous centre that has since been upfaulted among younger rocks, in response to isostatic forces acting at the margin of a tensional zone.

Gadamsa volcano with a 10-km-diameter caldera lies on the Gadamsa segment of the WFB some 20 km SSW of Adama[32]. An age of 0.85 Myr (Table 2) pinpoints the middle period of build-up of the Gadamsa cone. One surprise of our dating program is that an indistinguishable age, 0.83 Myr, applies to a late-stage obsidian lava from the similar-sized Boku caldera situated midway between Gadamsa and Adama (Fig. 1). Boku caldera is so severely broken that it required satellite imagery for its initial recognition[29,32]. It seems that the caldera–cone diameter ratio at Boku was probably close enough to unity that caldera collapse facilitated the destruction of the greater part of the remnant cone.

Zukwala volcano, 30 km north-west of Gadamsa, lies on an isolated (non-WFB) volcanotectonic line that projects NNE via a Holocene basalt lava cone to the Bishoftu maars[33]. The Zukwala trachytes yield a progressive sequence of ages, from 1.28 Myr for a basal exposed flow to 0.85 Myr for a crater rim flow (Table 1).

The Boseti segment of the en-echelon WFB is encountered east of Adama. The geology of this area is dominated by Boseti Gudda volcano[29,34], where a caldera is largely buried by a subsequent high cone of pantelleritic tuffs, pumice and subordinate lavas. Morbidelli *et al.*[11] obtained a K–Ar age of 1.6 Myr for a near-summit lava, but the fresh morphology of satellite cones and flows on the southern slopes of Boseti Gudda shows that parasitic activity continued into recent times. Boseti Gudda, and its comenditic neighbour Boseti Baricha immediately to the

Table 2 K-Ar age determinations on volcanic samples from the southeastern sector of the Addis Ababa–rift floor traverse

Sample	Location	Lat (grad N)	Long (grad E)	Analysed portion	% K_2O	Vol ^{40}Ar ($\times10^{-4}$ mm^3 g^{-1})	% atmospheric ^{40}Ar	Age (Myr)
Porphyritic obsidian (76–1)	Gara Mariam, Tedi	9.510	43.522	Feldspar	6.15	6.60	39.8	3.32 ± 0.06
Porphyritic obsidian (76–1)	Gara Mariam, Tedi	9.510	43.522	Matrix (excluding anorthoclase phenocrysts)	5.37	5.41	55.3	3.11 ± 0.06
Welded tuff (76–3)	3 km south-east of Tedi	9.500	43.541	Whole-rock	4.46	2.45	43.0	1.71 ± 0.04
Welded tuff (76–5)	Kimbibit horst, 4 km west of Adama	9.483	43.583	Whole-rock	5.10	0.850	91.2	0.51 ± 0.03
Olivine basalt (76–6)	Scarp, 2 km west of Adama	9.480	43.604	Matrix (excluding labradorite phenocrysts)	0.675	0.135	89.1	0.61 ± 0.03
Green welded tuff (76–10)	North rim, Gadamsa caldera	9.320	43.556	Whole-rock	0.669 0.810	0.185 0.218	94.8 93.5	0.85 ± 0.07
Olivine basalt (76–8)	Nagow cone, Gadamsa caldera	9.313	43.557	Whole-rock	1.25	0.168	94.6	0.41 ± 0.04
Olivine hyalo basalt (76–15)	Northeastern flanks, Gara Boku	9.417	43.650	Whole-rock	0.920	0.183	89.5	0.61 ± 0.04
Comenditic obsidian (76–16)	North summit, Gara Boku	9.415	43.645	Whole-rock	4.54	1.21	51.5	0.83 ± 0.02
Olivine basalt (76–7)	2 km south-west of Bofa	9.395	43.824	Whole-rock	1.28	0.185	90.8	0.44 ± 0.05
Olivine trachyte (76–12)	Jinjimma flow 1 km south-east of Wolenchiti	9.611	43.808	Whole-rock	4.74	0.325	78.9	0.21 ± 0.01
Hyalo-trachyte (76–13)	North flank, Gara Chisa	9.645	43.877	Whole-rock	2.14	0.161	94.4	0.23 ± 0.03
Aphyric obsidian (76–14)	Summit, Gara Chisa	9.654	43.873	Whole-rock	4.36	0.226	84.4	0.16 ± 0.01
Olivine mugearite (76–17)	Sogido, 7 km east of Wolenchiti	9.633	43.887	Whole-rock	1.82	0.212	91.1	0.36 ± 0.03
Holocene hawaiite flow (RV 337)	Tulu Moye volcanic centre	9.265	43.451	Whole-rock	n.d.	<0.010	100.0	0.0
19th century pyroxene hyalobasalt (G27)	Gariboldi volcanic centre	9.812	44.114	Whole-rock	1.15	<0.016	100.0	0.0
19th century hyalobasalt cinder cone (G203)	Gariboldi volcanic centre	9.81	44.11	Whole-rock	1.05	0.111	95.2	0.31 ± 0.06

Samples collected by P.M., analysed by J.G.M.
Constants as for Table 1.

north, lie on a line of major crustal fracturing from which mildly alkaline olivine basalts have erupted[34,35]. An extensive such flow sampled 2 km south of Bofa on the southern flank of Boseti Gudda yields an apparent age of 0.44 Myr (Table 2). Perfectly preserved end-Pleistocene cinder cones lie on a basalt fissure line displaced 7 km west from the Bofa–Boseti line[35,36].

On the lower western flanks of Boseti Baricha, the Jinjimma olivine trachyte cone issued a flow which we date at 0.21 Myr, consistent with rather well-preserved morphology[29,35]. The cone has been broken by a NNE-trending fault with an upthrow of ~200 m to the west[29], and smaller parallel faults cut the flow. 6 km farther NNE along this active and intensely sliced zone, trachyte obsidians from the spectacularly faulted and fissured Gara Chisa dome yield ages of 0.23 Myr (northern flank) and 0.16 Myr (summit). The Jinjimma–Chisa line of silicic volcanism thus seems to represent a recent westward side-stepping of silicic volcanism in the WFB. However, young mafic eruptions have continued from the main Boseti alignment, most notably voluminous and highly fluid mugearite flows from the col between Gudda and Baricha[34,35]. Our date of 0.36 Myr for a mugearite specimen from Sogido (Table 2) is certainly too old, as these lavas are stratigraphically younger than flanking obsidian flows from Gara Chisa dated at 0.23 Myr. If excess argon occurs at a level common to many Ethiopian rift basalts[37], then an age of 0.23 Myr would be indicated.

Conclusions

In summary, a general riftward younging of surficial volcanic units is a clear feature of the Addis Ababa sector of the Ethiopian rift valley. Exceptions to this pattern are interesting but second-order phenomena, such as the Zukwala volcanic line along the western margin of the rift, and young centres on the plateau west of Addis Ababa[27,40]. The younging phenomenon is consonant with the broad crustal downwarp developed between the Abbay basin and the rift axis[38,39]. Downwarping towards the future site of the rift valley began in late Jurassic time, continued throughout Oligocene–Miocene flood basalt extrusion, and was active together with block faulting during early–mid Pleistocene differential uplift of the Ethiopian plateaux.

But does the progressive confining of rift volcanism with time merely reflect topographic control, from deepening of the proto-rift trough that culminated in mid-Pleistocene downfaulting of the rift valley? Or does it reflect a fundamental control of eruptive sites from processes of crustal necking and separation[41]? Plate tectonic analysis of the Afar triple-junction has led to two estimates of Ethiopian rift 'opening' of 65 km (ref. 42) and 40 km (ref. 43). The latter better fits the geological and geophysical constraints. Geologically, there is continuous exposure of ~24 Myr-old volcanic units for at least 20 km riftwards down the Kassam gorge; and at the southern end of the Ethiopian rift, the floor is severely faulted but exposes Precambrian rocks[44]. Geophysically, crustal thickening occurs east across the Ethiopian plateau to Addis Ababa and possibly as far as Bishoftu[45,46]; however, gravity modelling of the crust within the rift implies drastic thinning and an extension of 25–40 km if stoping processes are ruled out[47,48].

Elucidation of the cause of progressive confinement is complicated by the changing character of rift volcanism with time, from voluminous flood basalt lavas through episodic but intimate mafic and silicic eruptions, to relatively voluminous silicic tuffs. Furthermore, earlier eruptive sites are now hidden. We conclude, on the basis of our work together with known geophysical constraints, that narrowing of the volcanic zone in the Ethiopian rift with time is due partly to crustal necking, but more significantly to topographical control[49].

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Sequences at the somatic recombination sites of immunoglobulin light-chain genes

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The entire nucleotide sequence of a 1.7-kilobase embryonic DNA fragment containing five joining (J) DNA segments for mouse immunoglobulin κ chain gene has been determined. Each J DNA segment can encode amino acid residues 96–108. Comparison of one of the five J DNA sequences with those of an embryonic variable (V) gene and a complete κ chain gene permitted localisation of a precise recombination site. The 5′-flanking regions of J DNA segments could form an inverted stem structure with the 3′-non-coding region of embryonic V genes. This hypothetical structure and gel-blotting analysis of total embryo and myeloma DNA suggest that the somatic recombination may be accompanied by excision of an entire DNA segment between a V gene and a J DNA segment. Antibody diversity may in part be generated by modulation of the precise recombination sites.

IMMUNOGLOBULIN polypeptide chains consist of two regions, the amino-terminal half, called the variable (V) region, and the carboxyl-terminal half, called the constant (C) region. In embryonic cells the two regions are encoded in widely separated DNA segments. During differentiation of bone marrow-derived (B) lymphocytes the two DNA segments are brought into proximity by site-specific recombination, producing a single transcription unit[1,2].

The details of the sequence organisation before and after recombination have been worked out in the mouse λI light-chain system by analysis of cloned DNA fragments [3,4]. These studies revealed that in embryo cells the conventionally defined V region is itself encoded in two separate DNA segments, one (V DNA) coding for the major part of the V region (residues 1–97) and the other (J DNA) coding for the rest of the V region (residues 98–109). Although the distance between the V and the C DNA segments is unknown, the J DNA segment has been mapped at 1.2 kilobases towards the 5′-side, relative to the direction of transcription, of the C DNA segment.

Somatic recombination takes place at the 3′-end of the V DNA segment and the 5′-end of the J DNA segment. Consequently, in a λI chain synthesising B lymphocytes or plasma cells, although the DNA segment coding for the entire V region is much closer to the C DNA segment, the two DNA segments are still separated by 1.2 kilobases. This untranslated sequence (intron)[5] separating the J and C DNA segments is thought to be removed at the RNA level by a process called splicing[6,7]. Hence, the J DNA segment and its flanking sequences seem to be involved in two key processes essential for the expression of an immunoglobulin gene: site-specific V–J recombination and J–C splicing[3].

More recently, we have analysed the organisation of mouse κ light-chain genes[8,9]. A comparison of the sequence organisation of the two light-chain gene systems revealed both common and differing features. As in the λ gene system, a secreted κ chain is encoded in three separate DNA segments in embryonic cells, Vκ, Jκ and Cκ. In addition, somatic recombination takes place between embryonic Vκ and Jκ DNA segments. However, unlike the λI gene system, the mouse genome contains multiple Vκ and Jκ DNA segments, the latter being clustered in the vicinity of a single copy of the Cκ DNA segment.

For a better understanding of the structural features of the recombination sites we have determined the nucleotide sequences of the Jκ cluster as well as the 3′-end region of an embryonic Vκ DNA. This information, in addition to Southern

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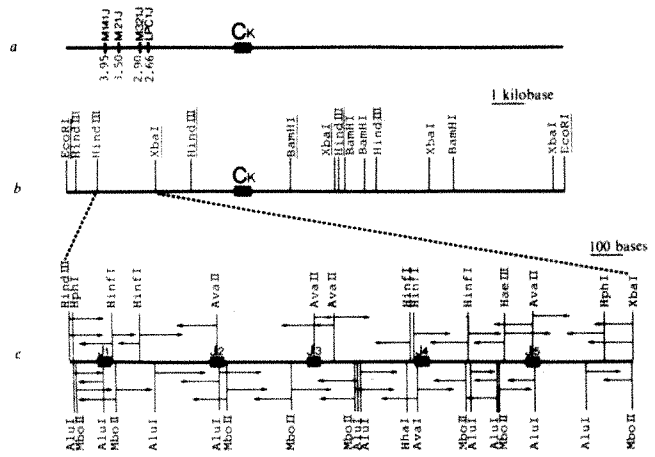


Fig. 1 *a*, R-loop map of the 15-kilobase insert of Ig146. κ mRNAs from four different myelomas, MOPC141, MOPC21, MOPC321 and LPC1, were individually annealed with the Ig146 *Eco*RI DNA fragment and the position of the corresponding J DNA segment was determined by electron microscopy (see ref. 9 for details). The bars represent the J DNA segments. The numbers indicate their distance from the Cκ DNA segment. *b*, Restriction enzyme cleavage map of the 15-kilobase insert of Ig146. The whole Ig146 phage DNA and the purified vector phage (λWES)[30] arm DNA (1 μg each) were digested either with one of the indicated enzymes or with that enzyme plus *Eco*RI, and separated by electrophoresis in a 1% agarose gel. Comparison of the single and double digestion patterns allowed localisation of the outermost cleavage sites of each enzyme on the 15-kilobase insert. Other cleavage sites were determined by comparing the pattern obtained by a single enzyme digestion (enzyme A or B) with that obtained by a double enzyme digestion (enzyme A plus B). The position of the Cκ DNA segment relative to the underlined cleavage sites was determined by examination of R-loop structures formed between MOPC321 κ mRNA and the Ig146 DNA predigested with the corresponding enzyme. *c*, Sequencing strategy and a restriction map of the 1.7-kilobase *Hin*dIII–*Xba*I fragment. The rightmost cleavage site of each enzyme was identified by comparing the cleavage pattern of the 1.7-kilobase *Hin*dIII–*Xba*I fragment with that of the 2.7-kilobase *Hin*dIII–*Hin*dIII fragment. For determination of additional cleavage sites the *Hin*dIII–*Xba*I fragment was end-labelled with [γ-^{32}P]ATP and separated into two fragments by *Hha*I digestion followed by electrophoresis. The purified *Hin*dIII–*Hha*I and *Hha*I–*Xba*I fragments were digested with one of the indicated enzymes in limited conditions and the cleavage sites were deduced according to the method of Smith and Birnstiel[31]. Horizontal arrows indicate the direction and the extent of sequence determination. The boxes indicate positions of J DNA segments as determined by the nucleotide sequence analysis.

gel-blotting experiments, was used to formulate a model for somatic recombination. We also discuss V–J joining with respect to generation of antibody diversity and evolution of immunoglobulin genes.

Sequencing strategy for the J DNA cluster

We have previously reported cloning of a 15-kilobase embryonic DNA fragment (clone Ig146κ) containing a single Cκ DNA copy[8]. Analyses of R-loops formed between this DNA fragment and five different κ mRNAs isolated from myelomas MOPC141, MOPC21, MOPC321, LPC1 and HOPC8 identified at least four different J DNA segments (HOPC8 J and LPC1 J may be identical) in the region 2.5–4.0 kilobases to the left of the Cκ DNA segment (Fig. 1*a*)[9]. To determine the nucleotide sequence of this region, we first constructed a restriction map of the 15-kilobase Ig146κ DNA using restriction enzymes *Hin*dIII, *Bam*HI, and *Xba*I (Fig. 1*b*). The position of the Cκ DNA segment in the restriction map was determined by examining R-loops formed between MOPC321κ mRNA and the Ig146κ DNA predigested with the enzymes listed above. From a comparison of this restriction map with the R-loop map previously constructed for J segments we concluded that the J DNA cluster is on the 1.7-kilobase *Hin*dIII–*Xba*I fragment (Fig. 1*a*,*b*). We then determined the entire nucleotide sequence of this fragment using a strategy shown in Fig. 1*c*.

Complete nucleotide sequence of the J-rich 1.7-kilobase *Hin*dIII–*Xba*I fragment

Figure 2 shows the complete nucleotide sequence of the *Hin*dIII–*Xba*I fragment. To date, about 30 mouse κ chains of BALB/c and NZB strains have been sequenced around the J region[10,11]. If one assumes that the Jκ region begins with residue 96 and ends with residue 108 (see below) based on the analogy to the Jλ region[4], the 30 Jκ regions fall into 10 different classes (Table 1). We therefore scanned the nucleotide sequence determined for each of the 10 predicted J sequences, and identified four (J1, J2, J4 and J5). Taking advantage of the fact that all known J regions are extensively homologous, we looked for additional J or J-like sequences and found one (J3) for which no sequenced κ chain is known. A systematic computer search for other J-like sequences that are related to the known or predicted J sequences by more than 50% homology revealed no more than the five Js in the 1.7-kilobase *Hin*dIII–*Xba*I fragment.

The amino acid sequences of the MOPC21 and MOPC321 κ chain J regions correspond to the predicted sequences of J2 and J4, respectively. Positions of these Js as determined by sequencing agree well with the positions of Js identified by R-loop mapping using the respective κ mRNA. We therefore conclude that J2 and J4 encode MOPC21 and MOPC321 J regions, respectively. Amino acid sequences of the MOPC141 and LPC1 (or HOPC8) J regions are unknown, but based on the R-loop map (Fig. 1*a*) we predict that these J regions are encoded in J1 and J5, respectively.

The distance between adjacent Js is quite constant. The five Js are arranged as follows: J1-310 base pairs-J2-246 base pairs-J3-268 base pairs-J4-299 base pairs-J5. We have recently completed sequencing a stretch of about 1 kilobase on each side of the *Hin*dIII–*Xba*I fragment and have found no J or J-like sequences. If all Js are clustered and regularly arranged every 300 or so base pairs, there should be no J sequences other than those identified here. On the other hand, if all J peptides (see Table 1) are germ-line encoded, there should be additional J DNA segments elsewhere. Alternatively, some J peptides may be somatically generated (see below).

Precise boundaries of J DNA segments

Our previous nucleotide sequencing studies of embryonic Vλ, embryonic Cλ and myeloma Vλ + Cλ DNA clones identified the site of V–J recombination within one of two alternative pairs of phosphodiester bonds[4]. To obtain similar information for a κ chain gene, it is necessary to identify a germ-line V DNA of a specific κ chain for which the J DNA sequence is known, and determine the nucleotide sequences of both germ-line V DNA and myeloma (V + C) DNA. To this end the germ-line V gene of MOPC321 κ chain, Vκ21-C, was cloned and sequenced (details to be published elsewhere). We also determined the partial nucleotide sequence of the cDNA clone of MOPC321 κ mRNA, 5D10 (ref. 3). Nucleotide sequences around the putative recombination sites on Vκ21-C and J4 (which encodes the Jκ peptide of MOPC321 κ chain, see above) as well as the sequence of the corresponding region of 5D10 are:

```
                    95
                    AsnGlnAspProPheThrPheGly
5D10                AATGAGGATCCATTCACGTTCGGC

Vκ21-C              AATGAGGATCCTCCCACAGTGGCT

J4                  GAATCACTGTGATTCACGTTCGGC
```

Examination of these sequences enabled us to conclude that the recombination is at the phosphodiester bond linking the second and third nucleotides of the proline codon at residue 95. This corresponds to residue 97 in the λ chain numbering system. In the case of one λ gene we have shown that recombination occurs either between the His(97) and Phe(98) codons or between the

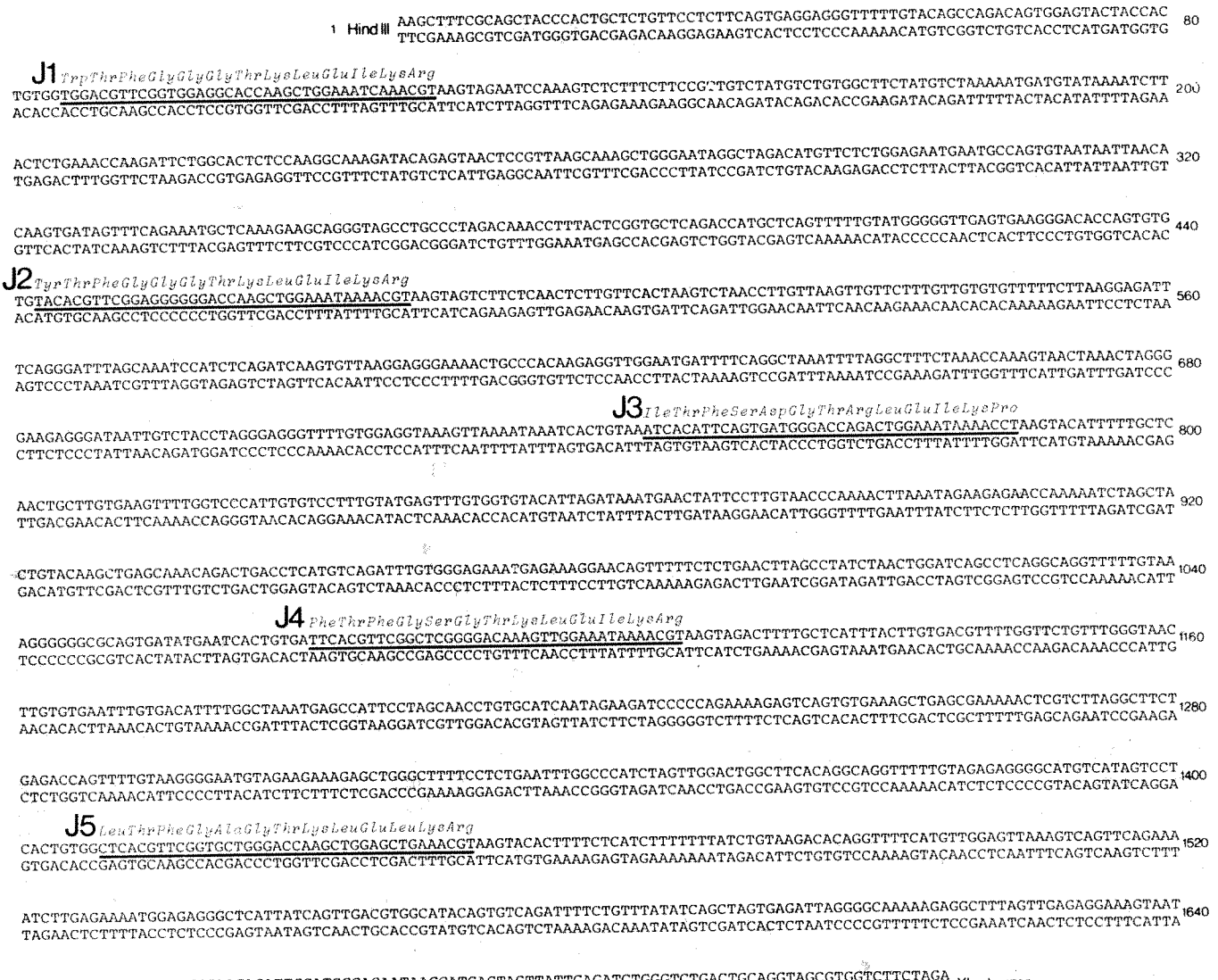


Fig. 2 Complete nucleotide sequence of the 1.7-kilobase *Hin*dIII–*Xba*I fragment containing the J cluster. For sequencing, a 2.7-kilobase *Hin*dIII–*Hin*dIII fragment carrying the J cluster (Fig. 1*b*) was subcloned into a plasmid vector pBR322 (ref. 32). A 300-μg portion of the chimaeric plasmid DNA was digested with a mixture of *Hin*dIII and *Xba*I and separated in a 6% polyacrylamide gel (20 × 40 × 0.5 cm); the 1.7-kilobase *Hin*dIII–*Xba*I fragment (Fig. 1*b*) was then purified. A 5-μg aliquot of this fragment was digested with *Hin*fI, *Mbo*II, *Alu*I, *Ava*I, *Hph*I, *Hae*III or *Ava*II, end-labelled and sequenced by the method of Maxam and Gilbert[33] as described previously[26]. In some experiments, 3′-ends of the DNA fragments were labelled with [α-^{32}P]nucleoside triphosphates (NTPs) using the Klenow fragment of *Escherichia coli* DNA polymerase I (ref. 34). For this, 1 μg of DNA was incubated with 20 μCi each of four [α-^{32}P]-NTPs (3,000 Ci mmol^{-1}, NEN) and 1 unit of the Klenow enzyme (Boehringer, Mannheim) at 25 °C for 20 min in 50 μl of 6 mM Tris-HCl, *p*H 7.5, 50 mM NaCl, 6 mM $MgCl_2$ and 6 mM β-mercaptoethanol. Aliquots (50 mM) of each of the four NTPs and 0.5 unit of the enzyme were then added to the reaction mix and the incubation was continued for another 10 min. The reaction was terminated by heating the mixture at 70 °C for 10 min and DNA was precipitated by ethanol. For strand separation the labelled DNA samples were dissolved in 20 μl of 50% (v/v) dimethyl sulphoxide, 10 mM Tris-borate, *p*H 8.3, 1 mM EDTA, 0.5% (v/v) xylene cyanol and 0.05% (v/v) bromphenol blue, heated at 90 °C for 3 min, chilled on ice and fractionated in a 6% or 8% strand-separation gel (bisacrylamide/acrylamide = 1/62.5) (A. Maxam, personal communication). Amino acid sequences predicted from the identified J DNA segments are shown in italics.

first and second bases of the latter codon[4]. Thus, the exact sites of recombination in the two cases seem to differ by one or two bases. It remains to be seen whether this difference is due to characteristics of the two recombination systems, one for κ chains and the other for λ chains. An alternative possibility is that the recombinases allow a limited flexibility in the sites of breakage and reunion regardless of the chain type (see below). In this respect it is relevant that all mouse κ chains sequenced to date carry proline at residue 95. As none of the Js reported here can code for this proline (Fig. 2) it is very likely that the first residue fully encoded in J is always at position 96. However, precise recombination sites can only be defined by comparative sequencing analysis of the three DNA segments involved.

Where does coding by J end? The sequences of J4 and 5D10 in the relevant regions are as follows:

```
                    108
                    LeuGluIleLysArgAlaAspAla
5D10                TTGGAAATAAAACGGGCTGATGCT
J4                  TTGGAAATAAAAC|GTAAGTAGACT
```

These sequences indicate that J4 can code for MOPC321 κ chain up to the second base of the Arg(108) codon, CGG. However, as J is followed by an intron preceding the C DNA segment and as most introns start with GT (ref. 12), we suggest that coding by J actually ends with the first base of the triplet CGT, as indicated by the vertical line. As shown in Fig. 3, sequences around the splice site are highly conserved among the five Js. In all cases except for J3, for which no κ chains are

Table 1 Classification of BALB/c and NZB myeloma κ chains

Group	Myeloma					Jκ Peptide (96 → 108)												Jκ DNA Segment
I	M141*	M63	B32	2880	7210	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	
	M70	M41	A17	1229	6308														J1
	C101	T111	X-44	7769															
II	M21	A22	9245	3741		Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	J2
III	M173	6684				Arg	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	
IV	M11	7940				Pro	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	
V	T124					Trp	Thr	Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	
VI	M321	7043				Phe	Thr	Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	J4
VII	X-24					Ile	Thr	Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	
VIII	LPC1*	M511	4045	3154		Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys	Arg	J5
	HOPC8*	7175	2485	7183															
IX	T601					Ile	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys	Arg	
X	2413					Trp	Thr	Phe	Gly	Gly	Gly	Thr	Asp	Leu	Glu	Ile	Glu	Arg	
XI						Ile	Thr	Phe	Ser	Asp	Gly	Thr	Arg	Leu	Glu	Ile	Lys	Pro	J3

BALB/c and NZB myeloma κ chains are classified into 10 groups according to the amino acid sequence of the Jκ peptide (residues 96–108)[10,11]. The DNA segments coding for four Jκ peptides, J1, J2, J4 and J5, have been identified on the embryonic Cκ clone, Ig146κ, in the present study. No myeloma κ chain is known for the fifth J DNA segment, J3, also identified in the present study. Those amino acid residues different from the corresponding residues of the J1 peptide are underlined.

* Myelomas classified by R-loop mapping rather than by amino acid sequencing (see text).

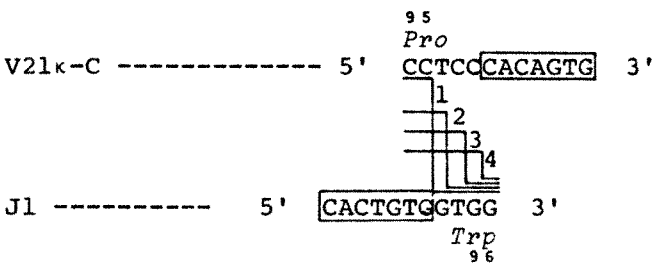
known, the doublet GT is present in the last J triplet (CGT) that can partially encode κ chains. It is likely, therefore, that coding by J always ends with the first base of this triplet. In the equivalent position of J3 a Pro codon, CCT, replaces the Arg codon, CGT. It is not clear whether J3 is a genuine J, and if so, where the coding precisely ends.

Inverted repeat-stem structure could be formed between embryonic V and J sequences

Figure 3 compares the sequences of five Js in the coding and flanking regions. Sequences are highly conserved in the coding regions and around the RNA splice sites. In contrast, sequences in the rest of the flanking regions are rather diverse, although the non-coding sequences between J3 and J4, and J4 and J5 are somewhat similar. Nevertheless, there are two short conserved sequences in the 5′-non-coding regions. One is a decamer, GGTTTTTGTA, located about 30 base pairs away from all Js, and the other is a palindromic hexamer interrupted by an AT base pair at the centre of symmetry, CACTGTG, immediately preceding the Js. The same or closely related sequences are also present at the equivalent position in the λI chain gene clone[4].

The significance of these conserved sequences with respect to V–J joining became apparent when the sequences of an embryonic Vκ gene clone, IgVκ21-C, and an embryonic VκI clone, Ig99λ (ref. 4), were examined. Both V DNA clones contain sequences closely related to the decamer and heptamer described above on the anti-sense strands in the 3′-non-coding regions. The presence of these sequences could permit the formation of an inverted repeat stem structure between the 3′-end of an embryonic V sequence and the 5′-end of a J sequence. Examples of such stem structures are shown in Fig. 4*a–d*. Vκ21-C can form a stem structure of similar stability with any one of the five Js. Another Vκ gene, VK-2 (ref. 13), which codes for a V region remotely related to the MOPC321 V region, can also form such a stem structure with any one of the five Jκs (Fig. 4*c*). We note that the majority of bases conserved in the 3′-non-coding regions of the two Vκ genes participate in base pairing with Jκs. A stem structure can also be constructed between embryonic Vλ and embryonic Jλ, although the homology is lower than that of the Vκ–Jκ stems (Fig. 4*d*). We propose that the hybrid stem structures are part of the recognition signals for the putative site-specific recombinase.

In all cases known, a few base pairs occur between either one or both cut points of recombination and the stem bases. Thus, for the V–J joining leading to the MOPC321 κ chain gene, the only case in which the exact cut points are known, three base pairs intervene between the cuts on V21κ-C and the stem bases, whereas the cuts on J4 are actually at the bases (Fig. 4*a*). We do not know whether this relationship between the cut points and the stem bases applies to other recombination events between various pairs of Vκs and Jκs, nor whether recombination between Vκ21-C and J4 always occurs at the same sites as shown in Fig. 4*a*. As residue 96 is a hot spot[10,11], one intriguing possibility is that the recombinase can cut and rejoin the DNA strands of a given pair of V and J at slightly different positions so that different triplet codons are reconstituted at the recombination site. For example, the hypothetical recombination events between V21κ-C and J1 (Fig. 4*b*) may occur in any one of the following four ways:



where recombinations 1 and 2 give the same dipeptide Pro(95)–Trp(96), whereas recombinations 3 and 4 generate Pro(95)–Arg(96) and Pro(95)–Pro(96), respectively. The latter two recombination events can account for the group III and group IV J peptides, respectively (see Table 1). It follows that the total number of Jκ DNA segments might be considerably less than the number of different Jκ peptides.

The inverted repeat structures described above have some resemblance to those found at the ends of prokaryotic insertion elements. Ohtsubo and Ohtsubo[14], and Grindley[15] determined sequences of IS1 and found that some 30 bases at one end are invertedly repeated on the other end. Inverted repeat sequences of similar length have also been found at the ends of most insertion elements. Integration of insertion elements into host DNA generates 5- or 9-base pair direct repeats at the outer ends of the inverted repeat sequences[15–22,36]. The conserved palindromic sequences, CACAGTG near V genes and CACTGTG near Js, can be considered to be equivalent to these direct repeats observed at the margins of integrated insertion elements. Although these structural similarities may be coincidental, they could reflect a common evolutionary origin or a common enzymatic mechanism for the two recombination systems, or both.

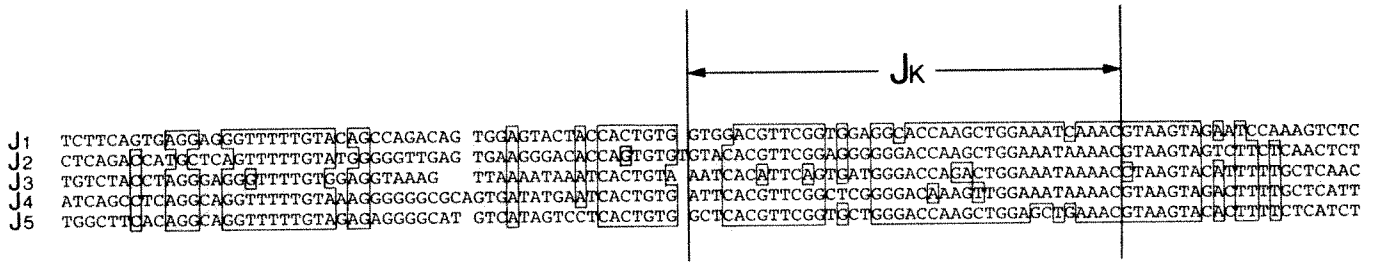


Fig. 3 Comparison of nucleotide sequences of the five J-coding and the flanking regions. Nucleotides common in at least four Js are outlined. Vertical lines indicate determined or predicted ends of the J-coding DNA segments.

V–J joining probably results in deletion

Several models have previously been proposed for recombination of immunoglobulin genes[2]. In the 'copy–insertion' model, a specific V DNA segment is duplicated and the copy is inserted at a site adjacent to a J DNA segment. The 'excision–insertion' model suggests that a specific V DNA segment is excised into an episome-like structure, and this in turn is integrated adjacent to a J DNA segment. According to the 'deletion model' the entire DNA in the interval between a particular pair of V and J DNA segments loops out, is excised, and is diluted out on subsequent cell multiplication. In the 'inversion' model the V and the C DNA (also J DNA) segments are arranged in opposite directions, and a segment of chromosome between a particular V DNA (inclusive) and a J DNA (exclusive) is inverted.

In the context of these models, let us consider the fate of the DNA sequences immediately adjacent to the 3′-end of V, or immediately adjacent to the 5′-end of J. In all the models except for the deletion model, either one of the two sequences or both should recombine with DNA sequences elsewhere in the genome on V–J joining. In contrast, the deletion model predicts that both sequences should be eliminated. To test these two alternatives, we investigated the λI chain gene in which both V and J are unique. We digested embryo and myeloma (H2020) DNA with various restriction enzymes, fractionated the digests in a 0.8% agarose gel, and analysed the DNA by Southern gel-blotting procedures. As the hybridisation probes we used a DNA fragment of about 300 base pairs from an embryonic VλI clone, Ig99λ, or from an embryonic CλI clone, Ig25λ, that includes the respective recombination site as shown in Fig. 5*E*. Both DNA fragments contain not only the sequence to be examined (*b* or *c*) but also an adjacent sequence (*a* or *d*) known to be incorporated into the rearranged λ chain gene. We deliberately chose such DNA fragments as hybridisation probes to let the *a* and *d* part serve as internal hybridisation controls.

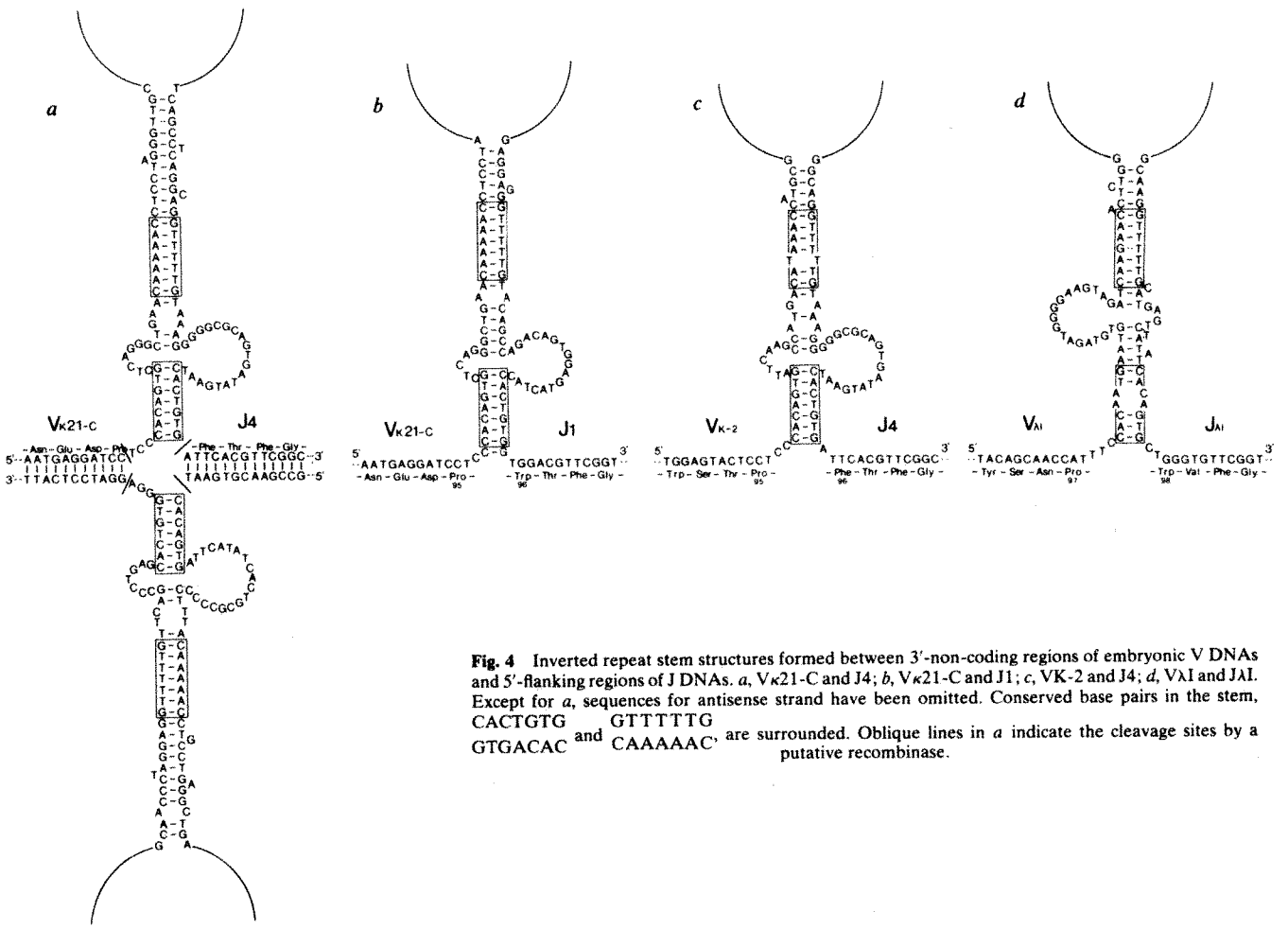


Fig. 4 Inverted repeat stem structures formed between 3′-non-coding regions of embryonic V DNAs and 5′-flanking regions of J DNAs. *a*, Vκ21-C and J4; *b*, Vκ21-C and J1; *c*, VK-2 and J4; *d*, VλI and JλI. Except for *a*, sequences for antisense strand have been omitted. Conserved base pairs in the stem, CACTGTG GTTTTTG / GTGACAC and CAAAAAC, are surrounded. Oblique lines in *a* indicate the cleavage sites by a putative recombinase.

Figure 5*A* compares hybridisation patterns of embryo and myeloma DNA that were digested with *Eco*RI and analysed with the *ab* probe. Embryo DNA gave two bands of 4.8 and 3.5 kilobases that had previously been identified as VλII- and VλI-containing fragments, respectively[3-5]. In the myeloma DNA an additional band of 7.4 kilobases known to contain the rearranged, complete λI gene[4] was observed, but no previously unidentified band was present, which would have been expected if the DNA sequence corresponding to the *b* part of the probe had recombined with another DNA sequence. Results of analogous experiments obtained with the *cd* probe are shown in Fig. 5*C*. Embryo DNA gave a band of 8.6 kilobases known to contain the CλI sequence, and another band, X. The latter band contained a sequence homologous to JλI and its flanking regions (unpublished observations). Myeloma DNA gave these two bands and an additional band of 7.4 kilobases containing the rearranged λI chain gene[3]. Again, we observed no myeloma-specific band that could be attributed to the hypothetically recombined DNA containing the *c* part of the prqbe. Similar results obtained with *Hin*dIII are shown in Fig. 5*B* and *D*. We did not observe myeloma-specific DNA fragments that could not be accounted for by the rearranged, complete λI chain gene with either probe. We have carried out similar experiments using *Kpn*I, *Bam*HI, *Hin*dII and *Hae*III, and obtained results that lead us to the same conclusion (data not shown). Although the results do not unequivocally prove the deletion model (more complicated models in which elements of the other models are combined cannot be ruled out), they suggest strongly that it is the correct one.

Evolution of light-chain genes

We thus suggest that V–J joining is a recombination event accompanied by excision of the DNA sequence between the V and J DNA segments. This DNA sequence, which we call *excison*, carries at its ends characteristic inverted repeat sequences consisting of about 30 bases that could form a stem-loop structure somewhat similar to those of prokaryotic insertion elements. In the light of these findings and the intron–exon structure of immunoglobulin genes we will consider some aspects of the evolution of light-chain genes.

The V and C regions of light chains show a weak sequence homology and are similar in their size, in arrangement of intra-chain disulphide bonds and in three-dimensional structure[23-25]. Based on these observations it has been generally thought that the DNA segments coding for the two regions arose by duplication of a primordial gene coding for a single homology unit (Fig. 6, step *a*). As we discussed elsewhere this duplication step probably generated a 'dimeric gene' in which the ancestral V- and C-coding DNA segments are separated by a spacer[26]. When this gene is expressed, the entire DNA is transcribed into a single RNA molecule and the spacer sequence is presumed to have been removed by splicing of the V- and C-coding RNA sequences. We assume that the J DNA segment was an integral part of the ancient V DNA because the J region is homologous to the carboxyl end of the C region[35].

The ancestral V DNA, together with some flanking sequences, duplicated, triplicated, and so on under pressure to increase V-region diversity (Fig. 6, step *b*). As splicing is most probably an intramolecular reaction, one requirement of such a 'polymeric gene' would be that the V and C DNA sequences are co-transcribed. In the long RNA transcript every V-coding sequence would have one half-pair of RNA splice sites at its 3′-end (the other half-pair is at the 5′-end of the C-coding sequence), so that a series of mature mRNAs containing different V-coding sequence and the same C-coding sequence could be generated. However, the V–C co-transcription requirement would impose a limit on the size of such a polymeric gene because the transcription unit would become intolerably long as the gene grows. To accommodate 200 V-coding DNA segments, a number estimated for mouse κ chains[2,8,13,27,28], the transcript would have to be 100 kilobases long even if no spacer

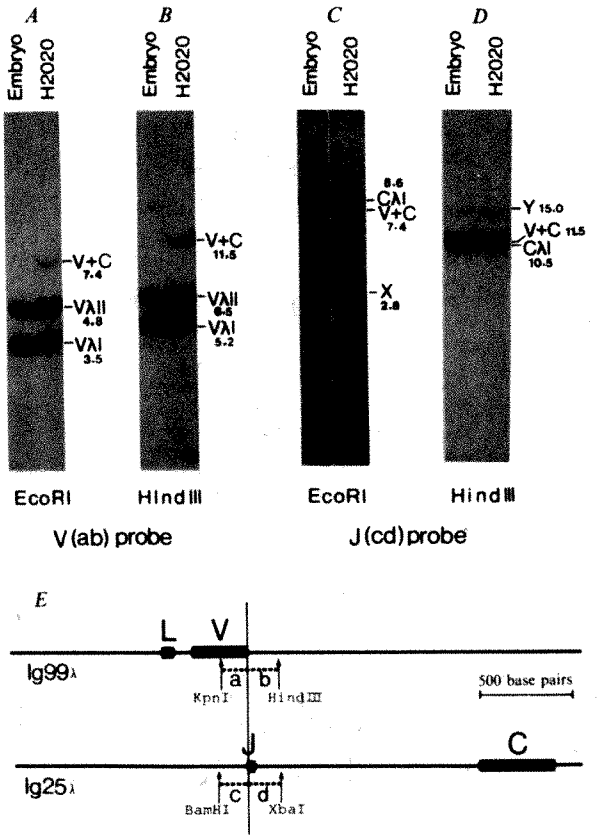


Fig. 5 Southern gel blots of total cellular DNA assayed with hybridisation probes containing the sequences around the recombination sites. High molecular weight DNA extracted from 13-d-old mouse (BALB/c) embryo or from λI light-chain-producing myeloma, H2020, was digested with *Eco*RI (*A* and *C*) or *Hin*dIII (*B* and *D*). The DNA fragments were separated in a 0.8% agarose gel and transferred to a nitrocellulose filter (Schleicher–Schuell, BA85). Filters were then incubated as previously described[3] in the presence of 2×10^7 c.p.m. of one of the two nick-translated DNA probes, $a+b$ and $c+d$, whose sequence compositions are indicated in *E*. *A* and *B*, hybridisation with the $a+b$ probe composed of the 330-base pair *Kpn*I–*Hin*dIII fragment purified from an embryonic VλI gene clone, Ig99λ. *C* and *D*, hybridisation with the $c+d$ probe composed of the 294-base pair *Bam*HI–*Xba*I fragment purified from an embryonic CλI gene clone, Ig25λ. Among the bands detected, those indicated to contain λ-coding sequences have previously been characterised by the analysis of DNA clones containing the corresponding DNA fragments. Band X was also cloned, and analysed by electron microscopy of heteroduplexes formed between the cloned DNA and the Ig25λ DNA. It contains a sequence homologous to JλI and its flanking region (unpublished observation). Band Y has not yet been characterised. Note that both band X and band Y are present not only in the myeloma DNA but also in the embryo DNA. Molecular sizes are indicated in kilobase pairs.

between two adjacent V DNA segments is postulated. The hypothetical transcript may be much larger because at least one such spacer seems to be 10 times longer than a V DNA segment[9]. One obvious solution is to duplicate the entire polymeric gene of a still allowable size. Indeed, all mammals studied so far seem to carry several unlinked sets of light-chain V DNA clusters each associated with a C-coding DNA segment.

An alternative solution is to rearrange DNA sequences somatically so that the V DNA to be expressed is brought in the vicinity of the C DNA in the lymphocytes. We propose that such a mechanism was initiated when an IS-like DNA element was accidentally inserted into one of the multiple V DNA copies of an ancestral polymeric gene, splitting it into two portions, one corresponding to the present day embryonic V DNA segment and the other to the J DNA segment (Fig. 6, step *c*). While this insertion was fixed in the germ-line genome, a mechanism to excise the inserted DNA and rejoin the split V-coding sequences was established in lymphocytes. As excision is a common and major manifestation of prokaryotic IS elements, the basic mechanism for such a process would probably have been

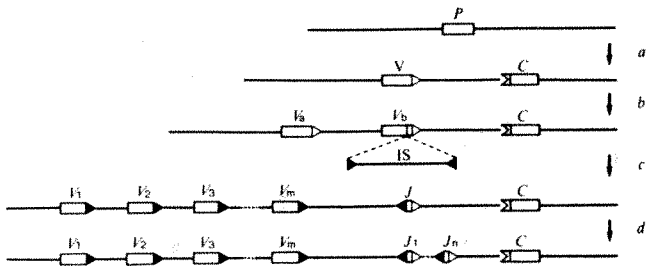


Fig. 6 A hypothetical scheme for the evolution of the κ-light-chain genes. *P*: a primordial gene coding for a single homology unit of immunoglobulin chains. *V*: an ancient variable-region gene. *C*: a constant-region gene. IS: a DNA element thought to be inserted into one of the *V* copies, splitting it to a 5′-portion, *V*, and a 3′-portion, *J*. ▷ and ▷◁ designate a pair of splice sites. ► and ◄ designate inverted repeat sequences at the ends of IS. Step *a*, duplication of *P* and generation of a pair of splice sites at or near the inward ends of *V* and *C*. Step *b*, limited multiplication of *V* and accidental insertion of IS into one of the multiplied *V*s, *V*b. Step *c*, multiplication of *V* and one half-pair of the inverted repeat sequences. Step *d*, multiplication of *J* and the other half-pair of the inverted repeat sequences. See text for details.

available in vertebrates. Once an ancestral V DNA is split, the major body of the V-coding sequence and the flanking sequences containing one of the two invertedly repeated sequences can multiply independently of the J DNA (Fig. 6, step *c*). Because every copy of the duplicated V DNA would have the excision–recombination site at its 3′-end, it can rejoin with the J DNA which is kept in the vicinity of the C DNA. Because the rearranged V DNA can bring in its own promoter, it is no longer necessary to confine the duplicated V DNA copies and the C DNA within the same transcription unit in the germ-line genome.

Multiplication of J DNA segments is an additional aspect of the evolution of light-chain genes (Fig. 6, step *d*). Amino acid sequencing studies of mouse κ chains suggest that a single J DNA segment can recombine with more than one V DNA segment in different lymphocyte clones. Conversely, a single V DNA segment seems to be capable of recombining with more than one J DNA segment[10,11]. In addition, a single J DNA segment can give rise to multiple J peptides by a slight shift of the recombination site between a given pair of V and J. Although the pressure under which J DNA segments multiplied is unknown, diversification of V regions may have been the primary source. As residue 96, the first amino acid fully encoded in J, is one of the third complementarity-determining residues[29], the J peptide could contribute to the antigen-binding activity of the antibody.

Thus, the V–J joining may well be a reversal of an ancient accidental insertion of an IS-like DNA element which was subsequently exploited by vertebrates to increase the V-region diversity within the general framework of the intron–exon structure of higher cell genes.

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Statistical mechanics of supercoils and the torsional stiffness of the DNA double helix

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The distribution of closed unknotted polymer chains over the writhing number is calculated by the Monte-Carlo method. For circular duplex DNA the variance of the distribution equals approximately half the observed variance of equilibrium distribution over the linking number. The balance which arises from fluctuations in DNA twisting makes it possible to estimate the torsional stiffness of the double helix.

MANY intriguing questions such as the spatial structure of superhelical DNA and the packaging of DNA inside virus particles and chromosomes, are often discussed in terms of the conformational flexibility of the double helix. In most cases changes in the DNA tertiary structure are thought to stem from small continuous local deformations of the double helix[1–7]. Within the framework of this model, DNA can be thought of as an elastic rod with properties completely characterised by its bending and torsional stiffnesses[8]. The resistance of DNA to the bending of the axis of the double helix is deduced from the experimentally determined DNA persistence length using the Bresler and Frenkel theory[9] (see also refs 10, 11).

Although in the past there has been an ambiguity in the value of the DNA persistence length, recent precise measurements using new techniques have provided a reliable estimate of about 600 Å in standard ionic conditions (0.2 M Na^+)[12–16]. This has made it possible to calculate the energy associated with the bending of the helix axis in DNA packaging within viruses[5], nucleosomes[6] and in the formation of the DNA superhelix[7].

The value of the torsional stiffness of the double helix, however, remains obscure. This is partly because the torsional stiffness does not affect any properties of linear DNA molecules. On the other hand, all properties of closed duplex DNA should depend in one way or another on the torsional stiffness of the double helix. But in most cases experimentally observed characteristics depend only indirectly on torsional stiffness, and recent attempts to evaluate the torsional stiffness have led to contradictory results[17,18].

In this article we demonstrate that the experimental data of Depew and Wang[19] and Pulleyblank *et al.*[20] offer a reliable estimation of the DNA torsional stiffness. These authors measured the equilibrium distribution of closed duplex DNA molecules over the number of titratable superhelical turns after enzymatic conversion of open circular (nicked) molecules into the closed form. Although this experimental distribution is determined by thermal fluctuations both in the torsional winding of the double helix and in the number of its tertiary turns[19,21], the latter may be theoretically calculated provided that the bending stiffness is known. The balance makes it possible to estimate the torsional stiffness of the double helix.

Rigorous formulation of the problem

Our treatment is based on the important results obtained in the field of differential topology during the last 20 years[22–25]. Fuller[26] was the first to emphasise the crucial significance of these findings for an analysis of closed duplex DNA and overtly introduced the important notion of the writhing number (Wr). This was defined as the difference between the topological characteristic of closed duplex DNA, the linking number (Lk) of the two complementary strands, and the total twist (Tw) of the strands measured along the helix axis.

The concepts of Lk, Tw and Wr are explained at length by Fuller[26,27] and Crick[28]. The central point is that the writhing number depends only on the spatial arrangement of the helix axis. Roughly speaking, this concept corresponds to our intuitive notion of the number of tertiary turns. However, even in the simplest case of a regular superhelix, the writhing number does not equal the number of superhelical turns[26–28].

In terms of the values of Lk, Tw and Wr, the problem of quantitative interpretation of the experimental results on equilibrium closing of nicked DNA[19,20] may be formulated as follows[21]. In nicked DNA the deviation ΔTw in twisting from the mean value and changes in the writhing number occur due to thermal motion. The experimentally determined value of ΔLk is expressed through these fluctuating characteristics:

$$\Delta Lk = \Delta Tw + Wr \qquad (1)$$

It is obvious that the averaged values $\langle Wr\rangle = \langle \Delta Tw\rangle = \langle \Delta Lk\rangle = 0$. However, the values of $\langle(\Delta Tw)^2\rangle$ and $\langle(Wr)^2\rangle$ should be nonzero and because in a nicked molecule the fluctuations in twisting and in the number of tertiary turns occur independently from each other[19,21]:

$$\langle(\Delta Lk)^2\rangle = \langle(\Delta Tw)^2\rangle + \langle(Wr)^2\rangle \qquad (2)$$

The value of $\langle(\Delta Lk)^2\rangle$ was determined experimentally[19,20,29]. On the other hand, the value of $\langle(Wr)^2\rangle$ is fully determined by the bending stiffness of the helix axis. So, if it were theoretically calculated using the known value of the DNA bending stiffness, the value of $\langle(\Delta Tw)^2\rangle$ would remain the only unknown quantity in equation (2). The value of $\langle(\Delta Tw)^2\rangle$ is connected with the value of the torsional stiffness in a simple way.

So, to determine the DNA torsional stiffness from the experimental data[19,20,29], one should calculate the $\langle(Wr)^2\rangle$ value. To this end we used the Monte-Carlo method. To obtain a reliable estimate of the desired value the calculation scheme should meet the following requirements: (1) An algorithm should make it possible to generate closed chains in space with a correct probability distribution. (2) A strict algorithm should make it possible to calculate the writhing number for an arbitrary spatial curve. (3) Since the experiments[19,20,29] were performed with molecules extracted from cells and virus particles in the closed duplex form, the samples most probably contained only unknotted molecules (in a general case the process of replication of a knotted DNA molecule should meet with obstacles[30]). However, when one randomly generates a closed chain it may prove to be knotted with some definite probability[30]. To discard such knotted chains in the course of the Monte-Carlo calculations a reliable computer algorithm is needed to recognise them. (4) Since the Monte-Carlo calculations are performed for some simplified models of the real DNA molecule, one should prove that the results are still valid when one passes to more realistic models.

Note that Benham[21] who recently attempted to solve the same problem used a calculation scheme that satisfied none of the above requirements. In contrast, the calculation scheme outlined below meets all the above requirements and makes it possible to obtain, for the first time, a reliable estimation of the $\langle(Wr)^2\rangle$ value.

The calculation scheme

To calculate the writhing number for a closed curve in space simulating the position of the central axis of a DNA molecule we have used two independent but equally rigorous approaches.

The first one is as follows[26]. A curve is projected onto a plane perpendicular to an arbitrary direction. For this projection the so-called directional writhing number[21,26] is calculated. The writhing number Wr is the average of the directional writhing

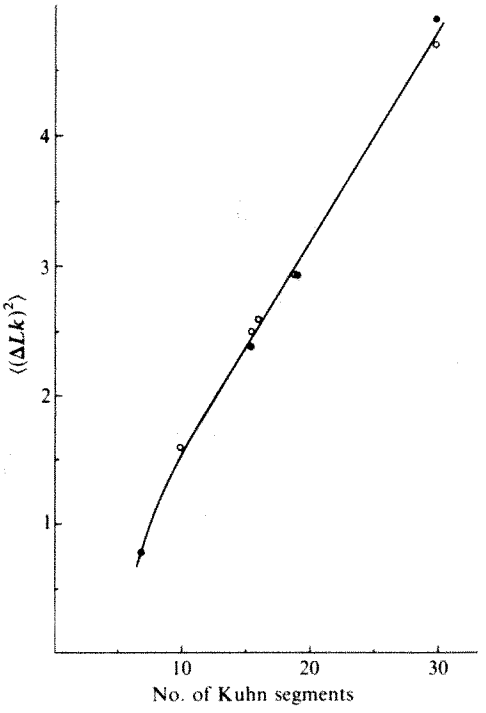


Fig. 1 Experimental dependence of the variance of equilibrium distribution over the linking number Lk on the number of Kuhn statistical segments in DNA molecules. The points correspond to the following DNAs (the values in parentheses are the numbers of base pairs): plasmid 15 of *E. coli* (2,300); minicol (3,300); SV40 (5,224); polyoma (5,380); Col E1 (6,360); fd (6,408); PM2 (10,050). The $\langle(\Delta Lk)^2\rangle$ values were taken from refs 20, 29 (○) and ref. 19 (●). The Kuhn statistical length was chosen to be equal to 1,150 Å (338 base pairs)[16].

number over all directions. A practical limitation of this method is that to obtain a reliable Wr value one has to analyse a great number of projections of a curve in different directions, so this is too time-consuming for routine Monte-Carlo calculations.

Another method based on a representation of the writhing number in terms of the gaussian integral is more convenient. It follows from the definition of the Wr value[26] and from the results in refs 22–25 that

$$Wr = \frac{1}{4\pi} \int\int \frac{(d\mathbf{r}_1 \times d\mathbf{r}_2)\mathbf{r}_{12}}{r_{12}^3} \qquad (3)$$

where $\mathbf{r}_1$ and $\mathbf{r}_2$ are the radius vectors whose ends run along the curve and $\mathbf{r}_{12} = \mathbf{r}_1 - \mathbf{r}_2$. Although in routine calculations we used the second method based on equation (3), our main results were also verified by the first method. Note that when the first method is used in Monte-Carlo calculations it is essential that the directional writhing number for each generated curve be averaged over its different projections before averaging the value $(Wr)^2$ over the different curves. Otherwise $\langle (Wr)^2 \rangle$ values are overestimated (this is one of Benham's[21] faults). A great advantage of the use of equation (3) in the Monte-Carlo calculations is that in these calculations the curves always consist of straight segments. We therefore represented the double integral in equation (3) for a total curve as the double sum over these segments and used an analytical expression for the value of the integral for a pair of straight segments.

To see if a randomly generated closed curve is knotted, we have calculated the topological invariant of knot (Alexander polinomial) as we did previously in our study on statistical mechanics of knots[30,31].

The Monte-Carlo calculations can be performed only for some simplified polymer models. These calculations are simplest for a model of freely joined straight segments, but actual DNA is most probably a continuous worm-like structure. It is known, however, that various mean characteristics of a polymer chain depend on the number of Kuhn statistical segments alone, and are independent of the particular polymer model, if the number of Kuhn segments is not too small. Special calculations show that this is also true for $\langle (Wr)^2 \rangle$. Indeed, the results hold as one passes from the free-joint model to a model with a free rotation of adjacent segments within a cone determined by a fixed angle between the segments. We compared the results obtained on the two models for the same number of Kuhn statistical segments. So we believe that our results should be valid for the worm-like model which is a limit of the model with free rotation.

We did not take into consideration the excluded volume effects because they are known to be negligible in ordinary conditions for the DNA chain lengths involved.

Finally, note that there is a very effective algorithm for the model of a freely joined chain (A. M. Dykhne, unpublished), making it possible to generate only closed chains in space. There is no such algorithm for the free-rotation model. To check the validity of our results for the free-rotation model we repeated some of the calculations using a very cumbersome method involving straightforward generation of arbitrary chains of a given length with a subsequent selection of those having a small enough distance between the ends.

Results

Figure 1 shows all the published experimental data on the variance of equilibrium distribution over the linking number $\langle (\Delta Lk)^2 \rangle$ as a function of DNA chain length. Chain length is expressed in terms of the number of Kuhn statistical segments, using the Kuhn statistical length of 1,150 Å given by Kovacic and van Holde[16]. Note that other recent estimations of this quantity[12–15] differ insignificantly from the chosen value (the Kuhn statistical length is double the persistence length). In the original papers[19,20] it was proved that the experimentally measured distribution over the linking number was in fact an equilibrium distribution. Note the high accuracy of the experimental data, as demonstrated by the very good agreement between results obtained in two different laboratories with several different DNA preparations and with two different enzyme systems (DNA ligase and a nicking-closing enzyme).

Figure 2 shows the results of our calculations of the variance of distribution over the writhing number $\langle (Wr)^2 \rangle$ as a function of the number of Kuhn segments in a closed polymer chain. The dotted line shows the results obtained when the $\langle (Wr)^2 \rangle$ value was calculated for all randomly generated closed chains both unknotted and knotted. The solid line corresponds to the results obtained for unknotted chains only. As mentioned above, it is probable that only the latter results correspond to the experimental situation. Note that although for the number of segments under study the percentage of knotted chains is very small (not more than about 5%, see refs 30, 31) they contribute appreciably to the $\langle (Wr)^2 \rangle$ value. Thus analysis of randomly generated chains on their topological state proved to be absolutely essential (see also below).

Figures 1 and 2 show that the calculated value of $\langle (Wr)^2 \rangle$ is almost equal to half the experimental $\langle (\Delta Lk)^2 \rangle$ value. Our data sharply contradict the data of Benham[21] who calculated a value for $\langle (Wr)^2 \rangle$ which was about four times larger than ours. In this connection it should be stressed that our calculations meet all the above-listed requirements and their statistical error is negligible. Our results can therefore be used for obtaining, from the experimental data summarised in Fig. 1, a reliable estimation of the variance of the twisting of DNA strands about the central axis $\langle (\Delta Tw)^2 \rangle$ which, according to equation (2), is simply the difference $\langle (\Delta Lk)^2 \rangle - \langle (Wr)^2 \rangle$. Figure 3 shows the results.

The result presented in Fig. 3 was obtained for a particular Kuhn statistical length (1,150 Å). The accuracy of the latter may be estimated at about 10% (see refs 12–16). It means that the possible error in the $\langle (\Delta Tw)^2 \rangle$ value estimation is about 20%.

The ΔLk value has a gaussian distribution[19,20,29]. Our calculations show that the same is true of the Wr value. It follows from these data and equation (1) that the ΔTw value should be normally distributed.

Let us now interpret the obtained value of $\langle (\Delta Tw)^2 \rangle$ in terms of the DNA torsional stiffness. We shall only consider the simplest model of independent variations of adjacent angles between nucleotide pairs. If a DNA molecule consists of $N+1$ base pairs it has N such independent angles. For each of them

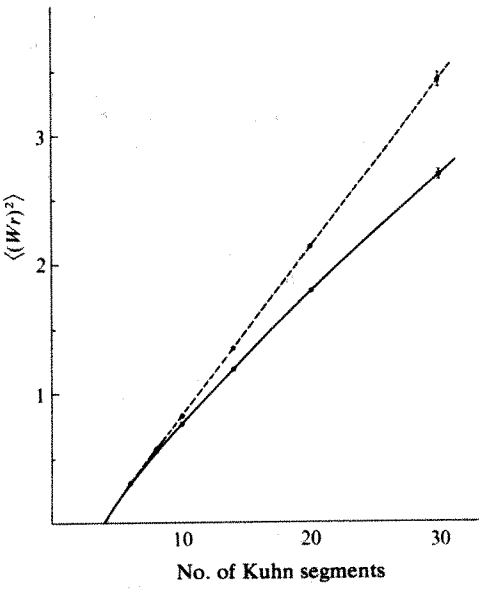


Fig. 2 Results of calculations of the variance of distribution over the writhing number Wr for a closed polymer chain as a function of the number of Kuhn statistical segments in it. The solid line refers to the case of unknotted chains only. The dotted line corresponds to the case when all randomly generated closed chains, both unknotted and knotted, are taken into consideration. The error bar is shown for the case of 30 segments. In all other cases the statistical error of the Monte-Carlo calculations is negligible.

one can use a parabolic dependence of the energy f on the deviation $\Delta\tau$ of the angle from its equilibrium position:

$$f = \tfrac{1}{2}g(\Delta\tau)^2 \quad (4)$$

where g is the value of torsional stiffness.

The probability of a given value of $\Delta\tau$ should conform to the Boltzmann equation

$$P(\Delta\tau) \sim \exp\left(-\frac{f}{RT}\right) = \exp\left(-\frac{g(\Delta\tau)^2}{2RT}\right) \quad (5)$$

As a result the variation in the total twisting angle of the molecule expressed in terms of the number of revolutions will have a gaussian distribution with the variance

$$\langle(\Delta Tw)^2\rangle = N\frac{RT}{g} \quad (6)$$

So, the value of $\langle(\Delta Tw)^2\rangle$ should be directly proportional to N and the slope of the straight line determines the value of torsional stiffness g. In accordance with equation (6) the calculated value of $\langle(\Delta Tw)^2\rangle$ is directly proportional to N (see solid line in Fig. 3). This result is by no means trivial and is valid only for precise calculations. This is demonstrated by the dotted line in Fig. 3, which was obtained from the same experimental data as Fig. 1, but using the theoretical curve for the $\langle(Wr)^2\rangle$ value calculated without the exclusive selection of unknotted chains (the dotted line in Fig. 2).

If the $\Delta\tau$ value is measured in angular degrees, then the numerical value of torsional stiffness which follows from the application of equation (6) to the data of Fig. 3, is

$$g = 0.036\,RT \quad (7)$$

It follows from the above estimation of the accuracy of determination of the $\langle(\Delta Tw)^2\rangle$ value that the obtained g value should be accurate to within 20%.

Note that we have restricted ourselves to considering only the simplest model of DNA torsional fluctuations. In principle, the same value of $\langle(\Delta Tw)^2\rangle$ may originate, for instance, from rare but marked changes in the winding angle of the double helix. An ultimate choice between different models cannot be made on the basis of the obtained $\langle(\Delta Tw)^2\rangle$ value alone. As mentioned in the introductory section, the above model which treats DNA as an elastic rod seems today preferable to other models. Note that there is no contradiction between this model and the concept of local fluctuational opening of the double helix[32,33], because the probability of the latter process in linear or nicked DNA is too small to account for the observed bending as well as torsional flexibility.

Discussion

The above results make it possible to clear up several intriguing questions. First the data lead directly to the conclusion that half of the total number of the titratable superhelical turns in closed duplex DNA is realised as tertiary turns (writhing number) and the other half is realised as the axial twisting (or untwisting) of the double helix. Note that all experimental techniques give the number of titratable superhelical turns only (see for example ref. 34). Our results make it possible for the first time to infer the number of tertiary turns (more exactly, the writhing number) from the experimentally determined number of titratable superhelical turns which is of great importance, for example, for a quantitative interpretation of the hydrodynamic properties of superhelical DNAs. The number of titratable superhelical turns remains strictly constant under fixed external conditions in a given molecule, but this constant value may be distributed in different ways between writhing and twisting. Using the results of theoretical treatment of the problem by Le Bret[17] and our results it is clear that the distributions over the writhing number and twisting in closed duplex DNA are virtually identical and their variance amounts to half the variance in nicked DNA calculated above.

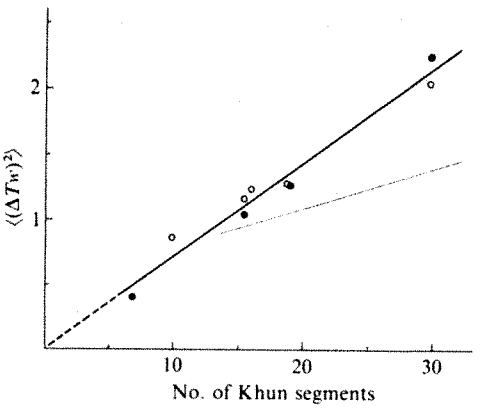


Fig. 3 The variance of the distribution over axial twisting Tw, calculated on the basis of Figs 1 and 2. Points are obtained from the experimental points in Fig. 1 and the solid line in Fig. 2 which corresponds to unknotted chains. The straight solid line is plotted through the resulting points. The dashed line is obtained from the same experimental data as for Fig. 1 but using the dotted line in Fig. 2 which was obtained without exclusive selection of unknotted chains.

For example, let us consider PM2 DNA. It has, in standard conditions, about 100 titratable superhelical turns[34]. So, the number of tertiary turns (the writhing number) for this DNA should be about 50, with the root-mean-square deviation of about unity. Note that the latter value does not contradict the fact that a sample containing the superhelical DNA molecules with a given number of topological turns gives a very sharp band in gel electrophoresis with a halfwidth much smaller than the distance between two bands which differ by one topological turn (that corresponds to only half a tertiary turn). As was emphasised by Depew and Wang[19], the width of a single band does not reflect the distribution of molecules with a given topology over different numbers of tertiary turns. So this width cannot be used for estimating the variance of the distribution as Le Bret[17] attempted to do.

Our estimation of the DNA torsional stiffness makes it possible to evaluate, for the first time, the free energy that is required to change the DNA winding angle at a given value. This free energy proved to be unexpectedly small. For instance, a change in the angle of 2.6°, which is the difference in this angle between the B and C forms of DNA, corresponds to a change in free energy of no more than 0.12 RT (72 cal mol^{-1} of base pairs).

Assuming that angles between different base pairs change independently one can estimate the amplitude (root-mean-square deviation) of thermal fluctuations in the value of the angle between adjacent base pairs that proves to be as large as ±5°. This may be directly estimated from the data in Fig. 3 without any additional assumptions (the amplitude is equal to $(\langle(\Delta Tw)^2\rangle/N)^{1/2}$). Note that, within the framework of the continuous model, this value may be overestimated only if there is a strong correlation between changes in the winding angle of sites far removed from each other. Of course, some correlation may exist but it is not very likely to be significant.

The large fluctuations in the winding angle that follow from our results most probably reflect more complex fluctuational changes in the DNA conformation. Theoretical conformational analysis suggests that the energy map of DNA has a long 'ravine' that corresponds to forms of the so-called B family[35]. It is natural to assume that the fluctuations in the winding angle correspond to a motion along the bottom of this ravine. In this case one can expect that along with the large fluctuations in the winding angle, other parameters, such as the inclination of base pairs (tilt) and the propeller of bases in the pairs should also markedly fluctuate. Presumably it is these fluctuations that lead to the considerable absorption of the light polarised along the helix axis[36]. Note that our concept does not contradict the results

of X-ray analysis of DNA fibres just because these fluctuations in fibres may be much smaller than those seen in solution due to intermolecular interaction.

Our conclusion that the large variability in DNA structural parameters can entail simple thermal motion may help clarify the mechanism of DNA–protein recognition. Indeed, these data throw great doubt on any concept that relates that recognition to small variations of DNA structure in sites with different sequences.

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letters

Weak low-latitude X-ray sources and local galactic structure

A LONG-STANDING problem of astronomy has been the elucidation of conditions that prevail in the local solar neighbourhood. Starting with Gould[1], work has continued to try to map out the morphology of the Milky Way in the vicinity of the Sun. It was noted a century ago[1] that the nearby OB stars were not distributed symmetrically with respect to the galactic plane. We report here some of the interesting contributions that X-ray sources make towards this picture.

Evidence is mounting that the low latitude ($|b^{II}| < 20°$), weak ($S < 200$ Uhuru c.p.s.) X-ray sources are situated in the solar neighbourhood. I have shown previously that the latitudinal distribution of these sources places them probably within ~ 2 kpc of the Sun, radiating between 10^{32} and 10^{34} erg s^{-1} in the 2–6 keV range[2]. This is corroborated by the discovery of stars such as Algol and γ Cas radiating in this energy band with this luminosity, as well as by models of Fabian, Pringle and Rees[3] that yield X-ray energies of this magnitude originating from accretion onto white dwarfs.

A remarkable statistic emerges when the galactic latitude of these sources ($5 < S < 50$ c.p.s.) is plotted from the 4U catalog[4]: 32 of the sources have negative galactic latitudes whereas only eight have positive values. The probability that this is a chance occurrence of a uniformly distributed population is 9×10^{-5}, calculated by

$$\sum_{i=8}^{n} \binom{n}{i} \times 0.5^n,$$

where $S = 32$ and $n = 40$. Thus, this is a statistically significant ($\approx 4\sigma$) indication of a non-random distribution of X-ray sources about the galactic plane. Furthermore, if one extends the analysis to 200 c.p.s. (where confusion will be caused by galactic centre sources), and to 4 c.p.s. (where confusion will be caused by extragalactic sources), the statistic remains $> 3\sigma$. For the inclusive population ($4 < S < 200$ c.p.s.), 50 sources have negative latitudes and 25 are positive. This is shown in Fig. 1, and summarised in Table 1. Note that the Ariel 5 satellite identified 22 new sources not in the Uhuru catalog: 15 are below the plane, and seven are above[5,6]. Unfortunately, the statistics are not adequate for a two-parameter fit to an equation of the type $b^{II} = A \sin l^{II} + B \cos l^{II}$ (see ref. 2), but a coincidence emerges when gross features are examined. For example, in the region $90° < l^{II} < 300°$, 22 sources ($4 < S < 200$) have negative galactic latitudes, and six are positive. This is close to the region where most local O and B stars are found to be below the galactic plane[7–9], and is further evidence that the weak sources reside in the solar neighbourhood. In fact, within 300 pc of the Sun, 115 O and B stars are located below the plane, and 79 are above[9], an asymmetry which is approximately 3σ. This result is subject to observational selection as it excludes parts of the southern Milky Way; thus, the asymmetry will, if anything, be enhanced.

Extending the anaology with local objects we can for the first time understand the log N log S slope of -0.4, discovered by Matilsky, *et al.*[10] and later verified by Forman *et al.*[4] using the improved data from the 4 Uhuru catalog. As pointed out by Johnson[11], the -0.4 slope does not arise simply from any spiral arm geometry. However, all previous analyses assumed a uniform density distribution, and this is probably not the case. Because of the unique position of the Sun in a locally dense

Table 1 Distribution of X-ray sources with $4 < S < 200$ c.p.s.

Uhuru intensity	No. of sources	Latitude interval	Probability
$5 < S < 50$	32	$b^{II} < 0°$	9×10^{-5}
	8	> 0	
$5 < S < 200$	38	< 0	3×10^{-3}
	17	> 0	
$4 < S < 200$	50	< 0	2.6×10^{-3}
	25	> 0	
$4 < S < 50$	44	< 0	2×10^{-4}
	16	> 0	

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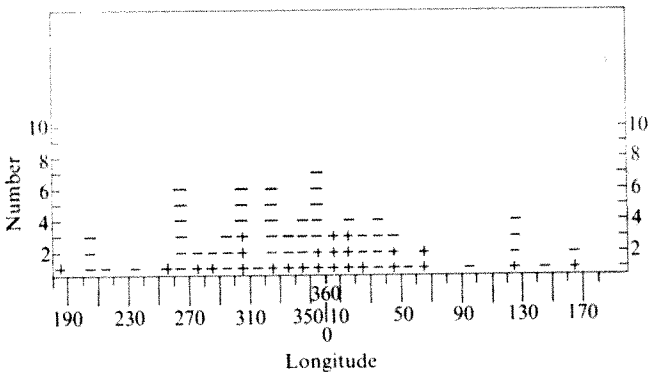


Fig. 1 Distribution of $|b^{II}| < 20°$ sources with $4 < S < 200$ Uhuru c.p.s. Number of sources in 10° longitude bins is plotted. '+', the source lies above the plane; '−', negative galactic latitude. Supernova remnants and clusters of galaxies are excluded.

region of a spiral arm, the density function of B stars *decreases* with increasing distance from the solar neighbourhood, in the region from 300 pc to ~2,000 pc. Although there are subtle variations with galactic longitude due to obscuration and other effects, McCuskey[8] has derived composite density functions which indicate an approximate density $D_s(r) \propto 1/r$ for early B stars. Thus it does not seem inappropriate to assume that a similar function exists for the local X-ray sources. This does not, of course, mean that these X-ray sources are members of typical OB star binary systems but merely that these sources probably have a local distribution function similar to that delineated by the nearby, early type stars. These may be single early type stars whose X-ray emission is due to a stellar wind interacting with the ambient interstellar medium[12], although the X-ray flux ought to be a bit softer than that of a Uhuru source. It is more probable, however, that these objects are similar to Wray 977 (4U1223–62), a 40 c.p.s. source, identified with a $m_v = 11$, B1 supergiant, which has a long (21 d) period, and whose smaller X-ray flux is due to the decreased mass transfer associated with the wide separation of the components. To arrive at a -0.4 slope for $d \log N/d \log S$, $D_s(r)$ will be proportional to $r^{-1.2}$, for the galactic sources distributed in the disk. There are, of course, possibilities that source confusion and source variability can obscure this straightforward view. However, these effects do not seem to change substantially our picture of the extragalactic component[13], which generally contains yet fainter sources and is represented by a steeper $\log N/\log S$ slope, which thereby ought to cause more severe problems than the galactic distribution. Thus it seems that a negative source density gradient in the solar neighbourhood is the most probable explanation of the distribution of the low luminosity X-ray sources.

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Differential solar rotation depends on solar activity

SUNSPOTS have long been used as tracers to determine the rotation rate of the Sun. Scheiner[1] noted 250 years ago that low-latitude sunspots rotate more rapidly than those at high latitude ('differential rotation'). In a recent review, Howard[2] stressed the limitations of the sunspot technique for determining rotation rate and differential rotation, as long-lived spots may have substantial individual motions, random and systematic, in both longitude and latitude. For example, leading and following spots of a sunspot group diverge early in the group's lifetime[3]; rotation rates for isolated ('unipolar') spots and groups differ, with group rates depending on the shape of the group[4]. Another problem in using sunspots is the Wilson effect, a surface depression of the spot causing errors in longitude estimates away from the central meridian. Despite these difficulties, spots have been used previously to establish that there is no secular variation in rotation rate and differential rotation, nor any variations with phase of the solar cycle[5], the only possible exception being during long-term disruptions in the solar activity cycle mechanism[6]. We present here evidence to suggest that the first of these conclusions is incorrect, and that differential rotation does depend on the amplitude of the solar cycle, at least for unipolar spots.

The time-averaged differential rotation law for sunspots may be written as

$$\langle\omega\rangle = \alpha + \beta \sin^2\phi + \gamma \sin^4\phi \qquad (1)$$

where $\langle\omega\rangle$ gives the daily angular sidereal motion in terms of solar latitude ϕ. Since sunspots are concentrated to within 30° latitude of the solar equator, the γ term is usually ignored. The most quoted determination for $\langle\omega\rangle$ is that of Newton and Nunn[5], who found from an analysis of sunspot data for the interval 1934 to 1944 that

$$\langle\omega\rangle = 14.38\ (\pm 0.01) - 2.96\ (\pm 0.09) \sin^2\phi \text{ deg per day} \qquad (2)$$

Newton and Nunn used stable recurrent spots in their determination: 174 unipolar spots dominated their analysis, although 89 leader and 8 follower spots of groups were also included. Because of what is known about non-uniform characteristic motions, particularly in groups, Newton and Nunn's comparison of the 1934–44 data with information on earlier cycles (given in refs 7 and 8) may be invalid. Data selection for the earlier cycles seems to have been based on the longevity of spots, irrespective of whether they were unipolar or leaders of groups—the actual fraction of each is not given, although reference is made to 'the large percentage of cases' where spots used were group leaders. Thus the evidence for the long-term invariance of differential rotation is inadequately founded. However Newton and Nunn's result for the invariance during a single cycle apparently stands, despite the small data sample near cycle minimum.

Since Ward[4] found that rotation estimates were a function of spot size and shape, with 'streamers' moving more quickly than 'bipolar' spots, which in turn move faster than unipolar spots, for comparison of daily angular sidereal motion from cycle to cycle it is necessary that only unipolar spots of restricted size range be used. We have done this for three years centred near the maxima of the solar cycles which peaked in 1883–85 and 1956–58, at the extremities of the 80-y 'Gleissberg Cycle'. The annual mean sunspot number at the maximum of the 11-y cycle, R_{max}, for 1956–58 was three times that for 1883–85, R_{max} having increased steadily between the two. Spot sizes were limited to 30 to 300 millionths of the visible solar disk, and latitudes to less than 30°. Although spot positions are available in the '*Daily Results*' and '*Ledgers*' of the Royal Observatory (based on plates taken at Greenwich, and subsequently Herstmonceux, and complemented with plates from Dehra Dûn, Mauritius, Cape of Good Hope, Kodaikanal, and Mt Wilson), all plates and

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prints for the two epochs were individually checked and where necessary spot positions remeasured. An additional selection criterion was spot longevity (at least 10 days). The only relaxation of these conditions was that the short-term appearance of a small companion to a long-lived unipolar spot was ignored. Spot positions within 10° of the limb were not used because of difficulties in determining accurate longitude from a foreshortened image of the spot. A more stringent criterion is needed for estimating the centre-of-gravity for sunspot groups; however we found that for unipolar spots positional estimates were reproducible and accurately determinable beyond 10° of the limb.

The strict application of the above selection criteria severely restricted the data sample to only 1 to 2 spots per month—50 long-lived unipolar spots were found suitable for 1883–85, and 67 for 1956–58. (Extending the time intervals to include spots from near cycle minima would not have significantly increased the data sample size because of the scarcity of long-lived unipolar spots near cycle minima.) The latitude ranges for the data used (combining northern and southern hemispheres, since there is no apparent difference in spot rotation rates, ref. 5) were—

	No. of spots	
Latitude	1883–85	1956–58
0–5°	6	3
5–10°	17	8
10–15°	13	17
15–20°	5	11
20–25°	7	20
25–30°	2	8

A total of 354 spot positions for 1883–85 and 525 positions for 1956–58 were included in the subsequent analysis. A least-squares analysis was used to determine the sunspot rotation relationship for $\phi \leq 30°$.

1883–85 $R_{max} \sim 60$

$$\langle\omega\rangle = 14.372\ (\pm 0.012) - 2.628\ (\pm 0.166)\ \sin^2\phi \quad \text{deg per day} \tag{3}$$

1956–58 $R_{max} \sim 180$

$$\langle\omega\rangle = 14.349\ (\pm 0.011) - 3.367\ (\pm 0.094)\ \sin^2\phi \quad \text{deg per day} \tag{4}$$

In this analysis it was assumed that the spots had a common proper motion in both longitude and latitude, and it was further assumed that the motion in latitude is directed towards the equator. Individual proper motions of the spots were ignored.

It seems that the equatorial rotation rate, α, remained essentially constant over the interval of interest, a result in accord with previous investigations (for example, refs 1–5) when standard errors are accounted for. However, Eddy (personal communication) has detected a variation in equatorial rotation rate between 1890 and 1960 by fitting all Greenwich sunspot group data to a modified rotation law

$$\langle\omega\rangle = a + b\phi + c\phi^2$$

and allowing for spot proper motions fitted to a quadratic. (b lies within the experimental uncertainty of value zero, and may be ignored.) By fitting our data to this same relationship, we confirm our initial finding of constant 'a' for the 1883–85 and 1956–58 maxima. Indeed, only the 1890–1900 cycle in the Eddy analysis shows a value markedly discrepant with our conclusion of constant equatorial rotation rate.

There seems to be a dramatic variation in differential rotation factor β, in the sense that high-latitude spots rotate more slowly with increasing R_{max}. Expression (2) is compatible with such an interpretation, since R_{max} for the 1938–39 maximum was ~110, although it must be stressed that the Newton and Nunn results did include a significant fraction (about one third) of leader and follower spots. A 20% higher estimate for our 'c' value for the 1956–58 maximum compared with Eddy's is compatible with Ward's conclusion regarding the differing behaviour of groups and unipolar spots.

All the above determinations depend on the adopted value of i, the inclination of the solar equator to the ecliptic, and Ω the longitude of the ascending node of the solar equator on the ecliptic. The values used today were estimated by Carrington[9] over a century ago. Recently Wöhl[11] has pointed out that there could be large errors in Carrington's values for i and Ω. The constant of differential rotation β would change if i and Ω were changed. Carrington used the latitude observations of individual sunspots covering an 8-yr interval to derive the constants. Since we have similar data we attempted to re-evaluate the solar elements and the results are shown together with estimates of the internal errors in the table below.

	Present evaluation	Carrington (1863)
i	7.22 ± 0.02	7.25
Ω_{1850}	73.94 ± 0.20	73.67

The present value of Ω is $\Omega_{1850} + 0.01396t°$ where t is the number of years since 1850. The error in Ω is larger than in i because the method enables the quantities $\Omega \sin i$ and i to be determined, and their errors are about equal. These results are in the same sense but much smaller than those predicted by Wöhl[11] who gives a table of previous determinations from sunspots and calculates his own set of elements from Doppler measurements of the solar plasma, which differs considerably from Carrington's values.

In view of the possibility of large systematic errors in the estimation of Carrington's elements, and the small difference in these new values it seems advisable to continue to use Carrington's values published in 1863 until techniques are further improved.

A satisfactory explanation of differential rotation remains a matter of debate—for a recent review see ref. 10. The evidence presented here suggests that any future theory must account for an invariant equatorial rotation rate α, but a differential factor β which varies with the amplitude of the sunspot cycle, at least in the case of unipolar spots.

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Origin of the jovian ring and the galilean satellites

THE discovery by Voyager I of a thin flat stream of rocks orbiting Jupiter at a radius of some 130,000 km, or $\sim 2R_P$ ($R_P = 6.677 \times 10^9$ cm = present polar radius), in its equatorial plane opens a new avenue of information in our understanding of the formation of the Solar System. Each of the major planets which possesses a regular satellite system also possesses a ring system. Theories for the formation of the major planets should

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therefore address themselves to this phenomenon of the co-appearance of rings and regular satellite systems, especially in considering the question whether Neptune, which has no regular satellites, may also not have a ring. We suggest here that Jupiter's rocky belt formed through condensation from a gaseous disk which was shed by the rotating gaseous envelope that originally contracted to form that planet. The four galilean satellites were formed from gaseous rings which were shed by the same envelope at an earlier and more turbulent stage in its evolution. It is possible that Jupiter may possess another rocky satellite belt at orbital radius $4R_P$[1].

The regular satellite systems of Jupiter, Saturn and Uranus are remarkable for the near coplanarity and circularity of their orbits. In addition, the orbital radii R_n, when numbered from the outermost members inwards ($n = 0, 1, 2, \ldots$), form nearly perfect geometric sequences

$$R_n/R_{n+1} = \text{constant} = \Gamma \tag{1}$$

where for Jupiter $\Gamma = 1.65$. We have recently[2] suggested that these regular satellite systems were formed in a manner similar to the hypothesis originally advanced by Laplace[3] for the formation of the planetary system; namely, through condensation from a set of gaseous rings which were shed by the primitive contracting gaseous envelopes, which make up the bulk of the mass of these planets[4,5]. Now, although Laplace's hypothesis was largely abandoned over a century ago because of a number of objections[6,7], one of us (A.J.R.P.) has shown these objections can be overcome if one takes into account a supersonic turbulent stress arising from the motions of overshooting convective elements within the cloud[8,9].

The turbulent stress has a radial component given by $\langle \rho_t v_t^2 \rangle = \beta \rho GM(r)/r$ where ρ is the gas density and $M(r)$ the mass interior to radius r, while β is the so-called turbulence parameter[10,11]. Typically, for $\beta \simeq 0.1$ the turbulent stress is some 30 times the normal gas pressure $\rho \mathcal{R}T/\mu$ just beneath the photosurface of the cloud. The main effect of this stress is to cause the gaseous envelope to become very centrally condensed; that is, it drastically lowers the moment of inertia $I \equiv fM_{env}R_e^2$, where M_{env} is the total envelope mass, and R_e is the equatorial radius. Typically, we find $f \simeq 0.01$–0.02. The turbulent stress also leads to the development of a very dense shell of non-turbulent gas at the surface of the cloud. As the cloud contracts to the fully rotating configuration at radius R_0, given where the centrifugal force at the equator first matches the gravitational force there, the dense outer shell evolves into a belt-like structure at the equator. This belt, of mass m, is left behind by the cloud while the latter withdraws dynamically to a smaller radius, stabilising itself rotationally through the extrusion of fresh material to the surface. The gas density of the detached ring diminishes with distance ξ from the mean Kepler orbit R_0 according to

$$\rho(\xi) = \rho_0 \exp(-\tfrac{1}{2}\alpha\xi^2/R_0^2) \tag{2}$$

where $\rho_0 = \alpha m/4\pi^2 R_0^3$ is the density on the mean orbit and $\alpha = \mu GM_{env}/\mathcal{R}T_0R_0 = \mathcal{O}(10^2)$.

The process of shedding gas rings repeats itself at the sequence of orbital radii R_n which satisfy the equations

$$R_n/R_{n+1} = [1 + m/M_{env}f]^2 \tag{3}$$

Thus if the contraction occurs homologously, meaning that both m/M_{env} and f are preserved, then R_n/R_{n+1} is constant and the sequence R_n is geometric. Next, taking $R_n/R_{n+1} = 1.65$ and $f = 0.02$, equation (3) tells us that the masses of the jovian rings were each about the same and equal to

$$m = 1.1 \times 10^{28}\ \text{g}$$

Such a mass of gas of solar composition contains rock and rock + H_2O ice components

$$m_{\text{rock}} \simeq 5 \times 10^{25}\ \text{g}, \quad m_{\text{rock+H}_2\text{O ice}} \simeq 12 \times 10^{25}\ \text{g}$$

The observed masses of the rocky satellites Io and Europa are 9×10^{25} and 5×10^{25} g, respectively, whilst the ice-like satellites Ganymede and Callisto have masses 15×10^{25} and 11×10^{25} g. This suggests that the protojovian envelope probably did shed rings of gas of roughly equal mass equal to that shown above.

To determine the chemistry of the galilean satellites, a turbulent polytropic numerical model of the fully rotating protojovian envelope was constructed having mass $M_{env} = M_J = 1.901 \times 10^{30}$ g. The turbulence parameter $\beta = 0.109$ is chosen so that the Titius–Bode 'constant' $\Gamma = \Gamma(\beta) = 1.65$. During uniform contraction the ratio of surface to central temperatures T_s/T_c is a fixed fraction θ_{ph} of the ratio of surface to central molecular weights μ_s/μ_c. Choosing $\theta_{ph} = 0.003$ we achieve a model where the condensation point for H_2O ice lies between the orbits of Europa and Ganymede, as required by the observational data.

The results of the calculations are summarised in Table 1. There we show the envelope moment-of-inertia coefficient f, the ratio P_s of turbulent stress to gas pressure just beneath the photosurface, and the temperature T_n and density ρ_n on the mean orbit of each gas ring just after the moment of detachment t_n from the planetary envelope, which is measured from initial radius $R_e = 30\ R_P$. We assume that a gas ring was shed at the orbit of each of the satellites and that Callisto's orbit marks the radius R_0 where the contracting envelope first became rotationally unstable. The cloud is energetically stable at each radius because of the turbulent support. The factor f increases slowly to 0.015 during the contraction owing to the steady dissociation of H_2. The final column details the composition of the condensate at each orbit, derived from the chemical condensation sequences computed by Lewis[12].

We note that Io and Europa form from a mixture of hydrous mineral silicates. For Io, the Fe combines with S to form troilite, whilst for Europa the Fe appears as FeO. As the various condensates draw together along the mean orbit of the gas ring to form the satellite, the heat released through their compaction

Table 1 Physical properties of the protojovian envelope and gas rings and the expected composition of the galilean satellites

Satellite	Orbital radius R_n/R_P	Moment-of-inertia coefficient f	Turbulent stress ratio P_s	Temperature T_n(K)	Gas density ρ_n(g cm^{-3})	Time of ring detachment t_n(10^6 yr)	Chemical composition of the condensate
Io	6.3	0.0150	26.1	533	4.4×10^{-4}	14.2	$CaTiO_3$, $MgSiO_3$, Ni, $(Na, K)AlSi_3O_8$, FeS, tremolite, talc
Europa	10.1	0.0148	25.9	338	1.1×10^{-4}	11.4	As above, with FeS → FeO (= 'rock')
Ganymede	16.0	0.0141	24.9	220	2.4×10^{-5}	7.3	rock, H_2O ice
Callisto	28.2	0.0130	23.5	133	4.0×10^{-6}	0.8	rock, H_2O ice

will cause the S as well as H_2O to leach out from the growing satellite's interior. This may account for the copius deposits of S observed on the surface of Io, as well as for the icy plains of Europa. For Europa, the black-body temperature due to the jovian envelope falls below the H_2O ice point some 2.5×10^7 yr after its formation, long after its gaseous ring has evaporated. The satellites Ganymede and Callisto condense below the H_2O ice point in this model and therefore consist of dirty ice.

According to the present theory, the contracting envelope also shed one or two more gaseous rings at orbital radii about $4R_P$ and $2.5R_P$. Why then do there appear to be no major satellites at these interior positions? The answer to this question lies in the rate of settling of the condensate particles compared with the dissipation rate of the gaseous ring. The gaseous rings thermally evaporate over a time scale $t_{evap}\sim10^4$ yr. The condensing particles settle onto the central keplerian orbit R_n of the gas ring over a time $t_{set}\propto 1/\rho_s a^2$ where, at Io's orbit, $t_{set}=10^3$ yr for particles of radius $a=10^{-2}$ cm and mass density $\rho_s=3$ g cm^{-3}. The galilean satellites are therefore expected to contain their full share of condensate. Inside Io, however, where the temperature is higher ($T_n\propto R_n^{-1}$), the particles are less hydrous and more dusty, and therefore more likely to remain suspended in the gas as a fine smoke[13]. In addition, the inner rings are most likely stirred by the increased envelope luminosity, which may also frustrate the settling out process. The mass of segregated rocky material at orbital radii $4R_P$ and $2.5R_P$ may therefore be only a small fraction of the total mass of condensate (about 5×10^{25} g) which existed at each of these orbits. If, further, this settled mass is less than $\sim2\times10^{18}$ g in the case of a protojovian envelope having $f=0.015$, or less than 2×10^{21} g for $f=0.02$, there will be insufficient time for the rocks to aggregate into a single body before the gaseous component of the ring disperses, taking with it the finer material. These mass bounds are consistent with the fact that so far no satellites have been detected at these orbits.

It is possible that the tiny satellite Amalthea (mass $\geq10^{21}$ g, orbital radius $\sim2.72R_P$) somehow formed from the ring near $2.5R_P$. But the dark red spectral features of this body[14], which suggest a composition similar to C-type asteroidal material, together with the absence of any corresponding satellite at $4R_P$, raises instead the more likely possibility that Amalthea is a stray asteroid captured by Jupiter at some time after its formation.

The process of shedding gas rings cannot proceed indefinitely as a uniform contraction of the outer layers of the cloud would lead to unrealistically high surface temperatures T_s. Instead we suggest, following the non-turbulent cloud calculations of Graboske *et al.*[15], that soon after radius $R_c=3R_P$ has been passed, where $T_s=1{,}120$ K, T_s begins to downturn and level off to ~600 K by radius $1.5R_P$. Now, a downturn in T_s implies a slowing down in the rate of release of gravitational energy $d\Omega/dt\propto R_c^2T_s^4$ and hence a diminution in the degree of supersonic turbulent convection in the cloud. But such a drop in turbulence also leads to a closer spacing between the rings of gas, as a weakly turbulent cloud is unable to support as much material at the equator. Computationally, we find that as T_s falls from 1,120 to 600 K, that β falls from 0.11 to 0.07 and $\Gamma=R_n/R_{n+1}$ drops sharply from 1.65 to 1.02 during the contraction from radius $R_c=3R_P$ to $1.5R_P$, for a cloud which maintains the same $f=0.015$. For $R_c\leq2.5R_P$ the rings are so closely spaced that they essentially merge together to form a continuous disk. Any largish grains condensing out in this dense ($\rho\sim3\times10^{-3}$ g cm^{-3}) gaseous keplerian disk settle onto the mid-plane to form a rocky sheet. Owing to the vigorous thermal stirring likely to be present in the gas, however, the mass of this solid disk is again likely to be much less than the total available condensate mass $\sim2\times10^{25}$ g. The disk is unable to coalesce into a few single bodies owing to its differential rotation[16,17]. After the gaseous component of the disk has dispersed, orbital resonances and other tidal phenomena[18] lead to evolutionary changes in its structure, which may account for the observed sharp inner edge at radius $1.9R_P$.

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Optical absorption coefficients of water

THE absorption spectrum of water in the visible region has been widely investigated[1–9] because of the basic, technological and environmental importance of liquid water. Despite these efforts, there are significant disagreements between experimental results: discrepancies of factors of ~2 in the absorption coefficients are not uncommon between various data. Possible reasons for the disagreements among the various studies are: (1) a lack of a reliable, sensitive technique for measuring small absorpton coefficients in liquids; (2) the presence of a significant amount of light scattering by particles (note that the amount of Rayleigh scattering by pure water is quite small and predictable in the visible[8]; and (3) measurements are often not done for pure distilled water stored in a noncontaminating vessel. We present here the first accurate measurement of the absorption coefficient of pure water at 21 °C in the 450–700-nm region. We have utilised a recently developed opto-acoustic (OA) technique[10], using pulsed dye lasers and immersed piezoelectric transducers. This technique is ideally suitable for measuring weak linear or nonlinear absorptions in nonfluorescing neat liquids or solutions[11,12]. Our present absorption spectra for water, having typical accuracies of ±5%, should be useful not only for a basic understanding of the properties of water, but also for practical applications like underwater light propagation or intracavity dye laser measurement of dissolved materials in aqueous solutions.

We used the OA cell shown in Fig. 1. There are several important changes to the cell previously used for benzene[10]. We have designed a special transducer with a stainless steel casing which has a flat polished inside surface against which a lead zirconate titanate (PZT) cylinder is pressed. (Details of the cell's construction and its operation will be published later.) This transducer is superior to the commercially available transducers

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which we previously used because the possibility of contamination of the water sample and the formation of small air bubbles on the transducer surface are eliminated. The OA cell is filled with triple-distilled deionised water. Since the coefficient of thermal expansion of water changes drastically with temperature[13], a small chromel–alumel thermocouple in contact with the water is used to monitor the water temperature which is stabilised at 21.5 ± 0.2 °C for the present experiments. This is necessary since the OA signal arising from the absorption of the optical radiation depends crucially on the coefficient of thermal expansion of the liquid as seen from equation (3) of ref. 10. An expansion tube is attached to the side of the OA cell to ensure that the water sample is at atmospheric pressure. With the above precautions, no contamination problems were encountered, and the OA spectrum was reproducible for a freshly distilled water sample as well as for the same sample stored in the OA cell for several weeks. To obtain the absorption spectrum, a flashlamp-pumped dye laser that can scan through the visible region (with various laser dyes) was used. The OA signal amplitude, A, is detected by the submersed transducer, and the laser pulse energy, E, is detected by a pyroelectric detector as the laser frequency, ν, is scanned by a stepping motor drive. A minicomputer collects the data and plots A/E as we scan ν. The normalised OA spectrum is directly proportional to the actual absorption spectrum[10] (note that conventional spectrophotometry measures absorption plus scattering, while here we only measure absorption). The proportionality constant can be obtained by a comparison method[11,12]; that is comparing the OA signal for pure water with the OA signal for an aqueous solution (water doped with a known small amount of nonfluorescing dye). In the present experiment, $KMnO_4$ is used as the nonfluorescing dye[14]. Absolute absorption coefficient at 659.9 nm is measured to be $(3.7 \pm 0.3) \times 10^{-3}$ cm^{-1}.

The OA absorption spectrum of distilled water at 21.5 °C is shown in Fig. 2, which exhibits a minimum at 21,050 cm^{-1} (475 nm) with a minimum value of α (21,050) = 1.7×10^{-4} cm^{-1}. It also exhibits distinct shoulders at 19,460 cm^{-1} and at 16,550 cm^{-1}. These shoulders, which are absent in heavy water[3], are due to sixth and fifth harmonics of the O—H stretch, respectively (being analogous to the absorption peaks observed in liquid benzene[15] due to the harmonics of the C—H stretch). We found that the nth harmonic absorption peak (or shoulder) in water is reasonably well given by the following simple anharmonic formula

$$\nu_n = n(3{,}620 - 63n) \quad \text{cm}^{-1} \qquad (1)$$

The comparison of experimental peak or shoulder positions in water with the predicted position by equation (1) is given in Table 1.

A comparison of our OA absorption spectrum with attenuation of absorption spectra given by other authors is given in Fig. 2. Of special interest is a comparison with the most recent data of Querry *et al.*[9] who performed a split laser pulse attenuation

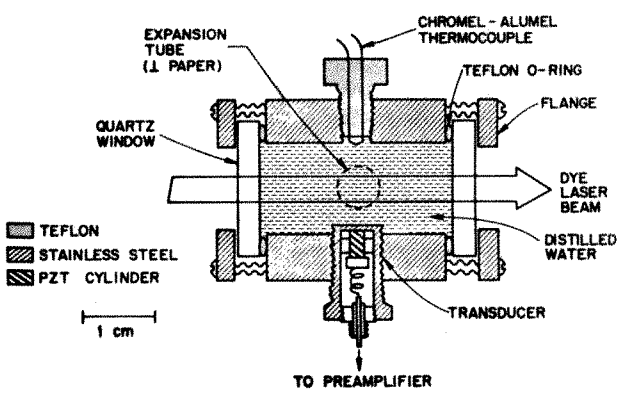


Fig. 1 Experimental OA cell for distilled water.

Table 1 Harmonics of the O—H stretch is liquid water. ν_n (cm^{-1}) in the position of an absorption peak (n = 1–4) or a shoulder (n = 5,6) for the nth harmonic

n	ν_n cm^{-1} (experimental)	ν_n cm^{-1} (predicated by equation (1))
1	3,401*	3,557
2	6,944*	6,988
3	10,309*	10,293
4	13,441*	13,472
5	16,550†	16,525
6	19,460†	19,452

* See for example ref. 6.
† Present work.

measurement on deionised filtered water. The spectral features exhibited by our data and the data of Querry *et al.* are strikingly similar, except that their attenuation coefficient is displaced upward by about 2×10^{-4} cm^{-1} from our absorption coefficient. Our data agree reasonably well with the review compilation of Irvine and Pollack[5], and of Hale and Querry[6] in the blue region (450–500 nm) but agreements at some points outside this region are sometimes poor. Agreements with Hulbert's[2] absorption data and Hass and Davisson's[8] adiabatic laser calorimetry measurements for distilled water in the blue are also good; however, their absorption coefficients are significantly lower than our values at longer wavelengths. The critical compilation of Kopelevich[7] seems strongly to underestimate the absorption in the 400–500-nm range.

In conclusion, we believe we have reported the first reliable absorption spectrum of pure distilled water at 21.5 °C. The absorption spectrum does not change appreciably from 20 to 30 °C. An overall comparison of the data by various authors indicates a broad absorption minimum near 475 nm. However, some values differ by as much as an order of magnitude. Agreement on the red side of the 600-nm region is much better and the existing data agree with our measurements to ±20%. Our presently reported data are believed to be the most reliable values so far. There are no clear-cut explanations for the large difference among the previous measurements themselves and for the differences between the previous measurements and our present ones. However, we suggest the following problems with the earlier optical transmission studies which measure extinction coefficients. (1) Conventional optical transmission measurements for pure water require long path lengths because of small absorption coefficients. To extract an absorption coefficient from such a transmission measurement, attenuation caused by scattering must be taken into account. Light scattering by suspended small particles can often be significant compared with the true absorption in the blue region. Accurate accounting of such light scattering effect is difficult. (2) Corrections are necessary for absorption and reflection losses at the optical windows. (3) Possible lateral displacements of the optical beam by water sample causes systematic errors. (4) Long cells may often require use of materials which may add contaminants to the pure water. The previous transmission measurements typically require corrections from (1) and (3) above and some authors probably over-correct their data while others under-correct their data. None of these problems limit the reliability of the measurements reported here since the OA technique measures true absorption directly and the careful construction of the OA cell out of primarily stainless steel has very nearly eliminated contamination problems.

The only possible source of error in our measurements exists in the fundamental requirement for quantitative OA spectroscopy that the optical absorption must only result in nonradiative decay producing heat (but no fluorescence or chemical changes). The above requirement is met in our present OA measurements of water and is justified on two considerations: first, vibrational relaxation of polyatomic molecules in liquid state at room temperature is known to proceed on a

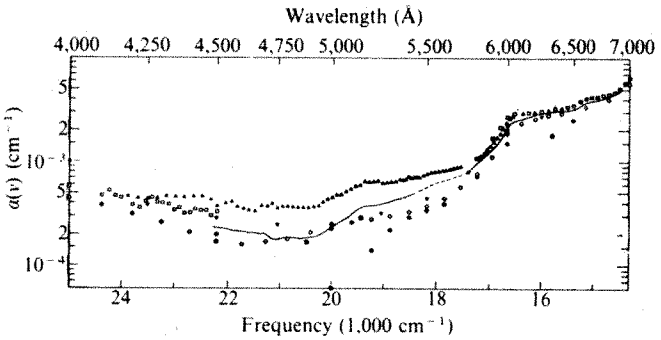


Fig. 2 Absorption spectrum of triple distilled deionised water by OA determination (solid line). The dashed parts are interpolations not covered by the tuning range of the laser dyes used. Also shown are experimental data of other authors for compaison: ◇, ref. 2; □, ref. 3; ◆, ref. 5; ▽, ref. 6; ●, ref. 7; ⊗, ref. 8; △, ref. 9. Statistical accuracy of our data is about ±5%. The uncertainty in the absolute calibration (which does not affect the shape of the curve) does not exceed ±10%.

picosecond time scale[16]. Molecules excited to high vibrational level (high harmonics) are expected to relax even faster as exemplified in the case of benzene[17]. Second, fluorescence yield is negligible unless there is a substantial Frank–London overlap between the singlet ground state and the singlet excited electronic state. The lowest known excited electronic state of H_2O is a triplet ($^3A_2$) situated at[18,19] about 36,300 cm^{-1} (275 nm). Thus the observed absorption in the visible region ($\lambda > 400$ nm) does not involve electronic excitation. Thus no excited electronic states of H_2O which could fluoresce to the ground state are produced in the present experiment. No fluorescence was experimentally observed in the water samples during our studies. Prominent spectral features are due to harmonics of the O—H stretch and seem to be represented reasonably well by the simple anharmonic formula given in equation (1). No other fine structures in the spectrum (which we measured in high resolution) are found, in agreement with Drummeter and Knestrick.[4] Detailed tabulation of the absorption coefficients of light and heavy water as well as further details of the comparison between existing data and our new measurements will be given elsewhere[20].

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Two-photon absorption spectra and cross-section measurements in liquids

THE recently developed high-sensitivity opto-acoustic (OA) absorption measurement technique[1] has proved ideal for the measurement of a variety of weak absorption phenomena in liquids[2–4]. We report here the results of an investigation of two-photon absorption in liquid benzene using the OA technique. Weak two-photon spectra (cross-section $\lesssim 10^{-51}$ cm^4 $molecule^{-1}$ $photon^{-1}$ s) in nonfluorescing liquids have been difficult to measure. The only previous published study for such a measurement used thermal blooming[5], which is difficult to interpret theoretically and apparently produces a poor signal-to-noise ratio. We demonstrate here that far superior spectra can be obtained by the simple and sensitive two-photon OA spectroscopy (TPOAS) technique. Polarisation dependence, vibrational intervals and electronic origin are obtained, and two-photon absorption cross-sections are derived for the first time in this study for the case of a weak two-photon absorption band in liquid benzene. Note that Bonch-Bruevich *et al.*[6] have recently attempted similar two-photon OA measurements, but they can only measure strong absorption (cross-section $\gtrsim 10^{-48}$ cm^4 $molecule^{-1}$ s) with a poor signal-to-noise ratio.

The two-photon absorption we study here is the $B_{2u} \leftarrow A_{1g}$ transition of benzene[7] which is symmetry forbidden but becomes allowed in two-photon absorption due to vibronic coupling. The $B_{2u} \leftarrow A_{1g}$ transitions have been extensively studied in vapour phase benzene[8–14] by fluorescence monitoring. In the liquid phase, however, fluorescence studies are severely hampered by the rapid nonradiative singlet to triplet relaxation[15,16] ($>4\times10^7$ s^{-1} in liquid benzene at room temperature). Quantum fluorescence yield[15] of the singlet excited state is small (<0.07) and decreases rapidly with vibrational energy[9,10]. The triplet state produced also undergoes rapid nonradiative decay[17–19] to the ground state ($\gtrsim 10^7$ s^{-1} in liquid benzene at room temperature). Thus no fluorescence monitoring of the $B_{2u} \leftarrow A_{1g}$ band in neat benzene at room temperature seems possible. TPOAS is ideal for measuring such weak two-photon spectra in nonfluorescing liquids. The $B_{2u} \leftarrow A_{1g}$ two-photon spectrum we obtain in the liquid resembles the corresponding vapour phase spectrum[8–13]; similarities include strong intensities of the $14_0^1 1_0^n$ vibronic transitions, laser polarisation dependence, and nearly identical values for ν_{18}, ν_{14} and ν_1 of the $^1B_{2u}$ state in the liquid as in the vapour case. However, the following differences are noted: (1) the electronic origin is 37,724 cm^{-1} in the liquid phase, compared with [7–11] 38,086 cm^{-1} in the vapour phase; (2) the absorption lines are broader (by a factor of ~10) in the liquid as compared with the vapour case[9,10] so that the rotational branches are unresolved; and (3) since fluorescence quantum efficiencies rapidly drop with higher vibrational excitation[9] even in the vapour phase, the two-photon spectra by fluorescence monitoring[8–13] shrink and vanish rapidly at higher vibrational excitation; this does not occur in our case.

The experimental apparatus used includes a scanable Chromatix CMX-4 flash-lamp pumped dye laser which provided the following tuning ranges with the Exciton dyes: Coumarin 2: 448–476 nm; LD 473: 469–500 nm; LD 490: 485–516 nm; Coumarin 504: 502–538 nm. Typical pulse energies of 2 mJ and bandwidth of 2 cm^{-1} with pulse durations of 1 μs were available at 10 pulses s^{-1}. Output beam diameter was 3 mm. A 5-cm focal length lens was used to focus the laser beam at the centre of a cylindrical stainless steel OA cell[1] (1 cm i.d. and 2 cm length), fitted with quartz windows and filled with distilled spectrograde benzene. A quarter-wave plate allows us to convert the normally linearly polarised laser beam to a circularly polarised one for appropriate studies. The transient ultrasonic signal produced by the two-photon absorption was detected by a submersed PZT transducer[1]. The transducer

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consisted of a 4 mm diameter × 4 mm high lead zirconate titanate (PZT) cylinder (LTZ-2 from Transducer Products, Connecticut). To obtain good reproducibility, it is essential that there should be no small microbubbles on the transducer face. The transducer signal and the square of the input laser pulse energy were measured with boxcar integrators. part of the dye laser output was analysed with a 2-m grating spectrometer. Either a Rb lamp or Ar laser lines provided markers for accurate calibration.

Figure 1*a* shows an OA spectrum of benzene in the 18,600 cm^{-1} to 22,200 cm^{-1} region obtained with linearly polarised radiation from the dye laser. The OA signal is normalised to the square of the input power. The seven peaks marked a, b, c, d, e, f, and g are identified as arising from the $B_{2u} \leftarrow A_{1g}$ two-photon absorption. The spacings between the peaks b, c, d, e, f, and g, are approximately 462 cm^{-1} which is in excellent agreement with the expected value of $\nu_1/2$. There are subsidiary small peaks between the major peaks and these are marked by A, B, C, D, and E. Table 1 compares the positions of these peaks with those observed in the high resolution study of two-photon absorption in vapour phase benzene. In order to make a satisfactory comparison, we have to choose the $B_{2u} \leftarrow A_{1g}$ electronic origin at 37,724 cm^{-1}. The identifications and the assignments seem to be consistent and reasonable.

The region of 18,600–22,200 cm^{-1} has two expected weak linear absorption regions arising from the seventh and the eighth harmonic of C—H stretch of benzene. We have studied the seventh harmonic at 18,810 cm^{-1} earlier[3]. This peak was mistaken as a two-photon peak by Twarowski and Kliger[5]. However, the input power dependence of this peak is found to be 1.05 ± 0.08 which indicates that input power dependence of the various peaks are necessary for correct identifications. Table 1 lists the measured input power dependence of the various peaks, obtained with the 5-cm focal length lens.

To further distinguish between the one-photon and two-photon absorption in benzene in our tuning range, we have also investigated the OA spectrum with no focusing lens. The resultant decrease in the light intensity (by a factor of 10^5) makes two-photon absorption unobservable while the linear absorption signal which is independent of intensity is unaffected. In these conditions only two absorption peaks are detected by the transducer with amplitudes depending linearly on input power and whose positions are $18{,}810 \pm 3$ cm^{-1} and $21{,}040 \pm 7$ cm^{-1}. These are identified as the seventh harmonic[3] and the eighth harmonic[20] of the C—H stretch of benzene, respectively. The peak height of the eighth harmonic at 21,040 cm^{-1} is $\leqslant 10\%$ of the two-photon peak at 21,031 cm^{-1} observed with the focusing lens. Thus the two-photon spectrum is essentially undistorted by the presence of the underlying single photon absorption.

We now estimate the absolute peak magnitude of the two-photon absorption cross-section for linear polarisation (peak c at 20,106 in Fig. 1*a*) by comparing it with the one-photon absorption peak X at 18,810 cm^{-1}. At a fixed laser pulse energy of 1.8 mJ, we observe that the ratio of the transducer signals U_c and U_X at peaks c and X respectively is

$$U_c/U_X \approx 3 \tag{1}$$

Since all the nonradiative lifetimes involved are shorter than 100 ns, while the laser pulse is 1 μs long, we assume that all the absorbed light energy is transformed into heat at the end of the laser pulse, causing local thermal expansion, which produces the transducer signal. The amplitude of the transducer signal depends linearly[1] on the light energy absorbed per unit length multiplied by an effective path length l. For the two-photon absorption case, l is taken to be twice the confocal length, that is $l_c \approx 0.05$ cm in our case. For the linear absorption case, l is taken as the laser path length directly in front of the transducer, that is $l_X \approx 0.4$ cm. Hence we have

$$U_c \approx K \times \sigma_c N l_c \int \Phi(t) P(t)\, dt \tag{2}$$

and

$$U_X \approx K \times \alpha_X l_X \int P(t)\, dt \tag{3}$$

where integrals are performed over the duration of the laser pulse, K is a constant, $N = 6 \times 10^{21}$ cm^{-3} is the molecular density, σ_c is the two-photon cross-section at peak c, $\Phi(t)$ is the instantaneous photon flux, $P(t)$ is the instantaneous power, and α_X is the peak absorption coefficient of the seventh harmonic of the C—H stretch, observed[3] to be $(4.5 \pm 1.0) \times 10^{-4}$ cm^{-1}. The

Table 1 Observed peaks by TPOAS for the $^1B_{2u} \leftarrow {}^1A_{1g}$ band in liquid benzene

Observed peaks Frequency ν (cm^{-1})	Fig. 1	Power dependence	Cross-section*	$2\nu - 37{,}724$ (cm^{-1})	Identification†	Calculated‡ position (cm^{-1})
18,810	X	1		1-photon 7th harmonic of C—H§		
19,323	a	2	2.1	922	18_0^1	923
19,644	b	2	6.6	1,564	14_0^1	1,564
19,792	A	2	1.5	1,860	$18_0^1 1_0^1$	1,843
20,106	c	2	10	2,488	$14_0^1 1_0^1$	2,488
20,241	B	2	1.8	2,758	$14_0^1 10_0^2$	2,731
					$18_0^1 1_0^2$	2,770
20,569	d	2	9.2	3,414	$14_0^1 1_0^2$	3,414
20,690	C	2	2.1	3,656	$14_0^1 10_0^2 1_0^1$	3,656
21,031	e	2	9.0	4,338	$14_0^1 1_0^3$	4,333
21,040	Y	1		1-photon 8th harmonic of C—H‖		
21,139	D	2	3.4	4,554	$14_0^1 10_0^2 1_0^2$	4,562
21,487	f	2	4.8	5,250	$14_0^1 1_0^4$	5,256
21,627	E	2	1.7	5,530	$14_0^1 10_0^2 1_0^3$	5,485
21,957	g	2	3.1	6,190	$14_0^1 1_0^5$	6,179

All values given are for linearly polarised laser light. Listed columns show laser frequency and notation of an absorption peak, the exponent of its laser energy dependence (±0.1), two-photon absorption cross-section, vibrational energy in the B_{2u} state (the electronic origin was set at 37,724 cm^{-1} in the liquid phase), identification of the transition, and calculated position (above the electronic origin) of the absorption peak assuming gas phase vibrational constants.

* 10^{-52} cm^4 molecule^{-1} photon^{-1} s. The relative values of these cross-sections are accurate to ±10% for the bigger peaks. The uncertainty in the absolute scale is a factor of 3 (see text).

† Using Wilson's notation[21].

‡ Using Wunsch *et al.*'s constants[9].

§ Ref. 3.

‖ This peak can be seen only without a focusing lens; details of this linear absorption peak is given in ref. 20.

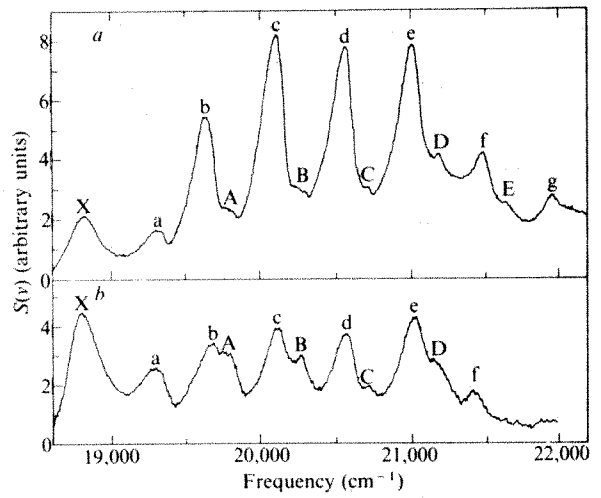


Fig. 1 Normalised two-photon spectra of liquid benzene for the $^1B_{2u} \leftarrow {}^1A_{1g}$ band. ν is the single photon energy, and $S(\nu)$ is the transducer signal amplitude normalised to the square of the laser pulse energy. Note that the absorption peak at 18,810 cm^{-1} is not a member of this band, but is the peak of the seventh harmonic of the C—H stretch. *a*, Spectrum for linear laser polarisation. *b*, Spectrum for circular laser polarisation, magnified by a factor of 2.5 compared with the spectrum in *a*.

laser pulse energy $E = \int P(t)\,dt$ is fixed at 1.8 mJ and the average $\Phi(t)$ is estimated to be 1.4×10^{27} photons $cm^2\,s^{-1}$. Note that U_c as given in equation (2) is independent of the focal length of the lens used, since tighter focusing results in a correspondingly shorter confocal length, l_c. This was verified experimentally for focal lengths of 2.5, 5 and 7.5 cm. Combining equations (1)–(3), we estimate that

$$\sigma_c \approx 1 \times 10^{-51}\ cm^4\ molecule^{-1}\ photon^{-1}\ s \qquad (4)$$

with an uncertainty of a factor of 3. The two-photon absorption cross-sections for other peaks in Fig. 1*a* can be scaled appropriately, as given in Table 1.

Judging from the signal-to-noise ratio in Fig. 1*a*, we note that the minimum two-photon cross-section presently detectable is $\sim 10^{-53}\ cm^4\ molecule^{-1}\ photon^{-1}\ s$. Also, since our OA signal becomes small near 18,500 cm^{-1} (Fig. 1*a*), we believe that possible broadband spurious effects (such as the laser electrostriction[22] effect) are negligible in our present study.

Finally, the use of circular polarisation (as opposed to linear polarisation used in the data reported above) has allowed us to obtain further check on our identification of the two photon transitions listed in Table 1. From vapour phase benzene two-photon absorption data we see that R, defined as the ratio of two photon absorption coefficient for linear polarisation to that for circular polarisation, has well defined values for various vibronic two-photon transitions in benzene. Theoretical considerations show[9,10,23] that the A_{1g} vibronic transitions should exhibit a ratio R of 5.7 (averaged over the rotational band) while the E_{2g} vibronic transitions should exhibit a ratio R of 0.66; the theory is well supported by experimental investigations in the gas phase[9,10] and in the crystalline phase[24]. For the liquid phase, the circularly-polarised TPOAS spectrum is shown in Fig. 1*b*, where the vertical scale is magnified by a factor of 2.5 compared with Fig. 1*a* to bring out the features. Note that (1) the relative heights of the various peaks are quite different and that for the A_{1g} vibronic transitions (for example, peak b or c) $R \approx 4 \pm 1$, while for the E_{2g} vibronic transitions (for example, peak a or A) $R \approx 1 \pm 0.3$ in reasonable agreement with theory[9,22]; and (2) the position of an A_{1g} transition peak is blueshifted (typically by 20 cm^{-1}) for circular polarisation as compared with the linear polarisation case, since different rotational branches are favoured for the different polarisations[9,10]; however, no shifts are observed for the E_{2g} transition peaks. However, we should emphasise that most theories of liquids do not treat molecules as being freely rotating and isolated as they would be in the gas phase, and hence the comparison between the effect of rotational structure in gas phase and liquid phase may not be strictly valid.

We have shown that TPOAS, utilising pulsed lasers and submersed transducers, represents a very powerful tool for the study of two-photon absorption spectra of non-fluorescing liquids. By this method, accurate two-photon spectroscopy of the $^1B_{2u}$ state of benzene in the neat liquid is studied with quantitative cross-sections derived for the first time. The selection rules for two-photon absorption are very different from those for one-photon absorption (at about twice the photon energy). Thus the TPOAS allows us to find and to determine the positions of vibronic levels not possible in single photon spectroscopy. We plan to extend these studies to multiphoton absorption and ionisation phenomena in various liquids.

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Photocatalytically induced fixation of molecular nitrogen by near UV radiation

A MAJOR method whereby molecular nitrogen is fixed in the soil is through microorganisms[1]. Recently, however, molecular nitrogen has been shown[2] to react with water vapour in the presence of TiO_2 under the stimulus of near UV radiation to produce ammonia and hydrazine according to the photoreducing reactions summarised as:

$$N_2 + 3H_2O \xrightarrow[TiO_2]{h\nu(\lambda \geq 365\ nm)} 2NH_3 + 3/2O_2 \qquad (1)$$

$$N_2 + 2H_2O \xrightarrow[TiO_2]{h\nu(\lambda \geq 365\ nm)} N_2H_4 + O_2 \qquad (2)$$

We report here the results of some recent experiments in which molecular nitrogen has been fixed, at room temperaure in oxidising conditions, on the surface of rutile (TiO_2) by low-energy UV photons.

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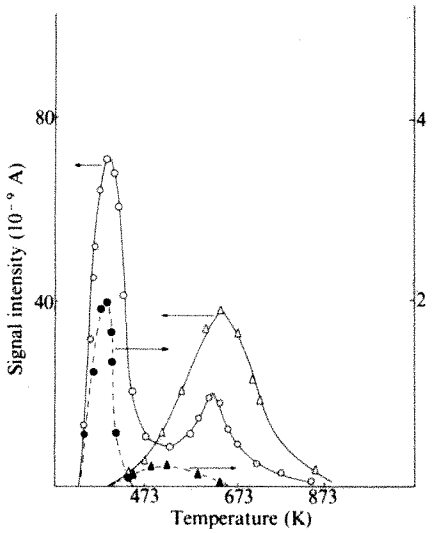


Fig. 1 Temperature programmed desorption profiles of TiO_2 surfaces: solid line, following the interaction of 20 Nm^{-2} of molecular nitrogen with a surface saturated with pre-adsorbed hydrogen peroxide; dashed line, following exposure to UV radiation ($\lambda \geq 365$ nm) in the presence of air. (The saturated surface containing $\sim 1.2 \times 10^{8}$ molecule m^{-2} of H_2O_2 was produced by adding an aqueous solution of H_2O_2 (Analar 30% w/v) to the outgassed rutile surface, followed by evacuation to remove the excess liquid.) ○●, $m/e = 32$; △▲, $m/e = 30$. For solid symbols, read right-hand scale; for open symbols, read left-hand scale.

TiO_2 powders, which contain adsorbed water, show significantly greater photochemical reactivity towards oxygen than specimens which have been dehydroxylated. It has been suggested[3] that the adsorbed hydroxyl groups, OH^-_{ads}, act as traps for the positive holes, h^+_{vb}, which are formed in the valence band of the TiO_2 when it is irradiated with photons of energy equal to or greater than the magnitude of the electronic band gap (E_g (TiO_2) ~ 3.2 eV) according to the mechanism:

$$TiO_2 + h\nu (E_g \geq 3.2 \text{ eV}) \rightarrow h^+_{vb} + e^+_{cb} \quad (3)$$

$$h^+_{vb} + OH^-_{ads} \rightarrow \dot{O}H_{ads} \quad (4)$$

The hole trapping leaves the conduction band electron, e^-_{cb}, available to interact with the ambient atmosphere and the chemisorption of molecular oxygen takes place according to:

$$e^-_{cb} + O_2 \rightarrow O^-_{2ads} \quad (5)$$

Much information has been obtained about the nature of the adsorbed species O^-_{2ads} (ref. 4), but the identification of the hydroxyl radical has been much more difficult to confirm due to its high reactivity, although there is indirect evidence[5] of its existence.

In a recent series of experiments we have used the technique of temperature programmed desorption (TPD) in conjunction with mass spectrometry and gas–liquid chromatography to investigate the surfaces of rutile powders, which contain pre-adsorbed water, following their exposure to prolonged irradiation (300 min) with near UV photons ($\lambda \geq 365$ nm) at room temperature in the presence of either pure oxygen or air. (These rutile powders, supplied by Tioxide International Ltd, contained 99% rutile with a specific surface area of 13 $m^2 g^{-1}$ as determined by krypton adsorption at 78 K.)

A comparison of the desorption spectra from these surfaces, following the irradiation treatment, with that arising from a TiO_2 surface containing pre-adsorbed hydrogen peroxide only, shows a correspondence between the molecular oxygen peaks in the respective profiles (Fig. 1). These results indicate that hydrogen peroxide is formed on the surface of the TiO_2 powder during the irradiation, probably through the direct interaction of the hydroxyl radicals which form during the hole trapping process:

$$H\dot{O}_{ads} + \dot{O}H_{ads} \rightarrow H_2O_{2ads} \quad (6)$$

A remarkable aspect of the desorption spectra from these surfaces is the appearance of a gaseous molecular species which from its mass cracking pattern ($m/e = 30$, 14, 16) and the relative peak intensity ratios of 100:6:1 has been identified as nitric oxide. In the case of surfaces containing pre-adsorbed hydrogen peroxide the nitric oxide appears only after exposing the surface to molecular nitrogen or to air. Similar effects are observed if the TiO_2 surfaces are irradiated in pure oxygen, whereas it seems that the nitric oxide forms directly if the irradiation takes place in air or a nitrogen–oxygen mixture. The quantity of nitric oxide desorbed from the surface saturated with pre-adsorbed hydrogen peroxide (Fig. 1) corresponds to a coverage of approximately 3×10^{17} molecule m^{-2}.

The surface species from which the nitric oxide arises has not yet been identified but it has been clearly demonstrated that molecular nitrogen reacts with adsorbed hydrogen peroxide (Fig. 2) and it is therefore most probable that nitric oxide arises from the direct interaction of molecular nitrogen with a species originating from the adsorbed hydrogen peroxide. Moreover it had been established that hydrogen peroxide is formed on the surface of TiO_2 (rutile) when oxygen, water and UV radiation are present simultaneously and that molecular nitrogen can be fixed at the surface of the TiO_2 through its interaction with the hydrogen peroxide which has been photosynthesised *in situ*.

Molecular nitrogen is thought to react with a species of atomic oxygen, either O_{ads} or O^-_{ads}, which originates from either the thermal or photochemical decomposition of the adsorbed hydrogen peroxide, as the interaction of molecular nitrogen with hydroxyl radicals is energetically very unfavourable[6],

$$N_2 + OH \rightarrow NO + NH \qquad \Delta H^{\ominus}_{298} = +387 \text{ kJ mol}^{-1} \quad (7)$$

We believe that the adsorbed species from which the gaseous nitric oxide originates is probably an adsorbed form of molecular nitric oxide, or one of its ions, and is created by a mechanism of the type shown in equations (8)–(11):

$$H_2O_{2ads} \rightarrow H_2O_{ads} + O^*_{ads} \quad (\text{where } O^*_{ads} \text{ is } O_{ads} \text{ or } O^-_{ads}) \quad (8)$$

$$N_2(g) + O^*_{ads} \rightarrow NO^*_{ads} + N_{ads} \quad (9)$$

$$N_{ads} + O^*_{ads} \rightarrow NO^*_{ads} \quad (\text{where NO}^* \text{ is } NO_{ads} \text{ or } NO^-_{ads}) \quad (10)$$

or

$$N_{ads} + O_{2ads} \rightarrow NO^*_{ads} + O_{ads} \quad (11)$$

X-ray photoelectron spectroscopic (XPS) examinations of the surfaces of these TiO_2 specimens show nitrogen (1s) signals at a

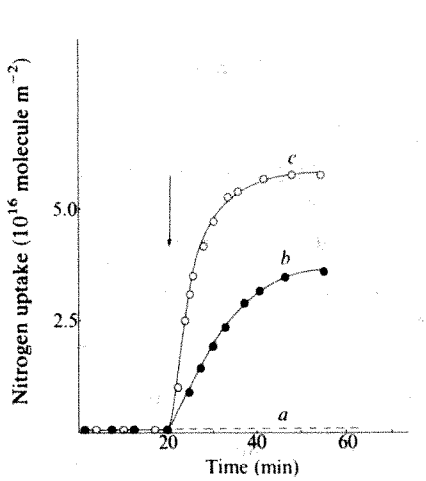


Fig. 2 The interaction of molecular nitrogen with hydrogen peroxide vapour at the surface of TiO_2 (rutile) at 298 K. *a*, TiO_2 absent; *b*, initial pressure of nitrogen = 3.3 N m^{-2}; *c*, initial pressure of nitrogen = 20.0 N m^{-2}. Arrow indicates hydrogen peroxide vapour admitted.

binding energy of 399.5 eV, confirming the presence of an adsorbed species containing nitrogen. In the experiment with pre-adsorbed hydrogen peroxide, the saturation coverage of the nitrogen species was estimated from the XPS data to be of the order of 10% of a monolayer, but it is not possible to decide whether the N(ls) signal originates from adsorbed nitric oxide or from a diatomic nitrogen species. Nitric oxide adsorption at 298 K (ref. 7), on oxidised TiO_2 surfaces, produces two irreversible states of adsorption having maxima in the desorption profile at 450 K and 660 K respectively. It is the stronger state of adsorption of nitric oxide to which the fixed nitrogen most closely corresponds. Electron paramagnetic resonance studies of the samples containing the fixed nitrogen failed to detect any species which could be ascribed to adsorbed molecular nitric oxide and we conclude that the adsorbed species is probably NO^-_{ads} (or $N_2O^{2-}_{2ads}$) as enhancement in the uptake of molecular nitrogen suppresses the formation of the species, O^-_{2ads} (ref. 7) which arises from the decomposition of the adsorbed hydrogen peroxide. In addition no mass spectrometric evidence has been found in the temperature programmed desorption profiles for the existence of molecules of N_2O or NO_2.

Titanium dioxide surfaces are probably not unique in being able to participate in the photocatalytic fixation of molecular nitrogen as many other solids exhibit photocatalytic properties[8]. The adsorbed nitrogen species formed during the fixation reaction may itself undergo further oxidation to a more stable species, such as the NO_2^- ion[9], and it seems likely that these processes can occur naturally at the Earth's surface under the stimulus of sunlight.

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CS_2 and COS in the stratospheric sulphur budget

THE persistence and pervasion of the stratospheric sulphate (Junge) layer[1,2] during periods of low volcanic activities have puzzled many aeronomers. Junge[3] suggested that sulphur dioxide, SO_2, is probably the gaseous precursor for the sulphate layer. The origin of stratospheric SO_2 is, however, uncertain. The gas may originate from the troposphere through upward diffusion processes[3], while Crutzen[4] recently suggested that the presence of SO_2 in the stratosphere may be attributed to photolysis of carbonyl sulphide, COS. Recent measurements[5] showed that the mixing ratio of SO_2 remains remarkably constant in the upper troposphere and the lower stratosphere region. We show here that such peculiar phenomena may be explained by oxidation of COS and carbon disulphide, CS_2. The gas, CS_2 has been detected by Lovelock[6] in seawater samples, and by Sandalls and Penkett[7] in the lower troposphere. We[8] recently argued that CS_2 may be a precursor for atmospheric COS. Here we investigate the possible role of COS and CS_2 in the stratospheric sulphur budget and account for the source for stratospheric sulphate, the observed distributions of SO_2 and COS.

Table 1 Chemical model

Ref	Reaction	Rate constant*
9, 10	$CS_2+OH \rightarrow COS+HS$	$R1 = 1.9(-13)$§
11	$CS_2+O \rightarrow CS+SO$	$R2 = 3.7(-11)\exp[-700/T]$
	$\rightarrow COS+S$	$(fa = 0.8, fb = 0.1, fc = 0.1)$†
	$\rightarrow CO+S_2$	
12	$CS_2+h\nu \rightarrow CS+S$	$J3$‡
26	$COS+h\nu \rightarrow CO+S$	$J4$‡
9, 10	$COS+OH \rightarrow CO_2+HS$	$R5 = 5.7(-14)$§
27	$COS+O \rightarrow CO+SO$	$R6 = 1.6(-11)\exp[-2250/T]$
17	$SO_2+OH+M \rightarrow HSO_3+M$	$R7 = \frac{8.2(-13)\times[M]}{7.9(+17)+[M]}$
28	$SO_2 \rightarrow$ precipitation and scavenging	$R8 = 5.8(-7)Z < 10$ km

* The rate constants are in units of $cm^3\ s^{-1}$ for two-body reaction, $cm^6\ s^{-1}$ for three-body reaction and s^{-1} for photolysis rates and $R8$.
† *fa*, *fb* and *fc* are the relative yields for the three branches.
‡ The photolysis rates are calculated from measured cross-sections allowing properly for diurnal variation of solar intensity.
§ These are room temperature values[9]. The activation energy for $R1$ and $R5$ should be negligible based on proposed reaction mechanism[10].

The atmospheric sinks for CS_2 are,

$$CS_2+OH \rightarrow \left[\begin{matrix}S-C-S\\ \quad\ |\\ \quad OH\end{matrix}\right]^* \rightarrow COS+HS \quad (1a)$$

$$\rightarrow CS+SOH \quad (1b)$$

$$CS_2+O \rightarrow CS+SO \quad (2a)$$

$$\rightarrow COS+S \quad (2b)$$

$$\rightarrow CO+S_2 \quad (2c)$$

and

$$CS_2+h\nu \rightarrow CS+S \qquad \lambda < 2{,}200\ \text{Å} \quad (3a)$$

$$\rightarrow CS_2^* \qquad \lambda > 2{,}200\ \text{Å} \quad (3b)$$

Reaction (1a) is exothermic while reaction (1b) is endothermic[9]. Recent work by Kurylo and Laufer[10] indirectly supports reaction (1a). Reaction (2) dominates in the stratosphere and proceeds[11] primarily through reaction (2a). Photolysis of CS_2 (reaction (3a)) seems to be relatively unimportant according to

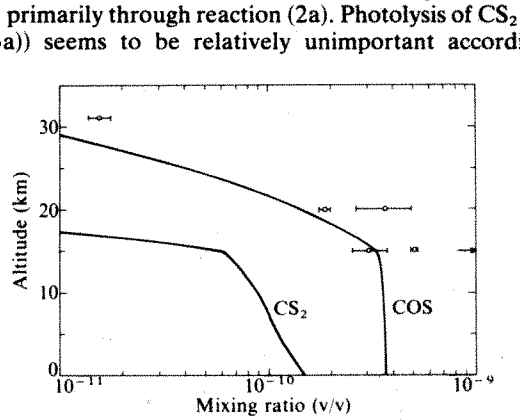


Fig. 1 Computed vertical profiles of CS_2 and COS. The profiles are calculated using a one-dimensional model described previously[20]. The rate constants for O—C—N—Cl—S chemistry are from ref. 29 and the main sulphur reactions are given in Table 1. The diffusion coefficients are from ref. 30. The boundary conditions at 0 km are 0.15 p.p.b. for CS_2 and zero flux for COS. Observed CS_2 and COS surface concentrations range from 0.07 to 0.37 p.p.b. (ref. 7) and 0.3 to 0.5 p.p.b. (refs 7, 21, 22) respectively. The stratospheric COS data[31] (○) are plotted for comparison. The arrow indicates the position of the tropopause.

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absorption cross-section data[12]. Photo-excitation of CS_2 (reaction (3b)) and the subsequent chemistry involving CS_2^* could lead to additional production of COS and SO_2 (refs 13, 14). Reactions involving CS_2^* have not been included in this study due to lack of laboratory data. Reaction (1a) could provide a major source for COS. We assume that reaction products such as HS, CS and S would ultimately be oxidised to form SO_2 over a relatively short time, although reaction of CS with O_2 could provide an additional source for COS (ref. 15). Chemical removal of SO_2 in the stratosphere is dominated[16–18] by the reaction,

$$OH + SO_2 + M \rightarrow HSO_3 + M \qquad (4)$$

The main chemical processes are summarised in Table 1. The rate constants $R1$ and $R5$ (Table 1) used are from Kurylo[9]. These values[9] apparently conflict with the corresponding upper limits set by Atkinson *et al.*[19], whose measurements were performed in less stringent conditions (higher reactant pressure). The observed[19] nonlinear dependence of first-order OH decay rates on reactant concentration may be attributed to complications[9] associated with secondary reactions.

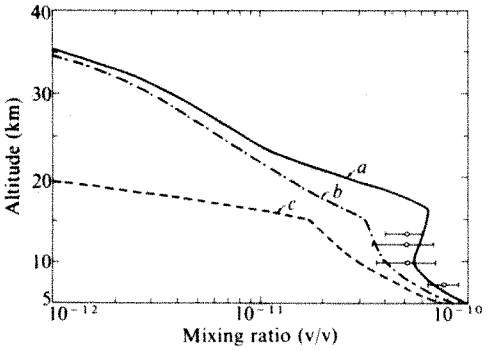


Fig. 2 Computed vertical profiles of SO_2, (lines) and measurements (circles)[5]. Curve *a* is calculated using the full model described in Table 1. Curve *b* is calculated with CS_2 chemistry omitted. Curve *c* is calculated with both COS and CS_2 chemistry omitted. In all three curves, a value of 0.3 p.p.b. is assumed for SO_2 at 1 km. This boundary is chosen such that the integrated tropospheric abundance of SO_2 is equal to 6×10^5 (S) ton, a value consistent with the global estimates of 4.5×10^5 and 5.2×10^5 (S) ton given by Mészáros[32] and Friend[33], respectively. The fixed mixing ratio boundary condition at 1 km implicitly incorporates[28] the effects of any production processes (for example, H_2S oxidation, anthropogenic and volcanic emissions) and removal processes (for example dry deposition) occurring below 1 km. Note that the reported data[5] were taken at ~50° N, while our model corresponds to 30° N. For proper comparison, the data points should be shifted upwards by ~2 km (ref. 30).

The vertical distribution of a minor species may be obtained by solving the appropriate one-dimensional continuity and flux equations[20]. Figure 1 shows the calculated altitude profiles of CS_2 and COS. The calculated mixing ratio of CS_2 drops abruptly above ~14 km altitude, reflecting the onset of the relatively fast reaction of CS_2 and O atoms. In calculating the COS profile, we specify a zero flux surface boundary condition instead of using its mixing ratio at the boundary, an assumption consistent with our premise that CS_2 is a precursor[8] of COS. The apparent agreement between the predicted and observed surface COS mixing ratios[7,21,22] of about 0.4 p.p.b. may be fortuitous in view of the possible ambiguities in rate constant data ($R1$ and $R5$ in Table 1) and paucity of CS_2 data. Nevertheless, an approximate balance in COS budget[8] may be achieved so long as the relationship

$$\frac{[CS_2]}{[COS]} \approx \frac{R5}{R1} \qquad (5)$$

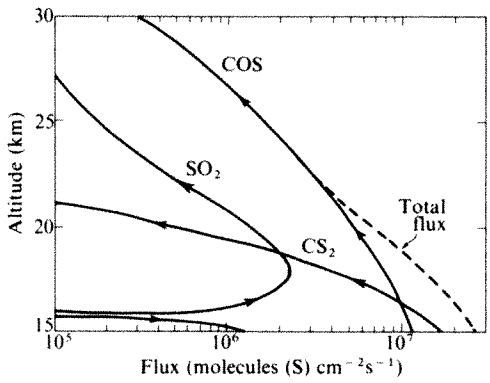


Fig. 3 Computed fluxes for CS_2, COS and SO_2. The flux of CS_2 has been multiplied by 2, reflecting that each molecule carries two sulphur atoms. The total flux Φ_t is set equal to $2\phi_{CS_2} + \phi_{COS} + \phi_{SO_2}$. The arrows indicate direction of flow.

holds in the lower troposphere. Otherwise, alternative sources and/or sinks for COS must be found to account for the observed surface concentrations of COS. If $R1$ were much less than the value[9] used here, CS_2 would remain relatively well mixed in the troposphere, making it a more potent source of stratospheric SO_2.

Figure 2 shows the altitude profiles of SO_2 and data reported by Jaeschke[5]. These three curves correspond to different assumptions concerning the atmospheric sources of SO_2. Curve *c* illustrates the case where there is no *in situ* source for SO_2. The rapid decrease in mixing ratio reflects the rapid removal of SO_2 by reaction (4). Curve *a* (standard model) represents the full model calculation where SO_2 is produced locally by reactions $R1$ through $R6$ in Table 1. Our calculation showed that SO_2 (curve *a*) is relatively well mixed in the region 10–15 km, agreeing with observed data[5], although such a feature could be accounted for by upwelling air motion during particular weather conditions of the day[5]. Note that in the absence of a CS_2 source, the calculated SO_2 distribution (curve *b*) lies somewhat below the observed data[5].

Figure 3 shows the combined flux, Φ_t, of CS_2, COS and SO_2 of about 2.5×10^7 molecules (S) $cm^{-2}\,s^{-1}$ or 2.1×10^5 ton (S) yr^{-1} into the stratosphere. This value should be compared with several independent estimates[3,23,24] of 5×10^6 to 2×10^7 molecules (S) $cm^{-2}\,s^{-1}$ necessary to sustain the sulphate layer. Note that the major contribution to Φ_t comes from CS_2 and COS, with each gas dominating at different altitude regions. The rapid increase of SO_2 flux with altitude between 15 and 17 km reflects the *in situ* production of SO_2 in the model rather than upward diffusion[3] of SO_2 from the troposphere. It seems that the persistence of the sulphate layer may be attributed partly to the oxidation of CS_2 below ~20 km and partly to the oxidation of COS above ~20 km, and that the observed altitude[3,23] (≈20 km) at which the sulphate layer peaks may be modulated by the relative strengths as well as the altitude dependence of these two sources.

The calculated global flux of CS_2 is about 6.4×10^8 molecules (S) $cm^{-2}\,s^{-1}$ or 5.4×10^6 ton (S) yr^{-1}, implying a CS_2 life time of about 0.2 yr compared with that of COS of about 0.6 yr. The calculated flux depends on the tropospheric OH concentration, CS_2 boundary condition and rate constant $R1$, all of which are subject to large uncertainties. The measured concentration of CS_2 in seawater samples reported by Lovelock[6] suggests that the ocean might provide a source of CS_2 of order 10^7 molecules (S) $cm^{-2}\,s^{-1}$ based on a simple piston model[25]. The role of the ocean in the atmospheric CS_2 budget has yet to be determined.

In conclusion, CS_2 could have an important role in the stratospheric sulphur budget. Clearly, more atmospheric measurements of SO_2, CS_2 and COS, as well as accurate determination of rate constants for $R1$ and $R5$, are needed for model validation. It is also evident that other sources of CS_2 and COS need to be identified.

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Thermohaline intrusions lie across isopycnals

A MAJOR task of physical oceanography is the determination of the fluxes of heat and salt and the identification of the processes responsible for producing the fluxes. For the development of realistic models of the ocean it is particularly important that fluxes across surfaces of constant density (isopycnal surfaces) be distinguished from those along isopycnals. We report here direct evidence that at least some of the 5–20-m thick intrusions commonly found in regions of strong horizontal variability (fronts) lie across isopycnals. The intrusions are formed by primarily lateral advection to produce complicated interleavings of the differing types of water across the fronts. The ubiquitous occurrence of intrusions in frontal regions and the fact that they cross isopycnal surfaces indicates that they are important factors in the vertical, as well as the horizontal, flux budgets of heat and salt. The low levels of cross-isopycnal fluxes due to small scale turbulence away from fronts[1] further suggests that these intrusions may be important in the overall global flux budgets of heat and salt.

The intrusions were studied at Ocean Station P (50° N, 145° W) in August 1977 during the mixed layer experiment (MILE). One of the reasons for selecting the site was its distance from known fronts. However, repeated surveys with a rapidly profiling towed vehicle revealed fronts in the weakly stratified region between 40 m (the base of the seasonal thermocline) and 100 m (the beginning of a strong salinity increase or halocline). The fronts were characterised by lateral changes along isopycnal-surfaces of 0.4 °C and 0.05% over distances <2 km.

Tongues of water up to 8 km long, with widths of 1 km and thicknesses of 10–20 m, were observed to intrude into the warmer, more saline water from the cool, low salinity side of one of the fronts. Figure 1 shows the down profiles from a tow section across a tongue. The section was roughly parallel to the front so the section shows the tongue in the direction of its minimum horizontal extent. Minima in temperature and salinity appear in the 7th profile between 0.5 and 0.55 MPa (these pressures correspond to nearly 50 m in depth) and slope downwards towards the right. The structure also becomes thicker as it deepens and shows splitting into double minima. The density changes due to the matched temperature and salinity structures are so nearly compensating that little evidence for the feature is apparent in the bottom set of profiles.

To be certain that the slope is not due to a changing internal wave displacement, the temperature and salinity contours must be examined as functions of potential density rather than of pressure. The quality of the density profiles is central to this argument. Careful laboratory tests of the temperature and conductivity sensors[2,3] show that the r.m.s. random noise in the data is equivalent to 2.1×10^{-4} kg m^{-3}, which is negligible for our purpose. The dynamic response of the temperature probe as it passed through a thin plume was obtained in the laboratory. The response of the conductivity cell, determined by comparison of the temperature–salinity diagrams from up and down depth cycles, showed that the cell response was closely matched to that of temperature. Even so, a numerical simulation of the sensors passing through features similar to those in Fig. 1 revealed that density errors equivalent to displacements of 1–3 m could occur as a consequence of microstructure in the profile. However, this is much less than the 15 m vertical displacement of feature across the tow path.

Contours of isotherms plotted in Fig. 2 with potential density as the vertical coordinate show the temperature minimum sloping with respect to lines of constant density. This figure includes data preceding that in Fig. 1, showing the nearly level isotherms in a region where an intrusion is not present. As a further test of the data, additional contours were made using only the up traces. If the slope were an artefact of a dynamic response

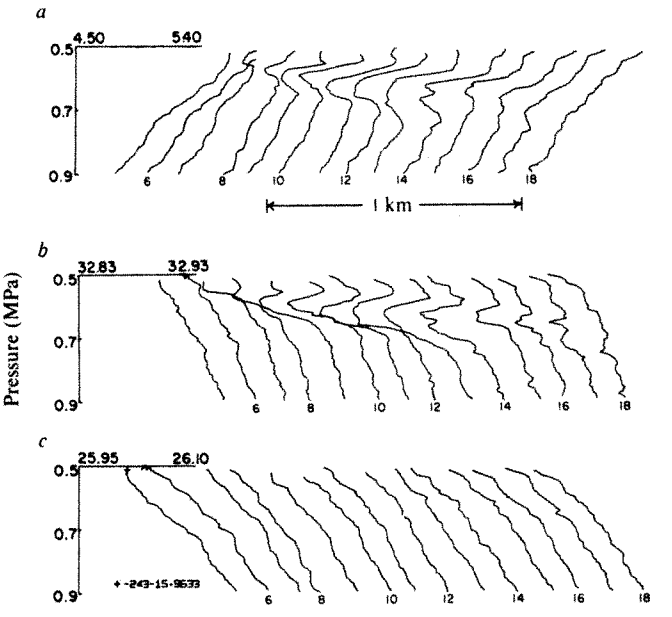


Fig. 1 Successive down profiles of temperature (*a*), salinity (*b*) and density (*c*) from a depth-cycling towed vehicle (1 MPa corresponds to a depth interval of 99.2 m). Pronounced minima in temperature and salinity slope downward from left to right and mark an intrusion of cool, low salinity water into the profile. The density effects of the cooler (an increase in density), low salinity (a decrease in density) water are so nearly compensating that there is little evidence for the feature in the bottom set of profiles.

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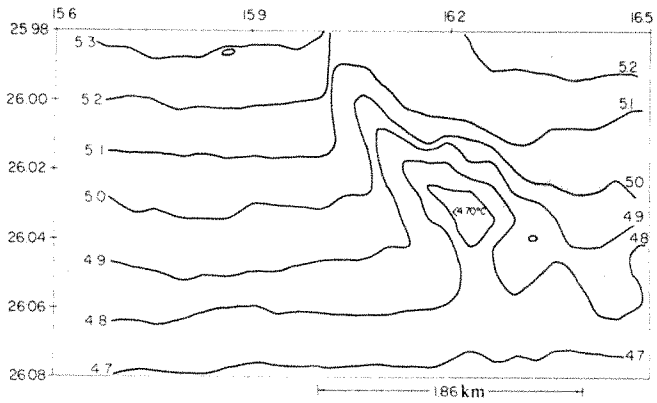


Fig. 2 Isotherms plotted with potential density (in kg m^{-3}) as the vertical coordinate. Internal wave distortions are removed from the contour. The remaining structure is an intrusion of cool, low salinity water that slopes downward from left to right.

problem, the slope should have reversed in the up casts. This is not the case and the maps are nearly identical to Fig. 2.

A section made at a 60° angle to that shown in the figures showed that the intrusion was also sloping downwards across isopycnal surfaces in a direction away from the front. Microstructure profiles through similar intrusions[5] have revealed distinctive signatures on the upper and lower boundaries of the minima that have been identified as the appropriate type of double-diffusive convection.

Double-diffusive convection is a consequence of the much lower molecular diffusivity of salt dissolved in water compared to the thermal diffusivity[6]. Consequently, when warm, saline water overlies cool, fresh water in a stratified profile small perturbations lead to a series of centimetre thick rising and descending columns known as salt fingers. When warm, saline water occurs beneath cooler, fresher water (known as the diffusive regime), a sequence of convecting regions separated by their interfaces is formed. Although the observations in Fig. 1 did not have the spatial resolution to detect the signatures of double diffusion, the inference from the microstructure observations is that salt fingering occurred above the minima and the diffusive regime below. Laboratory experiments[7] of diffusively unstable intrusions have shown that the increase in density below a salt fingering region dominates the decrease above the 'diffusive' regime, with the consequence that cool, fresh intrusions tend to sink as they move laterally. The thickening and formation multiple minima are also revealed in the laboratory work.

The cross-isopycnal slope of 100 m-thick intrusions across the Antarctic Front has been inferred[7] by a cross-spectral comparison of horizontally separated profiles. We have obtained the same result directly for much thinner features by mapping against density.

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Connectance in real biotic communities and critical values for stability of model ecosystems

BIOLOGICAL communities differ in the number of species that they contain; in the number of interactions between species (primarily feeding and competitive relationships); and in the intensity of their interactions. We present here the first extensive empirical data on the relationship between connectance (defined as the fraction of the pairs of species which directly interact[1,2]) and species richness in biological communities. Our results show that connectance may decline considerably as the number of species increases.

A decade ago, most ecologists firmly believed that increased ecosystem complexity (both the number of species and the number of interactions) would lead to greater ecosystem stability. For example, Odum[3] suggested that "Higher diversity, then, means longer food chains and more cases of symbiosis (mutualism, parasitism, commensalism, and so forth), and greater possibilities for negative feedback control, which reduces oscillations and hence increases stability". This attractive and widespread statement has been shaken dramatically by May[1,2,4,5] and his followers[6-13]. May examined the complexity–stability question in model ecosystems by constructing interaction matrices with each off-diagonal element (interaction coefficient) assigned from a distribution of random numbers, where distribution itself has mean value zero, and standard deviation value (interaction strength) s; the stability of the matrices was then evaluated in terms of their eigenvalues[14]. The central feature of May's results for model ecosystems is the sharp transition from stable to unstable behaviour as soon as either the species number m, or the connectance C, or the interaction strength s, exceeds a critical value. If the equation $s\sqrt{mC} > 1$ holds, the system will almost certainly be unstable. In general, by becoming more complex a system becomes less likely to be stable. Selected empirical evidence supports such a conclusion. According to May[4], "this inverts the naive, if well-intentioned, view that 'complexity begets stability', and its accompanying moral that we should preserve, or even create, complex systems as buffers against man's importunities". May's corollaries are useful for sharpening discussion but their application to natural resource management and optimum land use is not without danger.

Recently, Lawlor[15] has shown that severe constraints must be introduced on the structure of an interaction matrix if it is to represent a biologically acceptable system. He has suggested, *inter alia*, the hyperbolic reduction of the connectance C as the number of species m increases: $Cm = 10$. This relationship ensures stability if the standard deviation s of the interaction coefficients (not variance; this is the mistake of Lawlor), is less than about one-third. Lawlor's comments induce the urgent question: What is the true connectance of real communities?

The question seems to be unanswerable on the basis of very rough, speculative or fragmentary data in most of the literature, even if such data are sometimes used in theoretical considerations. The extensive data collected by one of us[16,17] have allowed reconstitution of the food webs in a great number of plant–aphid–parasitoid communities. The parasitoids belong to three families: Aphidiidae, Aphelinidae and Encyrtidae (Hymenoptera). All host–parasitoid relationships have been tested in the laboratory. The results for one representative community are demonstrated in Fig. 1. In the cannopy of an oak–pine–birch forest (the order Pino–Quercetalia Soó 1962 in the sense of the Zürich–Montpellier phytosociological school) 37 species have been recorded of vascular plants, aphids and their parasitoids; a trophic connectance of 0.059 has been calculated for this community. Some chains have been confirmed by numerous field assessments, for example, 23 geographically distinct observations for that of *Pinus silvestris–Schizolachnus pineti–Pauesia*

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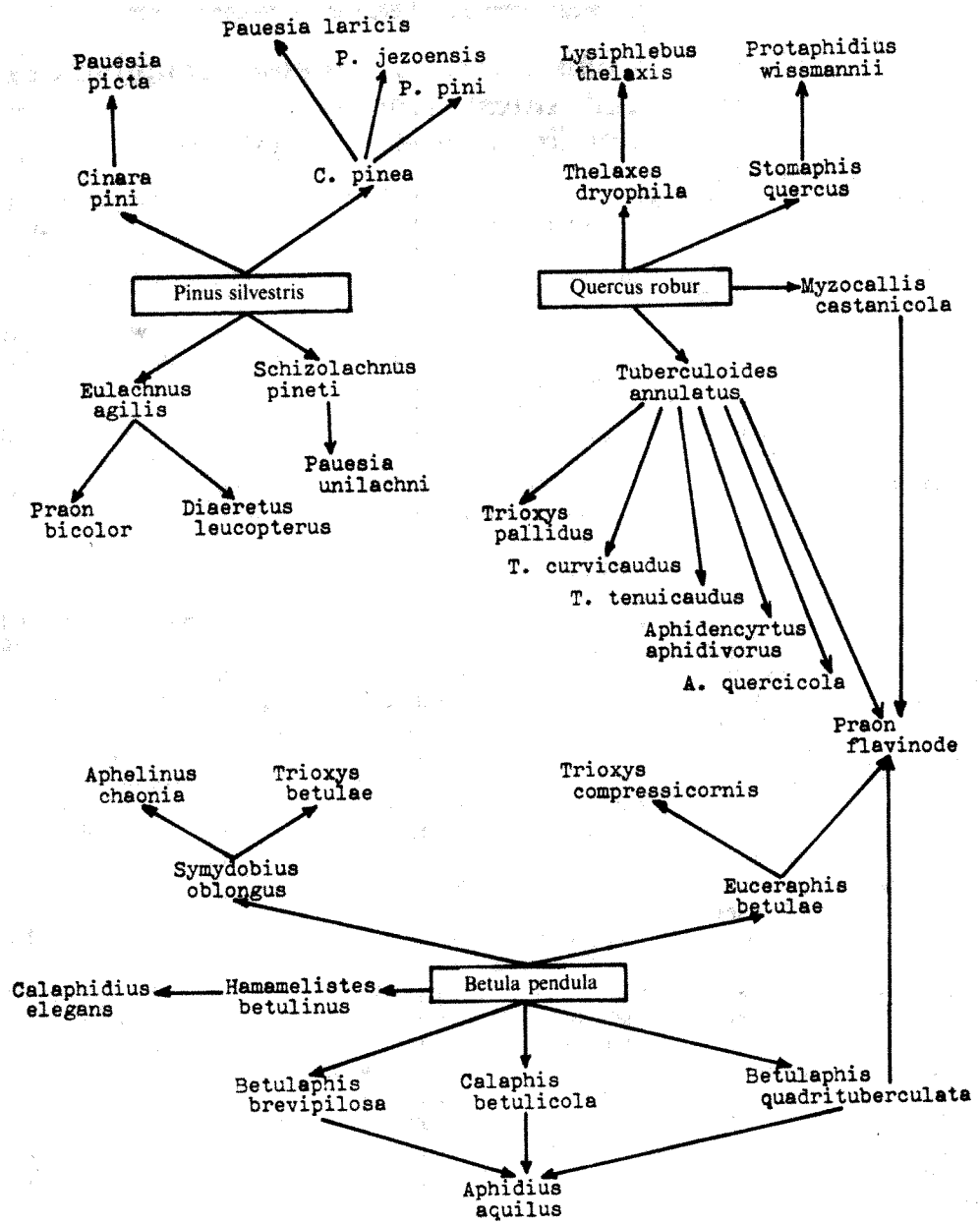


Fig. 1 An example of the plant–aphid–parasitoid food web: a cannopy of oak–pine–birch forest in Central Europe (number of species = 37, trophic connectance = 0.059, potential total connectance = 0.137).

unilachni. Other chains are not so remarkable, for example, that of *Betula pendula–Betulaphis brevipilosa–Aphidius aquilus*. The potential total connectance is highly overestimated because it has been calculated on the basis of both the discovered trophic connections and all potential competitive interactions derived from the structure of the food webs regardless of the biological reality. The aggregation of the populations[18] and the differentiation of the food niches considerably reduce the number of competitive interactions. For example, *Cinara pinea* can never directly compete with *Eulachnus agilis* or *Schizolachnus pineti* despite the common plant host.

Food webs have been reconstructed for 31 plant–aphid–parasitoid communities; the summary of the connectance analysis is presented in Fig. 2. The analysed communities cover a broad variety of Central European biotopes and successional stages. With regard to the previous comments, we may conclude that the decrease in total connectance with increased species richness fits very closely the relationship $Cm = 3$. This reduced connectance ensures the stability of model ecosystems if the standard deviation s of the interaction coefficients is less than 0.577, that is, the variance is less than one-third. It means that with this modest value of s, an increasing number of species does not cause a decrease of model stability: a conclusion which, at

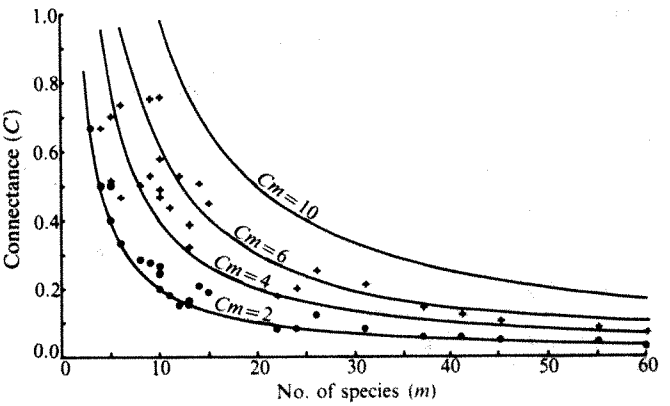


Fig. 2 Four hyperbolic approximations and the empirical relationships between connectance (c) and species number (m) in real plant–aphid–parasitoid communities. Potential total C (+) was calculated on the basis of both the discovered trophic connectance (●) ions and all potential competitive interactions derived from the structure of the food webs (see, for example, Fig. 1) regardless of the biological reality.

least, does not contradict the traditional ecological hypothesis. Connectance values for pure competition communities have recently been estimated by McNaughton[19]. His data for communities consisting of up to 20 plant species follow roughly the product $Cm = 4.7$.

The low connectance assessed by us in plant–aphid–parasitoid communities, arises undoubtedly from their organisation in guilds of species interacting within each guild, whereas their interactions with species in other guilds are only loose. Such an organisation may reduce the destabilising effect of greater system complexity[1,2]. It seems likely that the majority of the plant–aphid–parasitoid communities possesses structures which correspond to the boundary conditions of model stability. This is, of course, a rather speculative conclusion as we are unable to estimate the s values. On the other hand, there is no evidence for the existence of any plant–aphid–parasitoid communities outside the region $2 < Cm < 6$. Destabilising effects can be expected, through increased connectance caused by hyperparasitoids and predators, both with broader food spectra[20,21]; further study is required on this point.

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Abnormal visual resolution of cats reared in stroboscopic illumination

REARING animals in a visually restricted environment can lead to marked abnormalities of both the response properties of single visual neurones[1–4] and the visual capacities of the organism[5,6]. These findings have important implications for understanding the development of human vision and have led to many studies of visually deprived humans[7–9]. Although there are similarities between the effects of deprivation in animals and humans there are still major difficulties in extrapolating from experimental deprivation to clinically observed amblyopia. There is, therefore, a need for quantitative psychophysical studies of visually deprived animals using the same tests as those used with human patients. We have quantified the pattern vision of visually deprived cats by measuring their spatial contrast sensitivity, an approach which has frequently been used to characterise the vision of human amblyopes[7–9]. We report here that the spatial resolution of cats reared in stroboscopic illumination is seriously impaired and that changes in their contrast sensitivity are similar to those seen in certain types of amblyopia.

Stroboscopic illumination prevents the perception of motion while allowing rather restricted pattern vision. Animals reared in this illumination show greatly reduced directional selectivity and binocularity of visual neurones in the superior colliculus and visual cortex[10,11], impaired orientation tuning in cortical neurones[11], and marked difficulties in visually guided behaviour[12,13]. However, there is as yet no quantitative description of their visual capacities.

Six cats were used. Three were reared from birth in a room illuminated by a 3-μs strobe light, flashed at a frequency of 40 min^{-1} for 12 h per day. One control cat was reared in a long duration (750 ms) flash of the same frequency as the strobe light. This 750-ms flash permitted the perception of motion while providing a control for the periodicity and low luminance of the strobe environment. The effective luminance of these two types of illumination was equated using a behavioural test in which luminance thresholds for grating discrimination were determined in normal cats for each of these light sources (T.P. and W.H.M., in preparation). When the deprived cats were at least a year old they were removed from the visually restricted environment. Testing began after at least 2 months of adaptation to normal illumination. Two more cats were obtained from a supplier and presumably had normal visual experience during development.

Our behavioural testing techniques resembled those developed by Berkley[14]. The cats were trained to discriminate between a vertical grating of sinusoidal horizontal luminance profile and a uniform field, each at a mean luminance of 15.9 cd m^{-2}. They responded by pressing with their nose on one of two transparent response panels through which the stimuli were visible. The stimuli were 30 cm behind the response panels, subtended a visual angle of $16° \times 20°$, and were generated on display oscilloscopes by conventional techniques[15].

On each trial the stimulus was present for 2 s before the response panel could be activated. Correct responses were rewarded with beef purée and the next trial began 5 s later. Following incorrect responses, no food reward was delivered and the start of the inter-trial interval was delayed 10 s. The position of the correct stimulus varied randomly.

Once the cats mastered the task (4 consecutive days ≥80% correct, 200 trials a day), measurement of contrast thresholds over a range of spatial frequencies began. Performance at a single spatial frequency was tested during each daily session of 200 trials. We used the method of constant stimuli to generate a psychometric function in each session: five contrast levels chosen to bracket the threshold were presented in random order within each block of five trials. Contrast threshold was defined from the resulting psychometric function as the contrast corresponding to 75% correct.

The results are shown in Fig. 1*a*. At the frequency of peak sensitivity, normal cats and the 750-ms control cat can resolve gratings of ~1.4% contrast. Their sensitivity decreases at lower and higher spatial frequencies. The functions for the three strobe cats show greatly reduced sensitivity at all spatial frequencies and a peak shifted towards lower frequencies. The minimal resolvable contrast for the three cats ranged from 7.6 to 29%. Strobe cats also showed a reduction of about 1.5 octaves in the high-frequency cut-off. Figure 1*b* shows log sensitivity loss for the three strobe cats, relative to the 750-ms control. This plot clearly demonstrates increasing sensitivity loss with higher spatial frequencies.

The functions in Fig. 1*a* have shown little change over more than 2 years of testing despite continuing exposure of the cats to a normal laboratory environment. Thus, these deficits are relatively permanent. It is unlikely, moreover, that optical factors can account for these losses. We tested all cats at a distance of 30 cm, which is close to the near point of accommodation for

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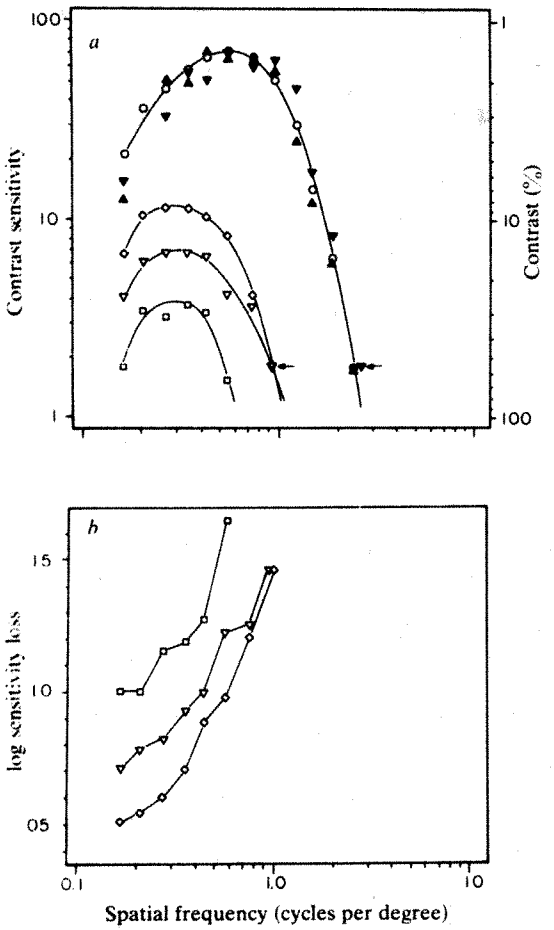


Fig. 1 *a*. Contrast sensitivity (reciprocal of threshold contrast which is shown on the right ordinate) as a function of spatial frequency. ○, 750-ms Control cat; △, □, ◇, three strobe-reared cats. Individual curves were fitted by eye. ▲, ▼, The two normal cats. Points indicated by the arrows are acuity measurements obtained by presenting a range of spatial frequencies at 55% contrast. Each point represents the reciprocal of the contrast value corresponding to 75% correct performance at that frequency and is based on at least 400 trials. Luminance, 15.9 cd m^{-2}; viewing distance, 30 cm; field size, 20° × 16°. *b*, Sensitivity loss for the three strobe-reared cats compared to the 750-ms control cat as a function of spatial frequency. The same symbol is used for each cat as in *a*. Each point represents the ratio of sensitivity of the 750-ms control cat to the sensitivity of the strobe-reared cat expressed in log units. 0.0 corresponds to no loss, 1.0 to a 10-fold reduction in sensitivity.

cats[16]. Refraction by split lamp retinoscopy showed our strobe cats to be slightly myopic (−0.5 to −1.5 dioptre), thus if their accommodative power was normal, they should have been able to focus at that distance. Moreover, optical blurring primarily degrades the resolution of high spatial frequencies[7] and could not account for the losses we observed at low frequencies. Therefore, we suggest that these deficits have a neural basis. Abnormalities in area 17, which have been reported in strobe-reared cats[11], could reduce behavioural contrast sensitivity as striate neurones are particularly sensitive to high spatial frequencies and have low contrast thresholds[17].

The profound visual loss of strobe-reared cats indicates that stroboscopic illumination, which has been used to examine the effects of motion deprivation, also severely restricts spatial vision. However, the normal contrast sensitivity of the cat reared in the long-duration flash demonstrates that the visual loss of strobe-reared cats is not due to either the low time-averaged luminance or periodicity of the strobe illumination. The pattern of visual loss, with the greatest impairment at high spatial frequencies, is similar to that found in strabismic[8,18] and anisometropic[8] amblyopia, suggesting common features between the visual environments of strobe-reared cats and human amblyopes. The visual deficit in these types of amblyopia has been attributed to the consistently blurred image in the amblyopic eye, which has its greatest impact on the visibility of high spatial frequencies. Optical blur may also be an important factor in strobe rearing as the extremely short-flash duration precludes normal accommodation, thus limiting the visibility of high spatial frequencies.

A greater loss at high than at low spatial frequencies in human amblyopes is commonly attributed to a selective impairment of sustained visual neurones[8,9], which, in contrast to transient neurones, respond better to higher spatial frequencies but prefer slower rates of temporal modulation[19]. The contrast sensitivity data reported here as well as the recent finding of reduced sensitivity to slow motion in strobe-reared cats[20] suggests that the major impact of strobe rearing is also on the sustained neurones. Consistent with this interpretation is the observation that sustained neurones are especially dependent on proper accommodation[21] and that they project exclusively to area 17 (ref. 22), which is highly abnormal in strobe-reared animals. Future measurements of the contrast sensitivity of cortical neurones in these cats will help clarify the relationship between neural and perceptual abnormalities in strobe rearing and may provide important insights into neural mechanisms involved in high spatial frequency loss.

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An intrinsic factor XII–prekallikrein-dependent pathway activates the human plasma renin–angiotensin system

EXPOSURE of normal human plasma to an activating (negatively charged) surface leads to the activation of the intrinsic coagulation system and the fibrinolytic system and the generation of vasoactive peptides (kinins)[1,2]. This contact activation mechanism is known to be initiated by factor XII (Hageman factor). Surface-activated factor XII converts prekallikrein (Fletcher factor) into kallikrein, which leads to further activation of factor XII. Prekallikrein circulates in plasma as a complex with the kallikrein substrate, high molecular weight kininogen, and the latter acts as a cofactor in the reciprocal activation of factor XII by kallikrein. The experiments described here demonstrate that the renin–angiotensin system is also activated by kallikrein through the factor XII-dependent pathway.

Normally, about 80% of the renin present in plasma is in an enzymatically inactive form[3–5]. It is called 'inactive renin' or 'prorenin', as opposed to naturally occurring 'active renin'. Both forms can be separated by ion-exchange chromatography[6]. Inactive renin is converted into the active form by limited proteolysis at neutral *p*H, either in acid-pretreated plasma by one or more endogenous serine proteases[7–9] and by urinary (renal) kallikrein[10], or in untreated plasma with exogenous trypsin[5,9]. Active renin is also formed during prolonged exposure of plasma to low temperature (cryoactivation)[11]. Recent reports have suggested that factor XII and prekallikrein might be involved in the generation of active renin, but the results were contradictory[12,13]. To investigate the possible involvement of the factor XII-dependent pathway in the activation of prorenin, we treated normal plasma ($n = 10$), factor XII-deficient plasma ($n = 2$) and prekallikrein-deficient plasma ($n = 1$) by (1) dialysis at *p*H 3.3 and 4 °C for 24 h with subsequent restoration of *p*H to 7.5 (ref. 14), or (2) incubation with Sepharose-bound trypsin at *p*H 7.5 and 4 °C. Renin was then measured by radioimmunoassay of angiotensin I formed after incubation of the plasma samples with an excess of purified sheep renin substrate at *p*H 7.5 and 37 °C for 3 h (ref. 4). Results in normal plasma showed identical amounts of activated renin after acid or trypsin treatment (Fig. 1, left-hand scale). Smaller increments of active renin were obtained during cryoactivation. In plasma from patients with Hageman trait, levels of naturally occurring active renin were 17 and 21 $\mu U\ ml^{-1}$, and those of inactive (trypsin-activable) renin 180 and 210 $\mu U\ ml^{-1}$. These values were within the normal range found in our laboratory, which is 12–45 $\mu U\ ml^{-1}$ for active renin and 70–230 $\mu U\ ml^{-1}$ for inactive renin ($n = 25$). In samples from the patient with Fletcher trait, active renin was low, 8.4 $\mu U\ ml^{-1}$, but inactive renin was markedly elevated, 510 $\mu U\ ml^{-1}$. In contrast to normal plasma, both factor XII- and prekallikrein-deficient plasma generated small amounts of active renin after acidification, whereas trypsin generated normal or increased amounts. There was no detectable cryoactivation of renin in the deficient plasmas. This suggested that acid activation and cryoactivation of plasma renin depend on the presence of both factor XII and prekallikrein.

Further experiments substantiated this hypothesis. Reconstitution of factor XII-deficient plasma and prekallikrein-deficient plasma with, respectively, purified factor XII and prekallikrein resulted in a correction of the generation of active renin after acidification. Prekallikrein in normal plasma is converted into active kallikrein after exposure of the plasma to low *p*H, trypsin or low temperature (cryoactivation)[15]. Simultaneous determinations of renin and kallikrein measured as amidolytic activity[16] showed parallel increments of both activities (Fig. 1, right-hand scale).

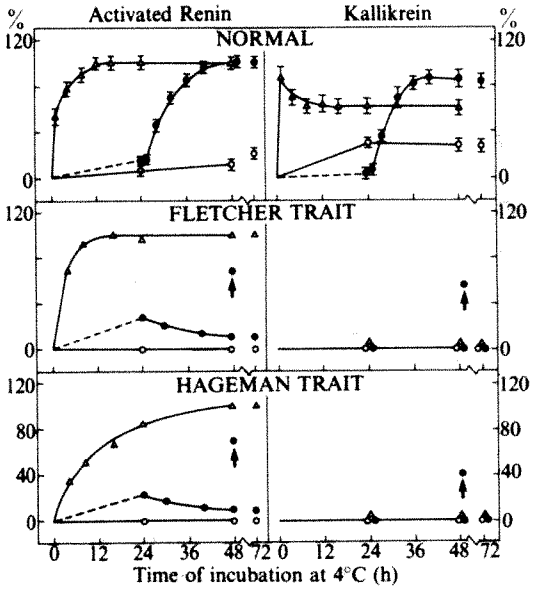


Fig. 1 Generation of active renin and kallikrein in normal plasma (mean ± s.e.m., $n = 10$), prekallikrein (Fletcher factor)-deficient plasma and factor XII (Hageman factor)-deficient plasma. Two procedures were used: acidification and addition of trypsin. Plasma, which contained 5×10^{-3} M EDTA, was acidified (- - - -) by dialysis against 0.15 M glycine-HCl buffer of *p*H 3.3 for 24 h (ref. 14). The *p*H was then restored to 7.5 with 1 M NaOH, and the generation of active renin and kallikrein was followed for 1–72 h (●—●). Trypsin (14,000 BAEE units per mg protein, Sigma) was covalently bound to CNBr-activated Sepharose (Pharmacia) in a ratio of 10 mg protein per g dry Sepharose. The immobilised enzyme was added to plasma (△—△) at a final concentration of 3,000 BAEE units per ml. After 1–48 h it was removed by centrifugation. All these procedures were carried out at 4 °C. Plasma that was kept at 4 °C without any other form of treatment served as a control (○—○). Pretreated and control plasma samples were then assayed for renin and kallikrein activity. For renin measurements the samples were incubated with purified sheep renin substrate (final concentration $6.7 \times K_m$)[4] at *p*H 7.5 and 37 °C for 3 h in the presence of the following protease (angiotensinase) inhibitors: 1×10^{-3} M EDTA, 1×10^{-3} M phenylmethylsulphonylfluoride, 3.4×10^{-3} M 8-hydroxyquinoline and aprotinin (Bayer) at a concentration of 100 kallikrein-inhibiting units per ml of incubate. The increment of renin after 48 h exposure to trypsin was taken as 100% activation. Kallikrein was measured as amidolytic activity using the chromogenic ester H-D-Pro-Phe-Arg-*p*-nitroanilide (PPAN, Kabi) as a substrate[16]. Plasma (15 μl) was mixed at 37 °C with 815 μl Tris buffer of *p*H 7.5 and 200 μl 4.1×10^{-3} M PPAN. The linear release of *p*-nitroanilide was followed at 405 nm for 1–2 min in a 1-cm semi-microcuvette at 37 °C. Complete activation of prekallikrein was obtained by mixing 100 μl of the untreated plasma with 100 μl dextran sulphate ($25\ mg\ l^{-1}$)[18]. After 7 min incubation at 4 °C, 30 μl of the mixture was added to 800 μl Tris buffer and 200 μl PPAN, and kallikrein was measured as before. The values obtained after dialysis at *p*H 3.3 and restoration of *p*H to 7.5 and with trypsin were expressed as a percentage of total activable prekallikrein as obtained with dextran sulphate. Lineweaver–Burk plots for the reactions of acid-activated renin, trypsin-activated renin and naturally occurring active renin with sheep renin substrate (1 h incubation) gave identical K_m values ($\sim 6 \times 10^{-7}\ mol\ l^{-1}$). Thus, the increase of renin activity after acidification and with trypsin is caused by a parallel change in the number of molecules of active renin rather than by changes in the enzymatic activity per molecule of active renin. When untreated normal plasma was kept at 4 °C, small increments in renin and kallikrein activity were observed ($P < 0.001$ for difference of values at 72 h from those at 0 h). Prekallikrein and factor XII were isolated from normal human plasma and purified[19]. They were added to, respectively, prekallikrein-deficient plasma (final concentration of prekallikrein ~ $50\ \mu g\ ml^{-1}$) and factor XII-deficient plasma (final concentration of factor XII ~ $28\ \mu g\ ml^{-1}$). Deficient plasmas to which Tris buffer of *p*H 7.5 was added, instead of prekallikrein or factor XII, served as controls. The reconstituted plasmas and controls were treated by dialysis at *p*H 3.3 for 24 h, and the renin and kallikrein activities were measured 24 h after the *p*H had been restored to 7.5 (results are indicated by arrows).

The acid activation of prekallikrein was confirmed by the parallel cleavage of ^{125}I-labelled prekallikrein added to normal plasma before acidification (Fig. 2). Previous experiments in which the activation of prekallikrein by activated factor XII was studied had shown that the generation of kallikrein activity was directly correlated with the extent of proteolytic cleavage[17]. Analysis of the reaction mixture by SDS-polyacrylamide gel electrophoresis in the presence of reducing agents demonstrated that the disappearance of prekallikrein of apparent molecular

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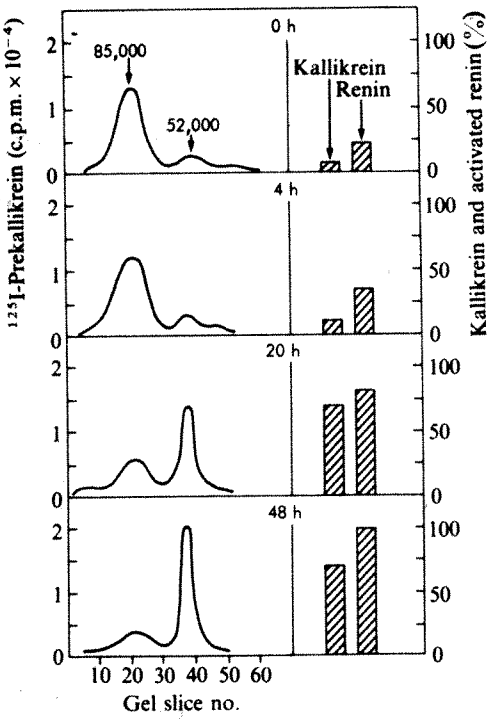


Fig. 2 Cleavage of ^{125}I-prekallikrein in acid-pretreated normal plasma. Prekallikrein was isolated from normal plasma[19] and labelled by the method of Bolton and Hunter[20]. ^{125}I-prekallikrein (~2.7 μCi in 15 μl) was added to 600 μl normal plasma. The mixture was dialysed at *p*H 3.3 for 24 h. Immediately (0 h) after restoration of *p*H to 7.5 with 1 M NaOH, and 4–48 h later, the reaction mixtures were analysed for the presence of cleavage products of ^{125}I-prekallikrein (left) and for kallikrein and renin activity (right). After reduction by β-mercaptoethanol the reaction mixture was subjected to SDS-polyacrylamide gel (7.5%) electrophoresis. The gels were sliced and the radioactivity in each slice (1.2 mm) was counted. The appearance of cleavage products with an apparent molecular weight of 52,000 indicates activation of prekallikrein[17].

weight 85,000 was associated with the simultaneous generation of cleavage fragments; the major radiolabelled fragment had an apparent molecular weight of 52,000.

Addition of a physiological amount of purified plasma kallikrein to factor XII-deficient plasma after the acidification step, caused complete activation of inactive renin (Fig. 3).

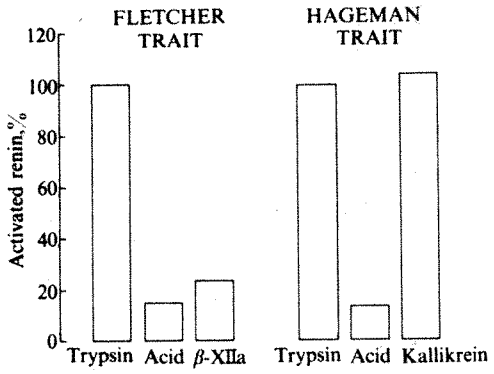


Fig. 3 Generation of active renin in prekallikrein (Fletcher factor)-deficient plasma and factor XII (Hageman factor)-deficient plasma. Plasma was treated with Sepharose-bound trypsin at *p*H 7.5 and 4 °C for 48 h or was dialysed at *p*H 3.3 for 24 h followed by dialysis at *p*H 7.5 and, 4 °C for 24 h. Factor β-XIIa (final concentration 10 μg ml^{-1}) was added to prekallikrein-deficient plasma after 24 h dialysis of the plasma at *p*H 3.3 and restoration of *p*H to 7.5. Plasma kallikrein (final concentration 32 μg ml^{-1}) was added to factor XII-deficient plasma again after 24 h dialysis of the plasma at *p*H 3.3 and restoration of *p*H to 7.5. The mixtures were incubated at 4 °C for 24 h. Factor β-XIIa (600 μg ml^{-1}) (from Dr John Griffin) gave a single band on SDS-polyacrylamide gel electrophoresis with an apparent MW of 28,000. Kallikrein (200 μg ml^{-1}) was prepared by activation of plasma prekallikrein by factor β-XIIa and was separated from factor β-XIIa by DEAE-Sephadex chromatography at *p*H 8.25[19].

Active factor XII fragment (β-XIIa) in a physiological concentration, however, was not capable of generating active renin in prekallikrein-deficient plasma. This indicates that in the factor XII-dependent pathway, plasma kallikrein is the major activator of inactive renin. The factor XII-dependency can be explained by the capacity of this factor to convert plasma prekallikrein into kallikrein.

We conclude that factor XII and prekallikrein are part of an endogenous pathway that activates renin *in vitro*. It is of interest that inactive (trypsin-activable) plasma renin was grossly elevated in the patient with Fletcher trait, whereas his plasma level of naturally occurring active renin was abnormally low. This may indicate that the factor XII–prekallikrein pathway is also important for the activation of renin *in vivo*. Possibly, inactive renin that is adsorbed or bound to the blood vessel wall, at sites that are inaccessible to circulating protease inhibitors, is activated through this pathway. The reported activation of prorenin after the addition of renal kallikrein to acid-pretreated plasma[10] suggests a further analogy with the clotting and fibrinolytic systems: plasma kallikrein is part of an intrinsic pathway for activating the renin–angiotensin system, whereas tissue (renal) kallikrein might be involved in extrinsic activation.

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Macrophage requirements for mouse B-cell stimulation differ according to the molecular form of anti-IgM antibodies

THYMUS-INDEPENDENT lymphocytes (B cells) comprise a heterogeneous population. Because of this heterogeneity, the precise nature of the signals which activate resting B cells to become antibody-forming cells is not known. It is likely that different subsets of B cells have different signalling requirements[1–3]. In addition, it has been proposed that B-cell proliferation and differentiation are controlled by separate and distinct signals[4]. Proliferation is thought to result from antigen binding with specific cell-surface immunoglobulin (Ig) receptors. However, due to the very low frequency of B cells reactive to a specific antigen, the activation process *per se* is difficult to study. This problem may be overcome by using reagents which mimic specific antigen binding to Ig receptors but act as poly-

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Table 1 Proliferative response of spleen cells to various stimulants

Stimulant	Dose (μg per well)	Cells cultured (c.p.m. ± s.e.m.) Unseparated spleen cells	Macrophage-depleted spleen cells Peritoneal macrophages added 0%*	2%	4%	8%	16%
NRG	100	1,710±90	370±30	1,350±29	1,320±95	1,210±113	1,440±81
Anti-IgM	100	6,650±1,430	510±38	12,850±1,473	7,320±50	5,242±369	1,710±145
$F(ab')_2$ NRG	20	2,700±125	1,100±53	2,380±80	2,760±171	3,120±254	2,890±599
$F(ab')_2$ anti-IgM	20	45,000±2,948	42,650±2,065	59,330±1,675	65,370±2,339	51,790±1,159	25,560±765
Control	—	3,500±368	640±148	1,920±59	3,510±140	3,240±466	2,820±499
LPS	1	66,020±4,459	51,650±2,065	30,200±872	32,400±838	21,440±495	11,570±684

Unseparated or macrophage-depleted spleen cells from 8-month-old CBA mice were cultured in triplicate for 3 d in flat-bottom microtitre plates at 4×10^5 cells per well. The cells were stimulated by addition of optimal doses of rabbit anti-IgM, $F(ab')_2$ anti-IgM or *Eschericha coli* 011B4 LPS. Control cultures received normal rabbit globulin (NRG), $F(ab')_2$ NRG or medium. Normal peritoneal macrophages were collected by lavage from CBA mice and added to macrophage-depleted cultures as indicated. All cultures were pulsed with 0.5 μCi of ^{3}H-thymidine for the last 24 h of culture. ^{3}H-thymidine incorporation is expressed as mean c.p.m. of triplicate cultures ± s.e.m.

* Macrophage-depleted spleen cells contained < 1% macrophages as measured by esterase staining.

clonal B-cell activators (PBAs). Anti-Ig antibodies are thought to function in this way and have been shown to be mitogenic for B cells[5–7]. Our laboratory has previously shown that soluble rabbit anti-mouse IgM or $F(ab')_2$ anti-IgM is mitogenic for mouse splenic B cells[8,9]. The response to intact anti-IgM only occurs with B cells from mice which are 7 months or older. In contrast, $F(ab')_2$ anti-IgM will stimulate B cells from both young and aged mice equally well suggesting that changes in B-cell Fc receptors may be involved in the age-dependent response to the intact anti-IgM molecule[10]. In all cases, the response rquires cross-linkage of surface μ heavy chains and is T-cell independent[10,11]. In the present study, we have investigated the macrophage requirement for anti-Ig stimulation. The requirement for macrophages in B-cell activation by PBAs, including anti-Ig reagents, is controversial[12,13]. We report that the proliferative response of B cells to intact anti-IgM is macrophage dependent whereas the response of the same cell population to $F(ab')_2$ anti-IgM is macrophage independent.

Spleen cells and peritonial cells were prepared from 8–10-month-old CBA/J mice. Peritoneal cells were obtained by lavage of the cavity with cold balanced salt solution containing 10% fetal calf serum (BSS-FCS). This procedure yielded $7–8\times10^6$ peritoneal cells per mouse of which at least 60% were macrophages as judged by esterase staining and which are hereafter referred to as peritoneal macrophages. When these cells were added to macrophage-depleted spleen cell cultures, the cell number was adjusted to compensate for non-macrophages. Spleen cells were prepared by gently pressing the tissue through a 60-mesh wire screen into cold BSS-FCS followed by two washes. Macrophages were depleted by a two-step procedure. First, the spleen cells were incubated for 90 min in BSS-FCS at 37 °C in plastic Petri dishes. Next, the non-adherent cells (2×10^8) were passed slowly through a Sephadex G-10 column[16] (18 cm^3 of packed Sephadex in a 20-cm^3 syringe) at 37 °C and eluted with warm BSS-FCS. Cells eluting in the first 5 ml were collected for culture. In several replicate experiments, this population contained 50–65% B cells and less than 1% macrophages as judged by immunofluorescent staining and esterase staining, respectively. Less than 0.1% of the G-10-passed cells were phagocytic for latex beads. Conditions for culture and stimulation with intact anti-IgM (anti-MC774 IgM myeloma)[8], $F(ab')_2$ anti-IgM[11] or lipopolysaccharide (LPS) are given in Table 1.

As shown in Table 1, unseparated spleen cells responded to all three stimulants. Macrophage-depleted spleen cells, however, failed to respond to intact anti-IgM but responded normally to $F(ab')_2$ anti-IgM and LPS. The lack of response to intact anti-IgM was not dose dependent; the cells remained unresponsive when cultured with a wide range of doses of anti-IgM (not shown). Addition of peritoneal macrophages (2%) to the macrophage-depleted spleen cell cultures completely restored, and actually enhanced, the response to intact anti-IgM. The response to $F(ab')_2$ anti-IgM was slightly increased whereas the response to LPS was somewhat inhibited. (Macrophages cultured alone at four different cell concentrations do not respond to any of the stimulants (data not shown).) Addition of increasing numbers of macrophages to the cultures (4% and 8%) reduced the response to intact anti-IgM to a level approximately equal to the response of unseparated spleen cells. At the same time, the response to $F(ab')_2$ anti-Ig remained relatively unchanged whereas the response to LPS was markedly inhibited. Responses to all three stimulants were significantly inhibited when 16% macrophages were added to the cultures. These results indicate that the macrophage requirements for stimulating B-cell proliferation with different anti-Ig reagents differ and depend, at least in part, on the molecular species of anti-Ig antibody used.

Mongini *et al.* have reported that the *in vitro* proliferative response of mouse B cells to soluble goat anti-IgM is macrophage dependent[15]. Our results confirm their findings, and we have extended the data to show that the response to $F(ab')_2$ anti-IgM is macrophage independent. In contrast, Sieckmann *et al.* have reported that intact anti-IgM stimulation of mouse B cells is macrophage independent[14]. However, as their culture media contained 2-mercaptoethanol (2-ME), that interpretation is not completely valid (see below).

It is not clear exactly why B cells require macrophages to respond to intact anti-IgM molecules but do not require accessory cells to respond to the $F(ab')_2$ form of the same molecule. Several possibilities must be considered. Additional experiments (not shown) have shown that 2-ME can substitute for macrophages and completely restore the response of macrophage-depleted spleen cells to intact anti-IgM. 2-ME is generally thought to replace supportive functions of macrophages related to cell viability[17]. It is unlikely, however, that the inability of macrophage-depleted cultures to respond to intact anti-IgM is due to problems of B-cell viability, as these same cultures respond normally to LPS and $F(ab')_2$ anti-IgM. Rather, 2-ME may simply potentiate the effect of the few remaining macrophages which are then able to support the induction of anti-IgM stimulation. This may involve the secondary or 'helper' signals provided by the macrophages. Alternatively, 2-ME may act independently and generate a cofactor from the serum which acts directly on the B cells[18]. Whatever the case, these secondary signals seem not to be required for stimulation by $F(ab')_2$ anti-IgM. A role for macrophage Fc receptors must also be considered. It is possible that macrophages bind the intact anti-IgM molecules through their Fc receptors and present them to the B cells in an insoluble matrix array. This would be similar to the stimulation of B cells by anti-Ig insolubilized on Sepharose beads as described by Parker[19]. However, this seems unlikely, as Mongini *et al.* reported that anti-IgM-pulsed and

washed macrophages could not stimulate macrophage-depleted B cells in the absence of additional soluble anti-IgM[15].

The interpretation we favour is that the differential requirement for macrophages is a reflection of different subsets of B cells responding to the two molecular forms of the anti-IgM antibodies. As these two stimulants differ only with respect to the presence of the Fc portion of the Ig molecules, differential sensitivity of the responding B cells to Fc receptor-mediated negative signals may also be involved. Recent experiments in our laboratory support these interpretations. We have found that complement receptor-positive (CR^+) B cells from aged mice (>7 months old) respond only to $F(ab')_2$ anti-IgM whereas CR^- B cells respond preferentially to intact anti-IgM[20]. The inability of CR^+ B cells to respond to the intact molecule is not reversed by addition of macrophages or 2-ME. Thus, it seems that intact anti-IgM and $F(ab')_2$ anti-IgM stimulate different populations of B cells and that these subsets have different signalling requirements for activation. In the context of this report, these findings suggest that depletion of macrophages from whole spleen cell populations selectively eliminates the ability of CR^- B cells to respond to intact anti-IgM.

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Increased intracellular cyclic GMP does not correlate with protein discharge from pancreatic acinar cells

THE pancreatic acinar cell discharges secretory proteins in response to cholecystokinin–pancreozymin (CCK-PZ) and its analogues, or to cholinergic agonists such as carbamylcholine (carbachol). The mechanisms for stimulus–secretion coupling in the acinar cell are not known, although intracellular and, to some extent, extracellular Ca^{2+} are required[1–4], and a transient 10- to 20-fold increase in intracellular levels of guanosine cyclic 3′5′-monophosphate (cyclic GMP) accompanies onset of

Table 1 Effect of MNNG on pancreatic acini

	% Discharge (2 h incubation)	Cyclic GMP level (2-min incubation) (fmol per μg DNA)
Experiment 1		
Unstimulated	10.1±2.5	19±2
Caerulein 10^{-9} M	56.8±1.0	120±37
MNNG 10^{-3} M	4.0±2.5	858±77
MNNG 10^{-4} M	9.1±2.2	1,111±236
MNNG 10^{-5} M	12.3±1.5	24±2
MNNG 10^{-6} M	13.4±1.8	11±2
Experiment 2		
Unstimulated	8.8±0.6	40±8
Caerulein 10^{-9} M	28.9±2.7	317±17
MNNG 10^{-4} M	11.2±0.7	2,989±694
MNNG 10^{-5} M	9.2±2.6	363±88
Caerulein 10^{-9} M +MNNG 10^{-4} M	28.8±2.9	4,017±751
MNNG 10^{-4} M added every 30 min	9.9±1.4	NA
Caerulein 10^{-9} M +MNNG 10^{-4} M, with MNNG added every 30 min	25.7±4.2	NA

Acini were prepared from pancreata of adult female guinea pigs using purified collagenase and mild mechanical shear[26,27]. Secretory proteins in acini to be used for measuring protein discharge were first pulse-labelled for 10 min at 37 °C with 4,5-^{3}H-L-leucine (5 μCi ml^{-1}, 40–60 Ci mmol^{-1}, Schwarz/Mann) in 10 ml of a solution (KRH) consisting of Krebs–Ringer's salts, 2% (v/v) essential and 1% non-essential amino acid concentrates (GIBCO), 2 mM L-glutamine, 14 mM D-glucose, 0.2% bovine plasma albumin (Reheis), 0.01% soybean trypsin inhibitor (Worthington) and 24 mM HEPES (Sigma), *p*H 7.4. Solutions were equilibrated with O_2. Following the pulse, unincorporated ^{3}H-leucine was removed by centrifugation and resuspension of acini in KRH. For determination of either secretory protein discharge or levels of cyclic GMP, acini from 1 g tissue were resuspended in 20 ml KRH containing an additional 3.8 mM unlabelled L-leucine, the substances indicated were added as 100-fold concentrates to 1-ml aliquots of the cell suspension, and incubation at 37 °C was continued for either 2 min (for cyclic GMP) or 2 h (for secretory protein discharge). MNNG was added to acini as a 100-fold concentrate in acetone. Addition of 1% acetone alone had no effect on either discharge or cyclic GMP response. Measurements of cyclic GMP were made on total acinar incubations (cells plus media). To monitor cyclic GMP recovery during purification, each incubation tube received 1.6 nCi ^{3}H-cyclic GMP (15 Ci mmol^{-1}, Schwarz/Mann) at the end of incubation and the reaction was stopped by addition of 0.5 ml 15% trichloroacetic acid (TCA) and freezing. The tubes were then thawed, sonicated for 30 s, centrifuged, and the supernatants passed over cation exchange resin[28]. To remove TCA, column fractions were extracted five times with eight volumes of water-saturated ether and lyophilised. Samples were redissolved in water and aliquots taken to determine cyclic GMP recovery (range: 40–90%). Cyclic GMP content was determined on the remainder of the sample by radioimmunoassay[29] with the modification that the antigen–antibody complex was precipitated with 7% TCA, *p*H 1.75. MNNG had no effect on antibody binding when added to the radioimmunoassay, and cyclic GMP from acini exposed to MNNG was sensitive to degradation by cyclic nucleotide phosphodiesterase (Boehringer, Mannheim). Total DNA in cell pellets was determined by the method of Burton[30]. Discharge of labelled secretory proteins was determined as described previously[31] following separation of acini and media by centrifugation at 800*g* for 5 min. Results are expressed as percentage discharge, defined as acid-precipitable d.p.m. in the media divided by total d.p.m. (cell pellets plus media) × 100. Values shown are means of triplicate determinations (±s.d.) from individual experiments. With 10^{-9} M caerulein, the average net percentage discharge (total minus resting) in 2 h was 26.4±2.3% (±s.e.m., n = 15), and the average cyclic GMP increase in 2 min was 20.8±4.8 times the basal level(±s.e.m., n = 9). Average responses to 10^{-4} M MNNG are listed in Table 2. NA, Not applicable.

induced discharge[5,6]. It has been proposed[5] that cyclic GMP may be a direct mediator of hormone-stimulated discharge, with the Ca^{2+}-dependent event either causing, or being caused by, increases in cyclic GMP[7,8]. To test this hypothesis, we have monitored the effects of several substances on secretory protein discharge and intracellular cyclic GMP levels in guinea pig

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pancreatic acini. Our results, reported here, are not consistent with a role for cyclic GMP in a direct causative pathway leading to discharge, as four of the compounds tested (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), sodium nitrite, hydroxylamine and sodium nitroprusside) elevated levels of cyclic GMP but did not trigger discharge, although acini treated with these agents remained competent to respond to natural secretagogues. Conversely, the phorbol ester 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) did not affect cyclic GMP levels, but did elicit discharge. In contrast to observations on cyclic GMP, the requirement for extracellular Ca^{2+} correlated well with discharge. Our findings therefore do not support the hypothesis that cyclic GMP is directly involved in stimulus–secretion coupling, but are consistent with such a role for Ca^{2+}. A preliminary report of this work has appeared[9].

Compounds were chosen because they increased cyclic GMP in other cell types. TPA, a tumour-promoting alkaloid[10], increases cyclic GMP in skin fibroblasts, with concomitant stimulation of DNA synthesis[11]. TPA also activates platelets and increases platelet cyclic GMP[12]. MNNG and hydroxylamine activate guanylate cyclase[13,14] and elevate intracellular cyclic GMP[13,15] in liver and other tissues. Similar effects occur with sodium nitrite[13,16], sodium nitroprusside[17] and ascorbic and dehydroascorbic acids[18–20]. These compounds may activate guanylate cyclase by direct oxidation of a reactive group on the enzyme, perhaps by a mechanism involving nitroxide or another free radical as an intermediate[20].

MNNG did not trigger discharge in pancreatic acini, but dramatically increased cyclic GMP levels (Table 1). In a representative experiment (Table 1, experiment 1), 10^{-9} M caerulein, a decapeptide analogue of CCK-PZ[21], triggered the net discharge (total discharge minus unstimulated discharge) of 47% of secretory proteins in 2 h. Caerulein also caused a sixfold increase in cyclic GMP after 2 min. In contrast, MNNG did not stimulate discharge, although 10^{-3} M and 10^{-4} M MNNG caused 45- and 58-fold increases, respectively, in cyclic GMP levels. Acini treated with MNNG are, however, still competent to discharge secretory proteins in response to caerulein (Table 1, experiment 2). The fact that extremely high cyclic GMP levels did not affect the cellular response to caerulein suggests that cyclic GMP does not inhibit Ca^{2+} entry into pancreatic acini, a mechanism that has been postulated for smooth muscle[17]. In addition, stimulation of discharge by 10^{-5} M carbachol was also unaffected by 10^{-4} M MNNG (data not shown). The lack of discharge with MNNG is probably not due to its inactivation, as repeated addition failed to elicit discharge (Table 1, experiment 2). We have also determined that 10^{-4} M MNNG does not stimulate discharge of amylase from acini. Therefore, MNNG does not cause zymogen granule release, as amylase discharge primarily reflects this final secretory step.

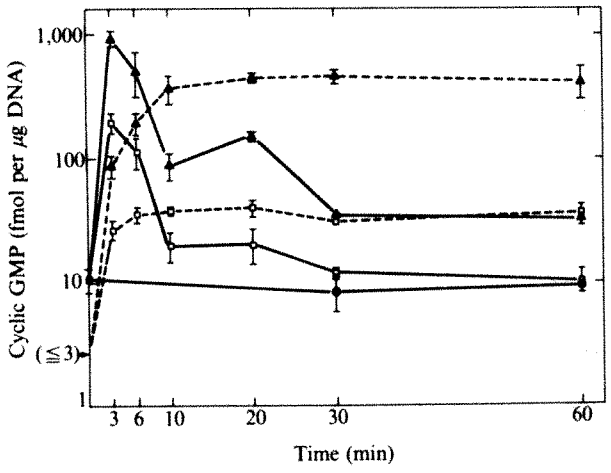


Fig. 1 Effect of MNNG 10^{-4} M and caerulein 10^{-9} M on intracellular and extracellular cyclic GMP levels in pancreatic acini as a function of incubation time. Acini were preincubated for 10 min at 37 °C in KRH (see Table 1) and then received either caerulein (□), MNNG (▲) or no additions (●). At the times indicated, the incubation mixtures were centrifuged and the media removed. Acini (——) and media (– – –) were analysed separately for cyclic GMP (see Table 1). Cyclic GMP levels in cells and media were normalised to DNA content of cell pellets. Note that cyclic GMP levels are plotted on a log scale. After 3 min, more than 95% of the total cyclic GMP is intracellular, justifying the procedure used in experiments shown in Tables 1 and 2 in which acini and media were not separated. Error bars represent ±1 s.d. for triplicate incubations from the same experiment.

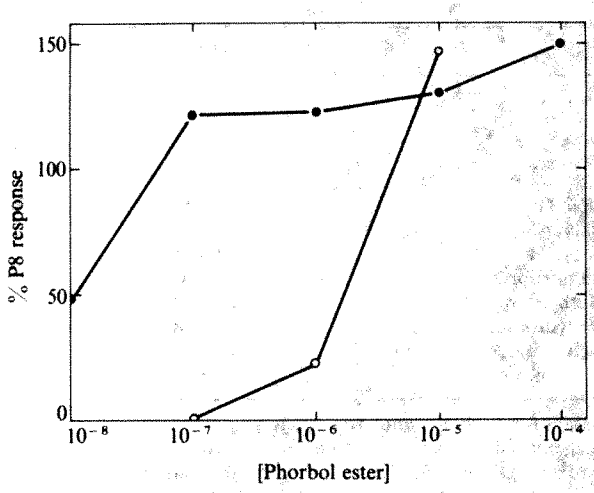


Fig. 2 Effect of TPA (●) and 4-*O*-methyl-TPA (○) on discharge from pancreatic acini. Discharge assays were carried out as described in Table 1. Phorbol esters were added from a 200-fold concentrated stock solution in dimethyl sulphoxide, which, when added alone at a concentration of 0.5%, had no effect on discharge. As experiments with TPA and 4-*O*-methyl-TPA were carried out on different days, the net discharge (total discharge minus resting discharge) was normalised to the net response elicited in the same experiment by the C-terminal octapeptide of CCK-PZ (P8), this being set at 100%. Data points are averages of duplicate incubations from single experiments. The normalised response to 10^{-7} M TPA in 2 h was 90 ± 17% (±s.e.m., $n = 3$). CCK-PZ octapeptide was from Dr Miguel Ondetti (Squibb) TPA and 4-*O*-methyl-TPA were from Consolidated Midland.

The cyclic GMP increase stimulated by MNNG is similar to that triggered by caerulein. Both are partially inhibited by the metabolic inhibitor carbonyl cyanide meta-chlorophenyl hydrazone (CCCP), and neither is affected by removing extracellular Ca^{2+}. The increase in cyclic GMP after 2 min in response to 10^{-9} M caerulein or to 10^{-4} M MNNG was inhibited by 83 ± 3% and 34 ± 4%, respectively, by 10^{-5} M CCCP (mean ±s.e.m., $n = 3$). CCCP also inhibits cyclic GMP increases stimulated by carbachol[5]. In contrast, removing extracellular Ca^{2+} (in the presence of 10^{-4} M EGTA) did not affect the increase in cyclic GMP caused by caerulein or MNNG. A Ca^{2+}-independent increase in cyclic GMP has also been observed in response to caerulein[7,8], carbachol[7,8] and the Ca^{2+} ionophore A23187 (ref. 8).

The time dependence for elevation of intracellular cyclic GMP by 10^{-9} M caerulein or 10^{-4} M MNNG is shown in Fig. 1 (solid lines). Within 3 min, caerulein stimulated an 18-fold increase in cyclic GMP, whereas the increase in response to MNNG was 87-fold. This larger increase with MNNG was consistently observed (see Table 1). Intracellular cyclic GMP levels decreased rapidly between 3 and 10 min after addition of the agents, and by 30 min the cyclic GMP concentration in caerulein-treated acini returned to unstimulated levels. The apparently higher level in MNNG-treated acini after 30 or 60 min may be due to cyclic GMP in the extracellular medium trapped within the cell pellets during centrifugation.

Cyclic GMP appears in the medium within 3 min after addition of caerulein or MNNG (Fig. 1, dotted lines) and plateaus

Table 2 Effect of various agents on cyclic GMP level and discharge response

Agent	Final concentration	Ratio of cyclic GMP (stimulated) to cyclic GMP (bkg) (2 min)	Normalised net discharge response (10^{-9} M caerulein = 100%) Agent alone (%)	Agent plus 10^{-9} M caerulein (%)
MNNG	10^{-4} M	79.9 ± 10.1 (12)	2.0 ± 1.6 (14)	112 ± 11 (4)
$NaNO_2$	10^{-3} M	22.4	−2.9	127
NH_2OH	10^{-3} M	162.0	−2.0	123
Na nitroprusside	10^{-3} M	93.3	4.5	113
Methylnitrosourea	10^{-3} M	1.9	0.2	97.6
Dehydroascorbic acid	10^{-2} M	0.8	0.03 ± 0.05 (3)	96.3
Dehydroascorbic acid	10^{-3} M	0.65 ± 0.05 (3)	−5.2 ± 12 (3)	81.3
Ascorbic acid	5×10^{-3} M	0.8	−0.9	92.8

Assays for discharge and cyclic GMP were as in Table 1. Incubation times were 2 min for cyclic GMP measurements and 2 h for discharge assays. The agents listed were added to acini as 100-fold concentrated stock solutions in acetone (MNNG (Aldrich), methylnitrosourea (K + K), dimethyl sulphoxide (DMSO) (for 10^{-3} M dehydroascorbate, K + K), or 0.15 M NaCl ($NaNO_2$, NH_2OH, sodium nitroprusside (all Fisher), ascorbate (Sigma)). A 1% concentration of acetone or DMSO had no effect on cyclic GMP levels or discharge rate. Dehydroascorbate 10^{-2} M was added as a twofold concentrated solution in KRH. Cyclic GMP levels in treated acini were divided by the level in untreated acini (bkg) in each experiment to yield the ratios listed in Table 2. The average cyclic GMP level in untreated acini was 23.0 ± 19.4 fmol per μg DNA (±s.e.m., $n = 19$). Discharge rates were corrected by subtracting the resting rate and normalised by setting the value for 10^{-9} M caerulein at 100%. Negative values indicate rates slightly below the resting rate. Where indicated, values are the mean ±s.e.m. for the number of experiments in parentheses. Otherwise, the data represent the mean of duplicate or triplicate incubations from one experiment.

after 10 min. Cyclic GMP is also released in response to carbachol[22]. Whatever the mechanism for release of cyclic GMP, in the case of MNNG it cannot be exocytosis of possible zymogen granule-associated cyclic GMP, as MNNG does not cause granule discharge.

The effects of six other compounds known to elevate cyclic GMP levels in other tissues[11–20] were also tested on pancreatic acini (Table 2). None of these substances elicited discharge, although sodium nitrite, hydroxylamine and sodium nitroprusside produced significant increases in cyclic GMP levels within 2 min. As is the case with MNNG, acini treated with each of the compounds remained responsive to caerulein.

In contrast to MNNG, TPA stimulated optimal discharge at doses from 10^{-7} M to 10^{-4} M (Fig. 2), but had no effect on cyclic GMP levels. TPA- and hormone-induced discharges were similar: both were inhibited by 10^{-5} M CCCP or by 10^{-4} M EGTA in Ca^{2+}-free medium. In addition, cell morphology after a 2-h incubation in 10^{-6} M TPA was consistent with normal stimulation when observed by electron microscopy. Amylase discharge in response to TPA paralleled discharge of labelled secretory proteins. The methylated TPA derivative, 4-*O*-methyl-TPA, was 100-fold less potent as a secretagogue (Fig. 2) and has greatly reduced tumour-promoting activity[10], suggesting that the mechanisms by which TPA acts as a tumour promoter and a secretagogue may be related. Finally, neither TPA nor 4-*O*-methyl-TPA significantly altered cyclic GMP levels, regardless of incubation time (10 s–1 h) or concentrations used (10^{-7}–10^{-4} M).

In conclusion, our experiments do not support the hypothesis[5] of a causal relationship between increased intracellular cyclic GMP and secretory protein discharge in pancreatic acinar cells. MNNG, hydroxylamine, sodium nitrite and sodium nitroprusside all increase cyclic GMP but do not elicit discharge (Table 1, Fig. 1, Table 2). Conversely, TPA triggers discharge (Fig. 2) but does not increase cyclic GMP. These findings are complemented by a recent report that cyclic nucleotide phosphodiesterase inhibitors elevate cyclic GMP but do not elicit discharge in rat pancreas[23]. We have also determined that neither MNNG, TPA nor caerulein affect cyclic AMP in guinea pig pancreatic acini. The possibility remains that cyclic GMP pools formed in response to MNNG and caerulein are located within different cellular compartments. This question could be resolved by immunocytochemistry[24].

Assuming that cyclic GMP is not a direct mediator of discharge, it seems that the most likely candidate is Ca^{2+}. Caerulein, carbachol and TPA failed to elicit discharge when Ca^{2+} was omitted from the medium and 10^{-4} M EGTA was included. Other secretagogues, including A23187 (ref. 25), require extracellular Ca^{2+}. Recently, Chandler and Williams[3] have suggested that secretagogues cause Ca^{2+} release from an intracellular membrane site which presumably must be replenished by Ca^{2+} influx across the plasma membrane. Whatever the mechanism for Ca^{2+} mobilisation, the question remains as to the nature of the proximal event following the increase in intracellular free Ca^{2+}.

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Rabbit skeletal myosin isoenzymes from fetal, fast-twitch and slow-twitch muscles

IT is generally accepted that myosin in vertebrate fast-twitch and slow-twitch muscles is structurally[1-4] and immunochemically[5-8] distinct and exists in several isoenzymatic forms[9-14]. The nature of myosin in developing mammalian skeletal muscles is controversial. Some authors[15,16] consider it to be identical to adult fast-twitch myosin on the basis of light-chain composition and histochemistry. Others[8] suggest that it may be a mixture of fast-twitch and slow-twitch myosins. Still others[4,17] maintain it is a distinct fetal form of myosin differing in primary structure from adult myosins. In this report we show that (1) myosin in both neonatal and adult fast-twitch muscles of the rabbit exists in three electrophoretically distinct isoenzymatic forms which differ in light-chain composition, and (2) the two-dimensional peptide map of the heavy chains of neonatal muscle myosin is distinct from maps of both fast-twitch and slow-twitch myosins. These results clearly establish the existence of fetal myosin isoenzymes in developing mammalian muscles and resolve apparent contradictions in the literature.

Pyrophosphate gel electrophoresis[9] has now been established as a sensitive and reliable method of resolving myosin isoenzymes differing in light-chain[13,18] or heavy-chain[19] composition. On analysis in this system, myosin from slow-twitch soleus muscle of the adult rabbit showed a single component (Fig. 1*a*). Adult fast-twitch longissimus dorsi muscle (Fig. 1*b*) showed three components (FM_1, FM_2 and FM_3) which migrated faster than the slow-twitch myosin. The longissimus dorsi muscle from a 2-day-old rabbit (Fig. 1*c*) also showed three components (fm_1, fm_2 and fm_3) which were electrophoretically faster than fast-twitch myosin components. However, a mixture of fast-twitch and fetal myosins (Fig. 1*d*) showed that fM_3 co-migrated with FM_1. Essentially the same profile of fetal components as shown in Fig. 1*c* was obtained from the developing slow-twitch soleus muscle of 2-day-old rabbits.

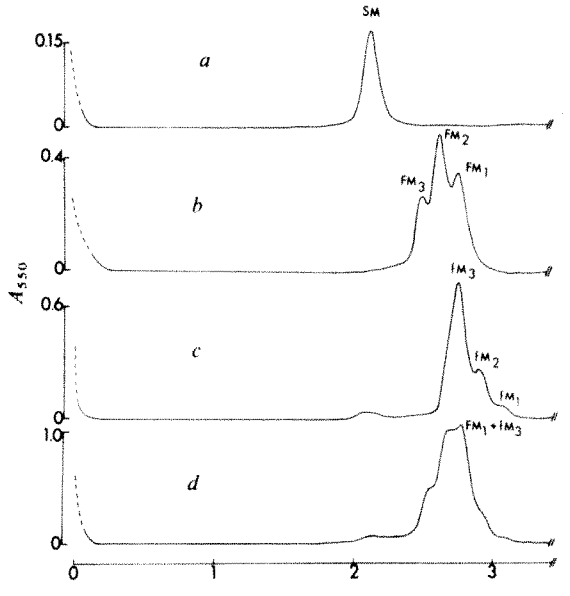


Fig. 1 Pyrophosphate gel electrophoresis of native myosins in polyacrylamide gels. Myosin was extracted and analysed in 4% polyacrylamide gels as described in refs 9 and 13. The curves show densitometer tracings of gels stained with Coomassie Brilliant Blue R. *a*, Slow-twitch myosin (SM) from adult rabbit soleus muscle; *b*, fast-twitch myosin components (FM_1, FM_2 and FM_3) from adult rabbit longissimus dorsi muscle; *c*, fetal myosin components (fM_1, fM_2 and fM_3) from the longissimus dorsi muscle of a 2-day-old rabbit; *d*, mixture of *b* and *c*, showing that FM_1 co-migrates with fM_3.

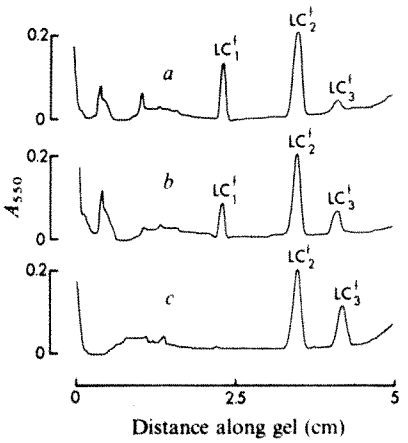


Fig. 2 SDS gel electrophoresis of myosin light chains from electrophoretically purified adult fast-twitch myosin components. After pyrophosphate gel electrophoresis, portions of the gel containing individual components were cut out and analysed for myosin light chains by SDS gel electrophoresis in 12.5% polyacrylamide gel as described in ref. 13. The curves are densitometer tracings showing the distribution of the three fast-twitch myosin light chains from three consecutive slices of the same pyrophosphate gel: *a*, LC_1^f (or A1), LC_2^f (DTNB light chain) and LC_3^f (A2), in FM_3; *b*, FM_2; *c*, FM_1. LC_1^f is absent from FM_1, the ratio $LC_2^f:LC_3^f$ for this component is approximately 1:1. In FM_2, all three light chains are present, the ratio $LC_1^f:LC_2^f:LC_3^f$ was approximately 1:2:1. A small amount of LC_3^f is present in the FM_3 slice from this particular pyrophosphate gel. Its presence is due to a slight contamination with FM_2 in this slice, as it is absent from the FM_3 slice of other gels. Because gels were sliced using a stained companion gel as a guide, it was difficult to avoid some contamination in one of the three consecutive slices. Where FM_3 was not contaminated by FM_2, the ratio $LC_1^f:LC_2^f$ was approximately 1:1. Apart from problems arising from contamination by neighbouring components, the distribution and stoichiometry of the light chains described here have been confirmed in several electrophoretic runs.

The isoenzymatic nature of the six components from adult fast-twitch and fetal myosins is shown by the analysis of their light-chain composition by methods described previously[13]. Figure 2 shows the result of light-chain analysis for FM_3 (Fig. 2*a*), FM_2 (Fig. 2*b*) and FM_1 (Fig. 2*c*). These results suggest that the light-chain composition in rabbit fast-twitch myosin isoenzymes is identical to the homologous chick fast-twitch myosin isoenzymes[13], being $(LC_2^f)_2(LC_3^f)_2$, $LC_1^f(LC_2^f)_2LC_3^f$ and $(LC_1^f)_2(LC_2^f)_2$, respectively.

Myosin from developing mammalian muscles contains three light chains which are indistinguishable from LC_1^f, LC_2^f and LC_3^f on SDS gel electrophoresis[15,20-23]. Analysis of light chains from individual fetal myosin components (Fig. 3) showed that fM_1, fM_2 and fM_3 are homologous to FM_1, FM_2 and FM_3, respectively, in light-chain composition. Although FM_1 co-migrated with fM_3, their different light-chain compositions show them to be different isoenzymes. As light chains constitute only a small fraction of the total molecular mass of native myosin, the difference between fetal and fast-twitch myosins with respect to their electrophoretic behaviour suggests that the heavy chains of these two types of myosin may be structurally different. This is borne out by peptide mapping of myosin heavy chains.

Cyanogen bromide digestion is a convenient method of treating myosin heavy chains for peptide mapping, as the number of peptide fragments is kept to a manageable level compared with the use of trypsin. Two-dimensional mapping of cyanogen bromide peptides has been used successfully to examine structural differences in the heavy chains of ventricular myosin isoenzymes in the rat[19] and is here applied to the analysis of the heavy chains of the three types of skeletal myosin. The maps

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(Fig. 4) for fast-twitch (adult longissimus dorsi, 3-day-old rabbit) and slow-twitch (adult soleus) myosins are clearly different. These maps are consistently reproducible from experiment to experiment. The greater complexity of the map for fast-twitch myosin heavy chains when compared with maps of the other two myosins may be related to the known heterogeneity of the fast-twitch myosin heavy chains[24,25]. Fetal peptides mapped in the more acidic regions of the gel compared with those of the other two myosins. This is consistent with the higher electrophoretic mobility of the fetal myosin isoenzymes in pyrophosphate gels. The maps suggest that the heavy chains of the three types of myosin are structurally distinct. This view is corroborated by earlier findings that (1) the patterns of release of tryptic peptides from these three types of myosins are different[4], and (2) a fetal myosin peptide, differing in amino acid sequence from its homologue obtained from adult muscle, can be isolated from neonatal rabbit muscle[17].

This work provides direct evidence for the existence of a new class of myosin isoenzymes in developing mammalian skeletal muscles which is distinct from those in adult fast-twitch and slow-twitch muscles. An unexpected structural feature of these fetal myosin isoenzymes is that their light-chain types and distribution are so similar to those of the fast-twitch myosin isoenzymes while heavy chains of these two types of myosins are structurally different. This fact has led to the erroneous conclusion that the myosin in developing mammalian muscle is identical to that of adult fast-twitch myosin[15,16]. It also explains the immunohistochemical reaction between anti-fast-twitch light-chain antibodies and developing fast-twitch and slow-twitch muscle fibres[8]. The reaction between antisera raised against slow-twitch myosin and developing muscle fibres[8] may be attributable to common antigenic determinants in fetal and slow-twitch myosins.

This work has helped to define the pattern of changes in myosin gene expression during muscle differentiation in the mammal. Contrary to earlier views[8,15,16], it shows that both fast-twitch and slow-twitch muscles synthesise the same fetal myosin isoenzymes early in development. Subsequently, fetal myosin isoenzymes are replaced by the appropriate adult fast-twitch or slow-twitch myosin isoenzymes. During the development of the fast-twitch muscle, this change may principally involve the genes for the heavy chains. Recent work[26] has

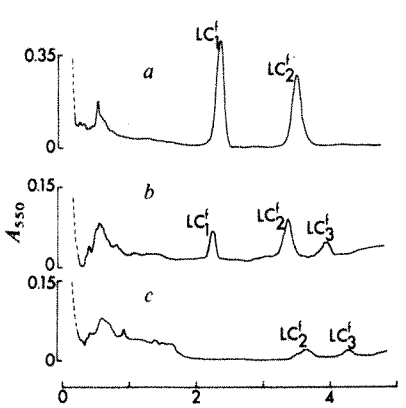


Fig. 3 SDS gel electrophoresis of myosin light chains from electrophoretically purified fetal myosin components. The methods used were the same as those described for Fig. 2. *a*, Light chains from fM_3 showing LC_1^f and LC_2^f only, occurring in approximately equal proportions; *b*, light chains in fM_2, in which $LC_1^f : LC_2^f : LC_3^f$ is approximately 1 : 2 : 1; *c*, light chains in fM_1, showing LC_2^f and LC_3^f only and occurring in approximately equal proportions. These results were obtained from consecutive slices of the same pyrophosphate gel. The results for fM_2 and fM_3 have been consistently obtained in many different runs, but fM_1 presents more difficulty as it constitutes only a small fraction of the fetal myosin. However, a similar result has been obtained in another experiment.

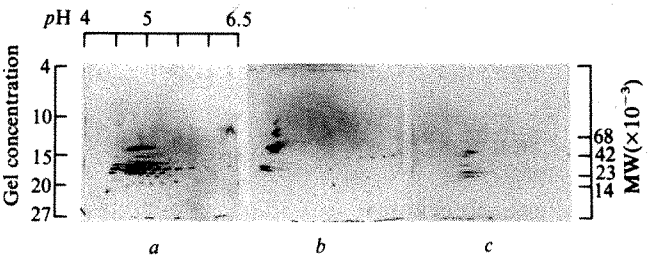


Fig. 4 Two-dimensional cyanogen bromide peptide maps of myosin heavy chains. These maps were obtained for adult fast-twitch myosin (*a*), fetal myosin (*b*) and adult slow-twitch myosin (*c*) using methods described in detail in ref. 19, and briefly outlined below. Myosin heavy chains were separated from carboxymethylated crude myosin extracts through a Sepharose 4B column in a denaturing solvent. The heavy-chain fraction was dialysed and then digested with cyanogen bromide in 70% formic acid at 4 °C for 60 h. The peptides were analysed by isoelectrofocusing in the first dimension between *p*H 4 and 6.5 and by SDS gel electrophoresis in gradient gels (Gradipore) in the second dimension. Gels were stained in Coomassie Brilliant Blue R. The three slabs were analysed simultaneously in the same electrophoresis unit. Gel concentration along the slab is indicated on the left. The scale on the right marks the positions of molecular weight markers, the numbers giving molecular weights in thousands.

demonstrated that the bulk of the light chains from developing mammalian muscle is indistinguishable in charge as well as in size from those of adult fast-twitch myosin, suggesting that they may be products of the same set of genes. However, in addition, a minor component, called embryonic LC_1, distinct in charge but similar in size to LC_1^f, has also been identified[26].

The existence of fetal isoenzymes of myosin in the mammal during the perinatal period is in apparent contrast to the situation in the chick[27]. Avian fast-twitch and slow-twitch muscles in the 11–14-day chick embryos apparently already synthesise some of the myosin isoenzymes found in the adult muscles. A distinct fetal myosin is apparently absent in both these types of avian muscle at about this time. A possible reason for this difference between rabbit and chick may be that chick fetal myosin is synthesised much earlier than the 14-day embryo. Alternatively, chicken fetal myosin homologous to those demonstrated here may be present in the 11–14-day embryos, but has not been resolved in pyrophosphate gels from adult myosin isoenzymes present during this period of development.

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Fast and slow muscles in tissue culture synthesise only fast myosin

ADULT fast and slow skeletal muscle fibres contain different myosins, a result of innervation by different classes of motoneurones[1]; innervation of one fibre type by the opposite motoneurone type leads to a switch in the kind of myosin synthesised[2,3] within pre-existing fibres[4,5]. In embryonic muscles, however, the particular myosin isozyme synthesised before and during the early stages of innervation has been a subject of controversy. Some investigators claim that all embryonic muscle fibres—whether destined to be fast or slow in the adult—initially synthesise both fast and slow myosins[6,7]. Proper innervation would then lead to a selection of one myosin type and repression of the other type within each cell. Others propose that the myosin of embryonic muscles is a distinct myosin, unique to the embryo[8,9]. On the other hand, we have presented evidence that all fetal muscles have an intrinsic programme which causes them to synthesise fast myosin initially[10,11]. After innervation, fast muscles continue synthesising fast myosin, while slow muscles switch to the synthesis of slow myosin. Some studies have corroborated our findings[12–14], but in all these studies the earliest fast and slow muscles that could be obtained were already innervated, and the slow muscles already contained a small amount of slow myosin. Accordingly, to examine the initial myosin synthesised in both fast and slow muscles before innervation, we have cultured tissue from chicken muscles destined to be fast or slow muscles in the adult. Muscle fibres in tissue culture are formed in the complete absence of innervation. Whether established from presumptive fast or presumptive slow muscles, all fibres in culture synthesised only fast myosin heavy and light chains.

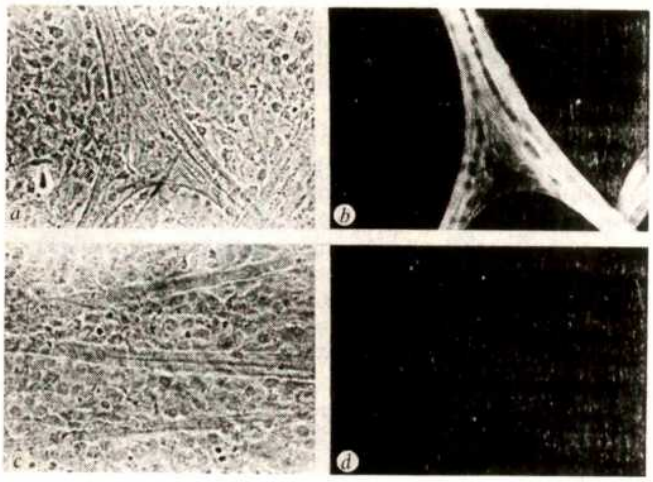


Fig. 1 Staining of cultured pectoralis muscle with fluorescent antimyosins. Muscle was cultured from an 11-d embryonic pectoralis muscle. After 7 d, cultures were fixed in acetone/formaldehyde, stained with affinity-purified, fluorescein-labelled AFHC or ASHC, destained, and photographed on a Zeiss Photomicroscope II equipped with epifluorescence. The production of anti-fast and anti-slow myosin heavy chain IgG has been described previously[15]. Affinity-purified AFHC and ASHC were prepared from the IgG by two rounds of absorption. First, each IgG was absorbed to its homologous myosin bound to PAB-cellulose (for example anti-fast IgG absorbed to fast myosin)[15]. The absorbed material was recovered and re-absorbed against the heterologous myosin type (for example anti-fast myosin antibody absorbed against slow myosin) to remove any cross-reacting antibodies. The final antibodies are specific to either fast or slow myosin heavy chains and show Ouchterlony reactions identical to those shown previously[10,15]. *a*, *c*, Phase micrographs of cultured pectoralis fibres surrounded by mononucleated non-muscle cells. *b*, Same field as (*a*), stained with fluorescent AFHC. *d*, Same field as (*c*), stained with fluorescent ASHC × 100.

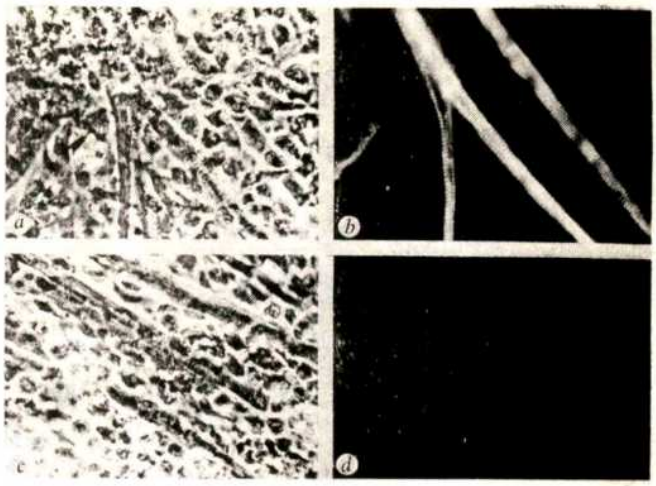


Fig. 2 Staining of cultured ALD muscle with fluorescent antimyosins. Muscle was cultured from a 12-d embryonic ALD muscle. After 7 d, cultures were fixed and stained as described in Fig. 1. *a*, *c*, Phase micrographs of cultured ALD fibres surrounded by non-muscle cells. (*b*), Same field as (*a*), stained with AFHC. (*d*), Same field as (*c*), stained with ASHC × 100.

Cultures of embryonic pectoralis (presumptive fast) and embryonic anterior latissimus dorsi (ALD) (presumptive slow) muscles were stained with affinity purified antibodies specific to adult chicken fast and slow myosin heavy chains. All fibres react only with the anti-fast myosin heavy chain antibody (AFHC) (Figs 1*b*, 2*b*). No reaction can be detected with the anti-slow myosin heavy chain antibody (ASHC) in either the pectoralis (Fig. 1*d*) or ALD (Fig. 2*d*) fibres. Replicating fibroblasts surrounding the fibres contain neither muscle-specific myosin.

These results do not agree with those of several other investigators. Masaki and Yoshizaki[6] claimed that cultured chicken pectoralis fibres reacted with antibodies against both fast and slow chicken myosins. Their failure to completely purify the myosin used as antigen and failure to affinity purify the antibodies[6,16] probably led to gross contamination of the anti-myosin antibodies with antibodies to non-myosin proteins, as others have demonstrated[17]. Similarly, Gauthier *et al.*[7] reported that when cross-sections of embryonic rat muscles were stained with antibodies to adult chicken fast and slow myosins, all fibres reacted with both antibodies. We have found, however, that affinity purified anti-chicken myosin antibodies, while staining rat muscle cross-sections, fail to stain the A bands of purified rat myofibrils or show a reaction against rat myosin on immunodiffusion gels (N.A.R. and A. Kelly, unpublished observations). Conversely, Bruggmann and Jenny[18] have shown that anti-rabbit myosin antibodies do not react with chicken myosin.

The ALD and pectoralis cultures might contain small amounts of slow myosin heavy chains not detectable by immunofluorescence. Therefore, cultures were labelled for 24 h with ^{35}S-methionine, washed, and each culture mixed with ~10-fold excess of both unlabelled fast and slow muscle from an

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adult chicken. The labelled myosins from the cultures and the unlabelled adult myosins of these mixtures were co-purified and diffused against AFHC and ASHC. As the pectoralis and ALD cultures had been mixed with adult unlabelled fast and slow muscles, each preparation contains unlabelled fast and slow myosin and each shows a reaction against both AFHC and ASHC when the immunodiffusion gels are stained (Fig. 3*a*, *b*). An autoradiograph of these gels, however, reveals that only the precipitin line against AFHC (Fig. 3*c*) contains radioactive myosin—myosin that must have been synthesised in the labelled ALD and pectoralis cultures. Thus, both ALD and pectoralis fibres in culture contain heavy chains reacting identically with AFHC on immunodiffusion plates. The fact that neither culture contains radioactive heavy chains reacting with ASHC (Fig. 3*d*) cannot be attributed to a failure to reach an equivalence point of antigen and antibody, since the stained gel clearly shows that an equivalence point of ASHC with slow myosin heavy chains was present. The absence of a radioactive precipitin line with ASHC must mean that no slow myosin heavy chains are synthesised in either culture.

Does the radioactive precipitin line in Fig. 3*c* really represent fast heavy chains or does it merely indicate some cross reaction with an 'embryonic' heavy chain? The radioactive myosin of the precipitin line represents cultured myosin which is less than 10% of the total myosin. This radioactive line exactly overlays the precipitin line seen after staining; the stained line, however, represents over 90% unlabelled adult fast heavy chain and less than 10% radioactive heavy chain from the cultures. That the equivalence point of AFHC with adult fast heavy chains and the equivalence point of labelled cultured myosin with 'cross-reacting' antibodies would coincide so exactly is unlikely. When these myosin samples were diluted 1:1 with only unlabelled adult myosin, the radioactive precipitin line still coincided exactly with the stained precipitin line (data not shown). Thus, although we halved the concentration of labelled myosin, its equivalence point was still the equivalence point of adult fast heavy chain with its antibody. Hence, it is unlikely that the radioactive heavy chains from the ALD and pectoralis cultures differ antigenically from adult fast myosin heavy chains.

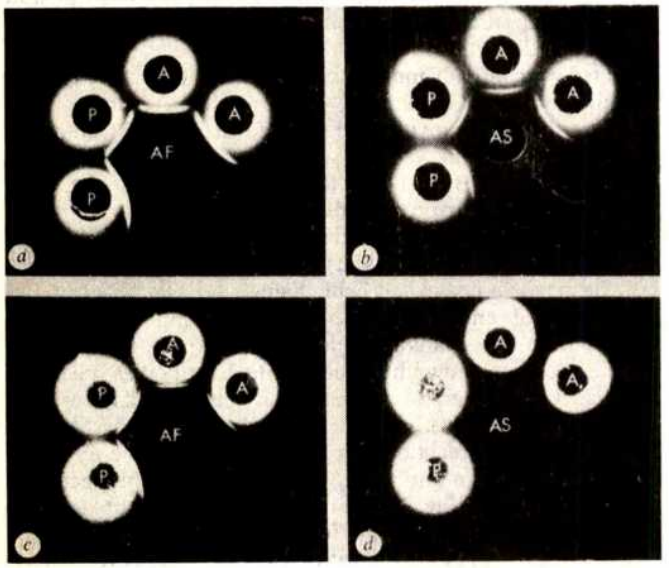


Fig. 3 Myosins from cultured ALD and pectoralis muscles diffused against specific anti-myosins. Myosins were isolated from mixtures of ^{35}S-methionine labelled cultures with unlabelled adult fast and slow chicken muscles. The co-purified myosins were diffused against AFHC and ASHC as previously described[10]. After diffusion, the gels were washed, stained with 1% thiazine red, and destained. The gels were then dried and autoradiographed with Royal X-Omat film for 48–72 h at −80°. In (*a*), AFHC was placed in the centre well. The left two wells (P) contained ^{35}S-methionine labelled myosin from pectoralis cultures mixed with unlabelled adult fast and slow myosins. The right two wells (A) contained ^{35}S-methionine labelled myosin from ALD cultures mixed with unlabelled adult fast and slow myosins. The total myosin concentration in each well was approximately 3 mg ml^{-1}. Since each preparation contained unlabelled adult fast myosin heavy chains, the AFHC reacted with each with a single precipitin line. *c*, Autoradiograph of (*a*). Both ALD and pectoralis cultures contained labelled myosin which reacted with AFHC. The precipitin line in (*c*) exactly coincides with the line in (*a*). In (*b*), ASHC was placed in the centre well, while the peripheral wells were filled as in (*a*). Since each preparation contained unlabelled adult slow myosin heavy chains, the ASHC reacted with each with a single precipitin line. *d*, Autoradiograph of (*b*). Neither the ALD nor the pectoralis cultures contained labelled myosin which reacted with ASHC.

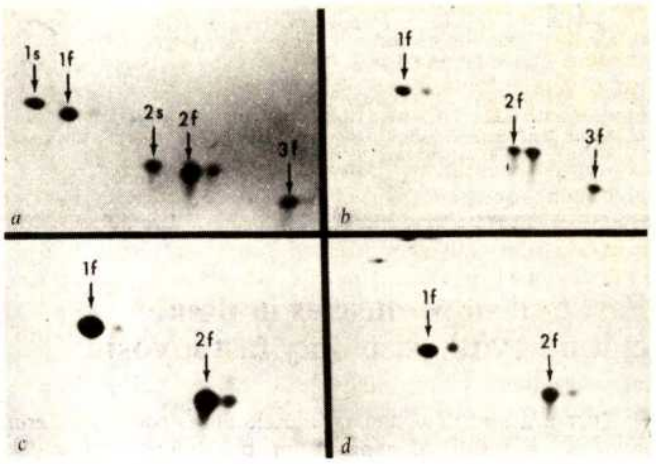


Fig. 4 Two-dimensional electrophoresis of myosin light chains. Myosins isolated by conventional methods[10] were subjected to electrophoresis on isoelectric focusing gels over *pH* range 4–6, followed by SDS-polyacrylamide gel electrophoresis on 15% acrylamide gels. In each figure, the more acidic region is on the right. *a*, *b*, Gels stained with Coomassie brilliant blue. *c*, *d*, Fluorographs of labelled cultured myosins which had been mixed with unlabelled adult fast and slow myosins as carriers and markers. Light chains were identified by comparison of fluorographs with the stained gels. Light chains were derived from adult chicken ALD (slow) and pectoralis (fast) myosins (*a*); adult pectoralis myosin alone (*b*); ^{35}S-methionine labelled pectoralis culture myosin (*c*); and ^{35}S-methionine labelled ALD culture myosin (*d*). Slow myosin light chains are 1s and 2s; fast myosin light chains are 1f, 2f, and 3f. The light chain to the right of 2f is the phosphorylated form of 2f. The chain to the right of 1f may be a secondary modification of 1f; others have claimed that it is a unique embryonic form of the myosin light chain[8]. It is present in both adult and cultured chicken myosins.

To examine the myosin light chains more closely, myosins from ^{35}S-methionine labelled ALD and pectoralis cultures (mixed with unlabelled adult fast and slow myosins as carriers and markers) were subjected to electrophoresis on two dimensional gels[19]. Figure 4*a* shows the light chains of a mixture of adult fast and slow light chains. When compared to previous one-dimensional gel analyses[10], which separate proteins only on the basis of molecular weight, some of these light chains show an increase in heterogeneity. Light chain 2f (LC_{2f}) appears as a doublet; the more acidic protein is the phosphorylated form of this light chain. Often, LC_{2s} is also present in its phosphorylated and non-phosphorylated forms, although only the non-phosphorylated chain is evident in Fig. 4.

LC_{1f} also appears as a doublet, with a dominant band accompanied by a minor band at its acidic side. This minor band, which varies in intensity in different preparations, can be seen in all our adult fast and cultured embryonic myosins (see below). Although barely detectable in this mixture of adult fast and slow

myosins (Fig. 4*a*), it is seen clearly in a different preparation of adult fast myosin alone (Fig. 4*b*).

When fluorographs are prepared from the cultured ALD (Fig. 4*d*) and cultured pectoralis (Fig. 4*c*) muscle myosins after two-dimensional electrophoresis, the dominant light chains co-migrate with adult LC_{1f} and LC_{2f}. In both figures, LC_{2f} is present in both the phosphorylated and non-phosphorylated forms. LC_{1f} is also present as a doublet. Whalen *et al.*[8] have seen a minor peptide similar to this satellite of LC_{1f} in myosins isolated from fetal, neonatal, and cultured rat fast muscles, although not in myosin from adult rat muscles; for this reason, they consider it an embryonic light chain. However, when RNA from cultured rat myotubes is translated in an *in vitro* system, this minor band is not seen (ref. 20, Fig. 6). In addition, this peptide has been detected in both adult and embryonic muscles of the chicken, cat, and rabbit (A. Kelly and N.A.R. in preparation). Hence, there is no compelling evidence to suggest that this is a distinct embryonic gene product, as proposed by Whalen *et al.*[8]

It has been shown previously that LC_{3f} is not present in cultured chicken muscle or in embryonic chicken muscles *in vivo*[10,21,22]. The bands migrating near the position of adult LC_{3f} and several others result from fibroblast contamination of the cultures. Neither ALD nor pectoralis cultures show any bands migrating with either LC_{1s} or LC_{2s}.

Muscle fibres in tissue culture, then, whether arising from potential fast or slow muscles *in vivo*, synthesise myosin heavy and light chains identical by several criteria to those in adult fast muscles. Combined with previous *in vivo* studies[10–14], this suggests that all pre-innervated skeletal muscle fibres, regardless of their destiny, synthesise only fast myosin. Thus, the initial expression of the fast myosin gene may be considered an endogenously programmed event in the development of both fast and slow muscles. Some motoneuronal contribution to myogenesis apparently can override this endogenous commitment and cue individual fibres to cease synthesising fast myosin and begin synthesising slow myosin, a process which occurs in normal slow muscle development. This contribution is most likely the specific pattern of impulses generated by the motoneurone[23–25].

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Filament geometry and the activation of insect flight muscles

HIGH wingbeat frequencies are required for flight in many insects, and have led to striking specialisations[1]. The thorax and wings form a resonant system whose oscillation is sustained by alternating contractions of antagonistic muscles. The action of these flight muscles is to produce only a small-amplitude deformation of the thorax, mechanically amplified into wing movements. The muscles are diverse in arrangement[1] and ultrastructure[2]. In several insect orders, they are of a characteristic 'fibrillar' type whose very regular architecture is well known[3]. As recognised largely through the work of Pringle[4], certain properties of fibrillar muscle permit more rapid wingbeats because the oscillation does not depend on pacemaking neural input: the muscles receive only slow and irregular stimulation, and the contraction of each is triggered, after a delay, directly by the stretch applied to it while its antagonist shortens. Studies of isolated glycerol-extracted fibrillar muscle fibres show that unusual properties underlying this sensitivity to stretch are intrinsic to the contractile apparatus itself[5]. ATPase levels and tension are low in calcium-activated fibres at rest length, but are increased by slight extension (0.1–5%). The muscles exhibit both actin-linked and myosin-linked regulation typical of many invertebrate muscles[6], and the additional regulatory effects of mechanical conditions are not understood. Stretch has been presumed to modify cross-bridge attachment by some unknown effect on the myosin filaments[4] (possibly transmitted through structures which connect them to the Z-line in certain insect muscles[7]). I suggest here that many aspects of stretch-activation in fibrillar muscles may be explained by the particular geometry of their myosin and actin filaments.

The staggered array of six actin filaments round each myosin filament in the lattice[3] is shown in Fig. 1*a*, 'unrolled' to form a radial projection. The solid bars are regions analogous to 'target areas' of the rigor state[3], assumed to be accessible to attachment of active bridges, and they occur every 385 Å along each filament. Two different sets of helical tracks with an axial separation of 385 Å are marked. Figure 1*b* shows the locations of myosin cross-bridges on the thick filament. These lie on helical tracks whose axial separation is again 385 Å, as indicated by the X-ray pattern of the relaxed muscle[8]; the number of such tracks is not yet firmly established, and Fig. 1*b* follows the suggestion[9] that there may be four rather than two[8] or six[10]. Superimposing *a* and *b*, the relationship between cross-bridges and their surrounding envelope of actin targets is seen to depend on the filament alignment. Thus, in *c*, all targets are beyond the 'reach' of the nearby bridges, whereas in *d* all are accessible. Different alignments of actin and myosin filaments are therefore not equivalent: the relationship between tracks of bridges and of targets does not vary throughout each half-sarcomere, but does alter when the alignment is changed by extending the muscle. (When actin and myosin periods are not related, changes occurring locally as a result of extension are largely 'averaged out' over the length of the sarcomere.)

The clear difference between *c* and *d* in Fig. 1 results because *a* and *b* have one set of helical tracks in common, with respect to number, pitch and hand (namely, the four-start tracks of pitch 385 Å), and because targets and bridges are both envisaged as having limited spatial extents. Similar effects result with certain other myosin structures: for example, the two-start myosin helix[8], if its hand is opposite to that of *b*. (The hand of the actin lattice is known, whereas that of the myosin helix is not[3].) In either of these cases, the alternation of match and mismatch repeats after 385 Å of relative movement of the filaments. The six-start myosin helix of Squire[10] permits a much less dramatic difference between match and mismatch, repeating after a movement of only 128 Å.

Filament sliding can therefore greatly influence opportunities for bridge attachment. To illustrate in a general way how these

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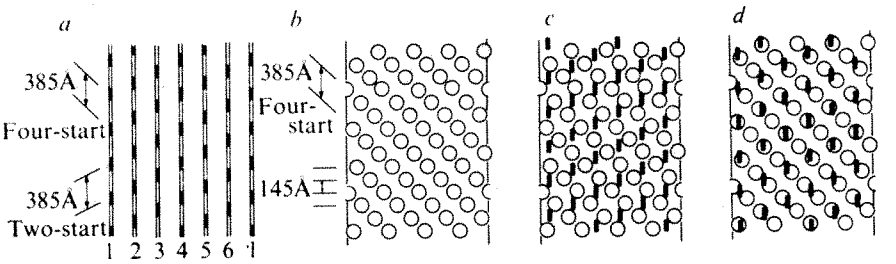


Fig. 1 Radial projections of actin envelope (*a*) and myosin surface lattice (*b*) in insect fibrillar muscle. Bars in *a* represent regions of thin filaments accessible to bridge attachment. Circles in *b* represent units of the cross-bridge array, presumably two myosin heads in an as yet unknown relationship. Matching of one set of helical tracks in the two lattices means that average proximity of bridges to attachment regions varies with alignment of the filaments (*c* and *d*). How far they can be completely segregated, as in *c*, depends on their respective dimensions. The size of circles in *b* reflects a substantial disorder in the cross-bridge array. The matching must have effects on the dynamics of the activated muscle which have no counterpart in cases where a 'vernier' relation exists between thick and thin filament periods. State *c* is considered the 'ground state' for the muscle at its rest length, whether activated or not. (This state is assumed to be maintained for some structural reason, such as a sterically preferred alignment of the myosin helices with the thin filament components. The preferred alignment will be attainable even in isometric conditions, if a slight dispersion of sarcomere lengths throughout the fibre can occur.) Other states than *c* will result following stretch, and in activated muscle will generate different levels of activity (see text); during small-amplitude oscillation, the alignment will vary cyclically over some part of the range *c*–*d*.

effects can modify conventional muscle dynamics, I assume the four-start matching shown in Fig. 1. The complete mismatch *c* might explain how rest-length fibrillar muscle fibres can be fully calcium-activated and yet develop little tension (see also Fig. 1, legend). Slight extension of the fibres will increase the accessibility of bridges to targets. The observed response to stretch, in both insect and vertebrate muscles, includes certain rapid transient effects[11] followed by delayed tension changes. The latter are generally understood to reflect the equilibration of cross-bridges between different attached and detached states in their new environments, at a rate governed by that of cross-bridge turnover[12]. Insect fibrillar muscles are distinctive in that the delayed tension change is large and does not ultimately reverse but instead approaches a different level at the new muscle length. In these terms Fig. 1 suggests a structural reason, hitherto only conjectural, that steady tension might be affected by a length change in fibrillar muscle. It is natural to expect the magnitude of the new tension to depend, perhaps not linearly, on the proximity of bridges to targets. This proximity in turn depends on the amount of extension; it alternately increases and declines with a repeat of 385 Å (about 3% of half-sarcomere length in *Lethocerus*) if Fig. 1 is interpreted literally. But other structural effects might accompany stretch, such as tension-induced changes in myosin helix pitch, non-uniform elongation of sarcomeres, and altered register across the filament lattice. Extension by 3% will then not restore the initial filament alignment immediately, and tension after a stretch will not vary in a periodic manner with the amount of extension. Nevertheless, a periodicity of about 3%, superimposed on other effects, is indeed observed when stretches are applied in sufficiently small and rapid steps[13], in contrast to earlier observations[14,15].

The rapid activation and inactivation of fibrillar muscles could thus be coupled to their small oscillatory length changes through their specialised filament structure. Certain geometrical relationships, accurately preserved throughout the highly regular filament lattice, would ensure that opportunities for bridge attachment can be modulated simultaneously throughout a bundle of muscle fibres in synchrony with the wingbeat cycle. The requisite matching of actin and myosin periods can be related to the unmatched structure in certain non-fibrillar crustacean and insect muscles, through small changes in the 'twist' of the myosin filament[9]. If further work confirms that such geometric effects indeed occur, the present description must be refined in the light of the complex non-linear dynamics of fibrillar muscles. The alternative geometries underlying the sensitivity of activation to stretch should perhaps be manifest also in the rigor state, or when ATP is replaced by its analogues. Finally, it will be interesting to consider how so specialised an adaptation to oscillatory activity can meet the widely varying requirements of flight in different insect species.

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Transition temperature of excitation–contraction coupling in frog twitch muscle fibres

THE mechanism by which depolarisation of the muscle fibre membrane leads to release of stored Ca^{2+} ions from the sarcoplasmic reticulum, although crucial, is perhaps the least understood stage in excitation–contraction coupling[1,2]. We report here our investigation of the temperature dependence of this mechanism, using intracellular injection of arsenazo III (refs 3, 4), a Ca^{2+} indicator dye with a fast response time[5]. We have found that the latency between depolarisation of the fibre and the onset of the rise in intracellular free Ca^{2+} is proportional to the reciprocal temperature, but that on an Arrhenius plot this relationship shows a change in slope at a temperature which depends upon the Ca^{2+} concentration in the bathing solution.

Experiments were carried out on the cutaneous pectoris muscle of *Rana temporaria*, stretched to a striation spacing of about 3.6 μm to reduce contraction. The bathing solution contained (in mM): NaCl, 120; KCl, 2; 0, 1.8 or 12 $CaCl_2$. In the 0 Ca^{2+} solution, 1 mM EGTA and 5 mM $MgCl_2$ were added; *p*H was adjusted to 7.2. The temperature of the solution was controlled by Peltier elements in the base of the chamber, and was measured by a thermistor close to the muscle. Fibres were penetrated with two micropipettes, for voltage recording and dye injection. The dye pipette also served as a current-passing electrode for initiating action potentials, or for voltage clamping. Intracellular Ca^{2+} changes were recorded as previously described[3,6,7]. Briefly, arsenazo III was injected into a fibre by ionophoresis, and light transmission through the fibre was measured at wavelengths of 532 and 602 nm from a spot of 80 μm diameter. Electrical subtraction of these two signals gave a

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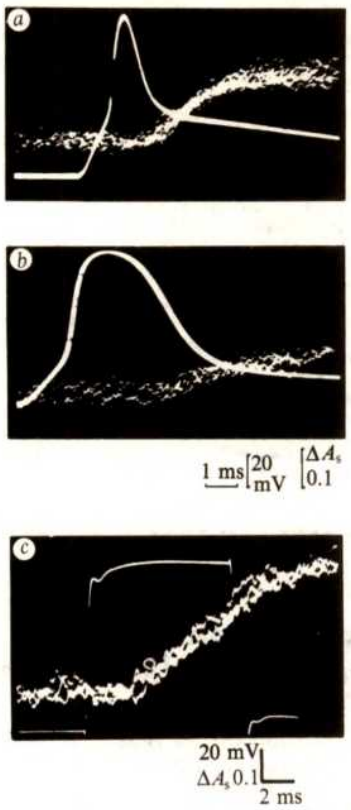


Fig. 1 Delay of the arsenazo III light response following stimulation by action potentials (*a*, *b*), and a voltage-clamped depolarising pulse (*c*). In each record the upper (noisy) trace shows light transmission changes from the arsenazo III-loaded fibre, at the wavelength pair 532–602 nm, and the calibration bars give the standardised change in light absorption, ΔA_s (see ref. 6). The lower traces show the membrane potential. Each record is a superposition of three to four sweeps. The records were obtained from different fibres, at the temperatures indicated. Ringer's solution contained 1.8 mM Ca^{2+} in all cases, and in *c* 2×10^{-6} tetrodotoxin and 30 mM tetraethyl ammonium bromide were also added to the solution. In *a* and *b* the fibres were stimulated by passing depolarising current pulses of 1 ms duration through the dye pipette. In *c* the fibre was voltage clamped, and stimulated by a 10-ms duration depolarising command pulse. Temperature: *a*, 29 °C; *b*, *c*, 7 °C.

record sensitive to Ca^{2+}-dependent changes in absorption of the dye, but in which movement artefacts were reduced.

When a fibre was depolarised to potentials above 0 mV, there was a definite latency between the rise in membrane potential and the onset of the Ca^{2+} response (Fig. 1 and refs 6, 8). This delay increased with decreasing temperature and was similar when the response was elicited by a depolarising pulse after blocking the action potential mechanism with tetrodotoxin, rather than by an action potential. For example, in 12 mM Ca^{2+} Ringer's solution, mean values of the delay-following action potentials and depolarising pulses were, respectively, 1.5 ± 0.2 ms (8) and 1.45 ± 0.42 ms (6) at a temperature of 15–16 °C, and 2.6 ± 0.8 ms (7) and 3.0 ± 0.6 ms (6) at 3–4 °C [mean, s.d. (number of fibres)]. Figure 2 shows the temperature dependence of the delay following an action potential, measured in muscle fibres bathed in Ringer's solutions containing various Ca^{2+} concentrations. The latency increased exponentially with the inverse of the absolute temperature (K) in all conditions studied, but at each Ca^{2+} concentration the slope of the relationship changed at a particular temperature. This transition temperature increased with increasing external Ca^{2+} concentration, and was 11 °C in 0 mM Ca^{2+}, 15 °C in 1.8 mM Ca^{2+} and 19.5 °C in 12 mM Ca^{2+}. This effect of Ca^{2+} concentration is probably specific to Ca^{2+} ions and not simply due to a change in divalent cation concentration, as the 0 Ca^{2+} solution contained 5 mM Mg^{2+}.

Another parameter of the excitation–contraction coupling system which displayed a similar temperature dependence to the initial latency, was the time during which a depolarised fibre could maintain an elevated Ca^{2+} level. Figure 3*a* shows a typical light absorbance record from a voltage-clamped fibre which was depolarised from −80 mV to +20 mV for 10 s. An initial large Ca^{2+} response was recorded which declined to the baseline over the duration of the pulse. Figure 3*b* shows an Arrhenius plot of the temperature dependence of the half-duration of this response, measured in a muscle bathed in 0 mM Ca^{2+} Ringer's solution. At temperatures below 11 °C the response half-duration remained constant at about 5.2 s, but at higher temperatures it decreased exponentially with the inverse of the absolute temperature. This transition temperature corresponds closely with that measured for the latency of the arsenazo signal during twitches on 0 mM Ca^{2+} solution. Technical difficulties prevented us from obtaining temperature dependence data at other Ca^{2+} concentrations, but at a temperature of 22.5 °C the response half-duration was slightly dependent on external Ca^{2+} concentration. Mean half-durations were 0 mM Ca^{2+}, 2.8 s (s.e.m. 0.14); 1.8 mM Ca^{2+}, 3.22 s (0.08); 12 mM Ca^{2+}, 3.8 s (0.12). This contrasts with frog slow muscle fibres, in which the duration of the maintained Ca^{2+} response is more strongly dependent on external Ca^{2+} concentration[9]. The peak sizes of the responses were not significantly affected by changes in temperature, suggesting that the shorter duration of the maintained Ca^{2+} response at high temperatures was not the result of exhaustion of Ca^{2+} reserves due to a higher rate of release. This agrees with the view that the amount of Ca^{2+} released during a prolonged depolarisation is controlled by a time-dependent inactivation of the release process[21,22].

Possible sources of the latency of the Ca^{2+} response include (1) response time of the dye, and diffusion time of Ca^{2+} within the myoplasm; (2) propagation time of the action potential inwards along the T-system; (3) a delay in the coupling mechanism between T-tubule depolarisation and Ca^{2+} release from the sarcoplasmic reticulum. The response time of arsenazo III is reported as faster than 400 μs at 21 °C (ref. 5) and the complex temperature dependence of the response latency makes it unlikely that the factors in (1) can account for the delay. Active propagation along the T-tubules has a velocity of about 7 cm s^{-1} at 7 °C in frog twitch fibres[10], and would therefore introduce a delay of about 1 ms for conduction to the centre of a

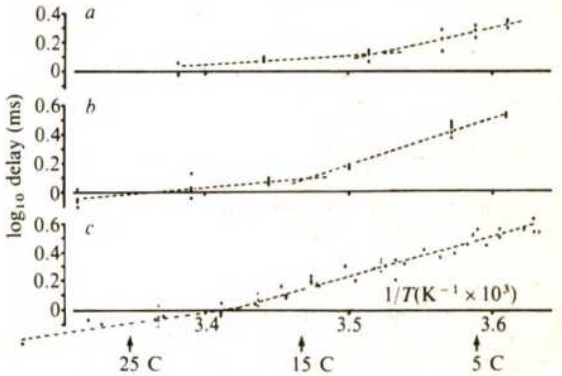


Fig. 2 Arrhenius plots of the log delay of the Ca^{2+} response against T^{-1}. The delay was measured by eliciting action potentials by a 1-ms depolarising pulse applied through the dye pipette, and measuring the latency between the foot of the action potential (about −40 mV) and the onset of the Ca^{2+} response (see Fig. 1). The three graphs show data from muscles bathed in Ringer's solution containing: *a*, 0 mM Ca^{2+} + 1 mM EGTA and 5 mM $MgCl_2$; *b*, 1.8 mM Ca^{2+}; *c*, 12 mM Ca^{2+}. A different muscle was used for each solution, and results from two muscles (●, and +) are shown in *c*. Each datum point was obtained from a different fibre, and is a mean of nine measurements. Readings were generally obtained from three or four fibres while holding the muscle at a given temperature, and different temperatures were tested in random order. Optical recording time constant was 0.5 ms. The lines drawn through the data were fitted by eye.

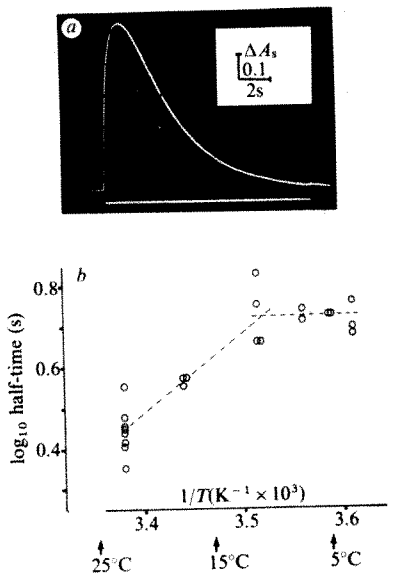


Fig. 3 *a*, Optical record showing the Ca^{2+}-induced change in differential light absorbance at 532–602 mn of an arsenazo III-loaded fibre, in response to a prolonged depolarisation. The fibre was voltage clamped to a potential of −80 mV at rest, and depolarised to +20 mV for the duration indicated by the bar. The vertical calibration, ΔA_s, gives the standardised change in light absorbance[3,6]. Ringer's solution contained 12 mM Ca^{2+}; temperature 5 °C; recording time constant 33 ms. *b*, Arrhenius plot of the log half-duration of the Ca^{2+} response during prolonged depolarisation. The half-duration was measured as the time for which the light absorbance record exceeded a value of one-half of the peak value. Each point is a single measurement from a different fibre from one muscle. Same experiment as Figs 2*a* and 3*a*.

100-μm fibre. However, the initial rise in the Ca^{2+} response probably results from activation of the sarcoplasmic reticulum at the periphery of the fibre, so that inward conduction time would not be an important factor in determining the minimal delay. A large part of the observed latency may therefore be attributed to the coupling mechanism.

The two component Arrhenius plots shown in Figs 1 and 2 resemble recent results on the elementary properties of drug receptor channels in muscle cells[11–15]. Possible causes of these temperature transitions include a membrane lipid phase transition[16–18], a conformational change in some protein[19] associated with the excitation–contraction coupling process, or different steps of a reaction sequence in the coupling process, being rate limiting in different parts of the temperature range[20]. Whatever the explanation, the influence of external Ca^{2+} concentration on the transition temperature of the latency in the rise of internal Ca^{2+} during a twitch, suggests that a delay in excitation–contraction coupling is introduced by a structure which is influenced by changes in Ca^{2+} concentration in the bathing medium, and is therefore probably located in the T-tubule membrane. The close similarity of transition temperature, and dependence on extracellular Ca^{2+}, of the duration of maintained Ca^{2+} response further suggests that this is also regulated by a structure in the T-tubule membrane[21,22].

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Inhomogeneous distribution of filipin–sterol complexes in smooth muscle cell plasma membrane

THE plasma membrane of the smooth muscle cell presents characteristic rows of micro-invaginations or caveolae arranged parallel to the longitudinal axis of the cell[1–4]. Previous studies have demonstrated that the plasma membrane in the rows contains a much higher density of intramembranous particles than the intervening membrane zones[2,4]. The polyene antibiotic, filipin, by specifically interacting with cholesterol[5–7], produces distinctive alterations in freeze-fractured membranes[8–11], and the addition of filipin to aldehyde fixatives has been recently introduced as a cytochemical technique for the freeze-fracture localisation of cholesterol in cell membranes[12,13]. By applying this technique to smooth muscle cells, we have now obtained morphological evidence that the invaginated, particle-rich bands of the plasma membrane react with filipin to a far greater extent than does the non-invaginated membrane. The two membrane zones may thus differ in cholesterol content.

Strips of smooth muscle from rat large intestine were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, *p*H 7.4, for 1 h, and incubated overnight at room temperature in the same fixative containing 300 μM filipin (Upjohn) dissolved in a drop of dimethyl sulphoxide (DMSO; final concentration 1%). Glutaraldehyde fixation is aimed at preserving cell ultrastructure by preventing the lysis resulting from filipin-induced permeability

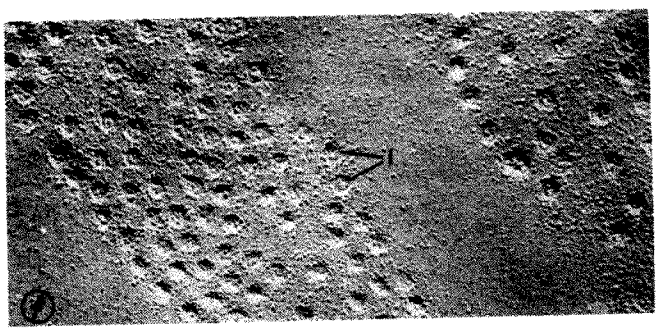


Fig. 1 Freeze-fracture replica of a smooth muscle cell fixed in glutaraldehyde alone. The plasma membrane of the cell has been split to reveal the protoplasmic leaflet or P-face[14]. The latter presents two parallel rows of crater-like circular depressions (i) representing the fractured necks of microinvaginations or caveolae. The density of intramembranous particles is higher within the rows than in the intervening membrane. The encircled arrows indicate the direction of platinum-carbon evaporation. ×47,000.

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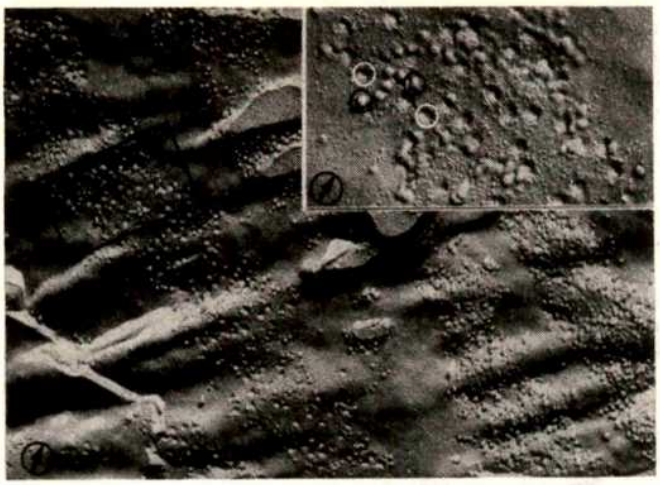


Fig. 2 Freeze-fracture replica of a smooth muscle cell fixed in glutaraldehyde-filipin mixture. The detail of a typical band of invaginations (black rectangle) is shown at high magnification in the inset. Numerous filipin–sterol complexes (black circles) are interspersed among the microinvaginations (white circles), whereas they are virtually absent outside the invaginated membrane regions. Filipin–sterol complexes appear as 25–30 nm protuberances which can be distinguished from the contiguous microinvaginations by the opposite polarity of platinum deposition (the direction of the shadowing is indicated by the encircled arrow). ×22,000. Inset: ×44,000.

changes in living cells[5]. Control muscle was fixed either in glutaraldehyde overnight, or in glutaraldehyde for 1 h and then in glutaraldehyde plus DMSO overnight. After rinsing in 0.1 M cacodylate buffer, *p*H 7.4, the tissues were cryoprotected with 30% glycerol in the same buffer, mounted on gold disks, and quickly frozen in Freon 22 cooled with liquid nitrogen. Fracturing and shadowing were carried out at −100 °C in Balzers BAF 300 and BAF 301 units. Replicas were cleaned by digestion in sodium hypochlorite, washed in distilled water, and recovered on coated 150-mesh copper grids. Freeze-fracture replicas were examined using a Philips EM 300 electron microscope.

Figure 1 illustrates the characteristic freeze-fracture appearance of the smooth muscle cell plasma membrane after aldehyde fixation alone[1–4]. The protoplasmic or P fracture face[14] shows many crater-like depressions, each one representing the fractured neck of an invagination. These craters are disposed in an orderly manner in parallel bands separated by regions of non-invaginated membrane. As has been previously observed[2,4], intramembranous particles are more numerous in the rows of invaginations. Fixation in glutaraldehyde plus 1% DMSO produces freeze-fracture images similar to those obtained with glutaraldehyde alone. When the smooth muscle is i in a filipin-containing glutaraldehyde solution, filipin–sterol complexes become recognisable as 25–30 nm protuberances[8–13] on the fractured plasma membranes (Fig. 2, inset). Filipin–sterol complexes are abundant in the particle-rich regions of the membrane, in which they are interspersed among the invaginations. By contrast, such complexes are virtually absent in the intercalated, particle-poor bands (Fig. 2). Due to the poor penetration of filipin within compact tissues, filipin–sterol complexes were usually confined to the most peripheral portions of the fractured specimens. Nevertheless, whenever found on a smooth muscle cell plasma membrane, they occurred with the selective distribution outlined above.

As filipin specifically binds to cholesterol and other related 3-β-hydroxysterols in cell membranes[6,7,15], our observation suggests heterogeneous distribution of these lipids in the smooth muscle cell plasma membrane. The functional significance of this heterogeneity is not clear, but it is worth noting that in the cells studied a higher sterol content is associated with both a higher density of intramembranous particles and morphological specialisation (invaginations) of the plasma membrane. It should be emphasised here that filipin labelling only represents qualitative evidence for sterol topographical inhomogeneity, and does not permit evaluation of the absolute sterol content of a discrete membrane region. In particular, the virtual lack of filipin reactivity in non-invaginated bands (which are the sites of insertion for actin myofilaments[16]) should not necessarily mean that there is no cholesterol there. In these membrane areas, the cholesterol level might be too low to induce the formation of detectable complexes to the same extent as found in invaginated regions.

The presence of a marked heterogeneity in sterol content between contiguous bands of membrane suggests that some restraint must exist in the lateral diffusion of cholesterol in the plane of the native membrane.

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Visualisation of ^{11}C-flunitrazepam displacement in the brain of the live baboon

THE presence of specific benzodiazepine receptors in the brain of rat[1,2], baboon[3] and man[4,5] has been demonstrated *in vitro*. These receptors have also been observed *in vivo* for diazepam[6] and flunitrazepam[7]. However in such experiments, although the radioactive ligand is injected in the living animal the demonstration of the presence of specific receptors requires brain tissue sampling for quantitative measurement *in vitro*. The fact that carbon-11 is a positron emitter with a half life of 20 min which can be used for very high specific activity labelling of drugs, raises the possibility of external detection of radioactivity in a living subject by positron emission tomography[8,9]. Using this new methodology the present study demonstrates the displacement of ^{11}C-flunitrazepam by lorazepam in the brain of live baboons.

Eight experiments were carried out with two female baboons (*Papio papio*) weighing 11 and 12 kg. After anaesthetisation with ketamine (15 mg per kg) the animals were intubated, ventilated and curarised by injection of nortoxiferin chlorhydrate (0.2 mg per kg as a starting dose followed by 0.1 mg per kg injections when necessary). At the end of the experiments the animals were decurarised using prostigmine. The interval between two experiments on the same animal was a minimum of 2 weeks. The animals were placed supine in a painless position under the positron camera (ECAT-ORTEC), the head inside a

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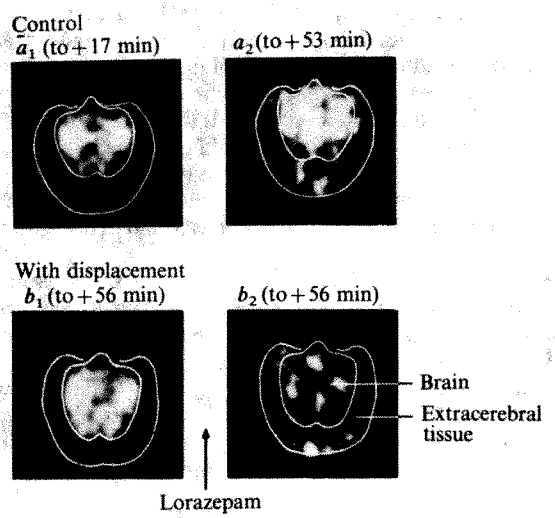


Fig. 1 Brain distribution at different times after i.v. injection of ^{11}C-flunitrazepam observed by positron emission tomography. Comparison of b_2 obtained 26 min after administration of a load of lorazepam (1.3 μM per kg) with a_2 (control experiment) reveals a displacement of the radioactive ligand. See Fig. 2 and Table 1 for quantitative data.

hammock in such a way that a 2-cm thick slice, parallel to and 2 cm above the inion orbital line, was observed by the camera. Flunitrazepam was labelled by ^{11}C-methyl iodide methylation of the nor derivative at a specific radioactivity of 300–900 Ci mmol^{-1} at the injection time[10]. The labelled drug (20–25 mCi), corresponding to 2–6 nM per kg weight, was injected intravenously (i.v.) at least 5 h after the ketamine anaesthesia, and the head scans were repeated over 1 h. Arterial blood and expired air samples were taken at given times to measure the radioactivity. In four experiments, lorazepam (1.3 μM per kg) was injected i.v. 30 min after ^{11}C-flunitrazepam to displace the radioactive tracer from the specific receptors. This benzodiazepine was used because of its high capacity to displace flunitrazepam from its specific binding sites[4]. The scans were visualised on a TV monitor and two regions of interest corresponding to cerebral and extracerebral tissues were selected. Quantitative data were obtained by comparing the '*in vivo*' activity with that of a control containing a known amount of radioactivity. Results in brain extracerebral tissues and blood were expressed as a percentage of the injected dose per g of tissue. Expired $^{11}CO_2$ activity was expressed as percentage injected dose. Blood pressure was recorded in some experiments by catheterisation of the femoral artery.

Figure 1 gives the pictures corresponding to one control (a) and one displacement experiment (b). Pictures a_1 and b_1 were obtained 17 and 20 min after ^{11}C-flunitrazepam injection. They show a high uptake of the labelled drug in the brain as compared with the extracerebral tissue, which cannot be seen on these pictures. On the other hand, 53 min after the tracer injection in the control experiment (a_2) the brain activity is still high but has decreased more rapidly than the extracerebral radioactivity, which is now visible. More important is the difference seen in b_2 taken at 56 min, 26 min after lorazepam injection. The contrast between brain and extracerebral tissue is less in this picture than in a_2. Figure 2 illustrates the kinetics of ^{11}C-flunitrazepam with and without displacement. In the control experiment the brain radioactivity curve (corrected for physical decay of ^{11}C) shows a sharp decrease during the first 20 min, followed by a more slowly decreasing phase during the next 30 min. In the displacement study lorazepam was injected at the beginning of the low-slope phase of the activity curve and led to a faster brain activity decrease than in the control. By contrast, extracerebral activity remained nearly constant throughout the experiment. Blood activity increased slightly after lorazepam injection.

Table 1 gives the data obtained in four control and four displacement studies. For each experiment the specific radioactivity (when measured) of the ligand at the time of injection, the cerebral, extracerebral, blood and expired $^{11}CO_2$ activities at the end of the experiment (50–60 min) are indicated. In all tests the extracerebral and blood activities are of the same order of magnitude, unlike that of the brain which is significantly lower after displacement. The presence of $^{11}CO_2$ in expired air indicates a demethylation of the radioactive ligand.

Modifications in the brain kinetics of high specific activity ^{11}C-flunitrazepam were definitely observed when a load of lorazepam was injected. The amount of radioactive ligand administered (2–6 nM per kg) is of the same order of magnitude as that used by other authors for displacement studies '*in vivo*' (ref. 7). Because of the low efficiency of radioactivity detection by external counting on the live animal, the specific radioactivity of the ligand had to be much higher than is generally used for '*in vitro*' measurements with tritiated molecules. Although theoretically a specific activity of 10^4 Ci μmol^{-1} can be reached when labelling with carbon-11, the best value obtained so far did not exceed 0.8–1.0 Ci μmol^{-1} at the time of injection[10]. A variation in displacement for different anatomical areas could not be observed because of the resolution (12 mm) of the positron camera. Furthermore, the thickness of the slice in the field of view of the detector is 20 mm. In these technical conditions it is difficult in a baboon's brain to differentiate the cortex, in which the density of receptors is high, from the other structures where the concentration is lower.

Chang *et al.*[7] have shown that after i.v. injection of ^{3}H-flunitrazepam only that fraction of the ligand bound to membranes is likely to be displaceable. Although bound and free fractions can be separated *in vitro* this is not the case *in vivo*

Table 1 Results of control and displacement studies of ^{11}C-flunitrazepam

	Specific activity (Ci mmol^{-1})	Brain uptake (% per g × 10^4)	Extracerebral uptake (% per g × 10^4)	Brain to extracerebral uptake ratio	Blood (% per g × 10^4)	Expired $^{11}CO_2$ (% in first hour)
Controls						
1	—	75	58	1.29	—	—
2	650	75	47	1.60	—	—
3	300	100	55	1.80	60	3.2
4	880	120	60	2.0	80	13
Mean ± s.d.	610	93 ± 22	55 ± 6	1.67 ± 0.3	—	
After displacement						
1	500	45	45	1.0	50	—
2	—	60	50	1.20	50	10.8
3	570	70	60	1.15	70	13.6
4	620	70	65	1.10	60	9.6
Mean ± s.d.	563	61 ± 12	55 ± 9	1.11 ± 0.09	58 ± 9.6	

Specific activity is given at the time of ^{11}C-flunitrazepam injection. Values are given as a percentage of the dose 50–60 min after injection of the radioactive ligand (20–25 mCi). In displacement studies lorazepam (1.3 μM per kg) was injected 30 min after ^{11}C-flunitrazepam. The average brain to extracerebral tissue uptake ratio was significantly lower (at the 2% level) after lorazepam injection and shows an apparent 35% displacement.

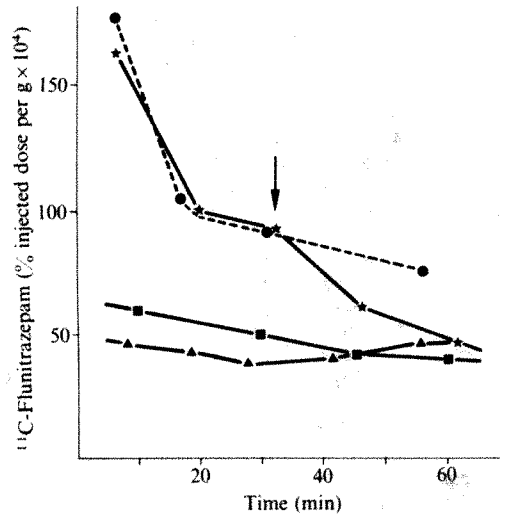


Fig. 2 ^{11}C-Flunitrazepam kinetics. ●, Cerebral uptake in a control experiment. ★, Cerebral uptake in a displacement experiment. Lorazepam (1.3 μM per kg) was injected (arrow) 30 min after ^{11}C-flunitrazepam. ■, Extracerebral uptake of the displacement experiment. The region measured is shown in Fig. 1. ▲, Blood in the displacement study.

and only the overall activity is measurable, giving an apparent displacement lower than observable when measured *in vitro*. Other physiological parameters, such as blood pressure and blood flow change after administration of a therapeutic dose of lorazepam, redistribution of the tracer in other organs which might modify its availability for brain tissue and the metabolism of the labelled drug itself with loss of the tracer by demethylation, might affect the amplitude of the displacement. In our experiments no modification of arterial blood pressure was observed. A redistribution of the radioactive ligand after displacement can be suspected as a slight increase in blood radioactivity following lorazepam injection was observed in all cases. However, this increase did not modify the extracerebral tissue uptake. Demethylation of the tracer was a significant feature, being variable from one experiment to another. As the *N*-demethylated product, which is not detected, has a pharmacological activity comparable with that of the parent compound, its possible binding to the same specific receptors will contribute to an underestimation of the observed displacement. Because of these points, an accurate quantification of the effect of a pharmacological dose of drug on the displacement of a radioactive ligand is more difficult *in vivo* than *in vitro*. However the data observed in this study correlate well with *in vitro* or semi-'*in vivo*' experiments. An apparent 35% displacement of ^{11}C-flunitrazepam by 1.3 μM per kg lorazepam agrees well with the ID_{50} values of Chang and Snyder[7] for clonazepam, flunitrazepam and diazepam in mice. The rapidity with which the displacement is observed, as shown in Fig. 2, corresponds well to the rapid anti-epileptic activity of the benzodiazepines.

These first results indicate that benzodiazepine brain receptors are detected in the live baboon in a non-traumatic way and that the methodology described may be applied to human beings for whom the detector used is better adapted than for animals. The development of new labelling methods with the short-lived radioisotope carbon-11 might also permit the detection in the living brain of other specific receptors and the evaluation of their properties as a function of pharmacological and physiopathological changes.

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Partial agonists for brain GABA/benzodiazepine receptor complex

BENZODIAZEPINE receptors in the brain[1] are activated by γ-aminobutyric acid (GABA) and by the GABA agonist muscimol *in vitro*[2]. This activation may be related to the GABA-potentiating effects of benzodiazepines observed in electrophysiological studies (see ref. 3 for review). The enhancement of specific ^{3}H-diazepam binding by GABA agonists is inhibited by bicuculline and thus offers a unique high-affinity binding system for investigations *in vitro* of agonist–antagonist interactions at GABA receptors in the central nervous system. We report here that two GABA-mimetic compounds, 3-aminopropanesulphonic acid (APS) and isoguvacine, are partial agonists (mixed agonists/antagonists) with intermediate efficacies. Imidazoleacetic acid (IAA) is also a partial agonist but with only marginal agonist activity, and the new GABA-mimetics piperidine-4-sulphonic acid (PSA) and 4,5,6,7-tetrahydroisoxazolo-(5,4-*c*)pyridin-3-ol (THIP) are competitive antagonists to the GABA/benzodiazepine receptor complex.

One group of GABA agonists increases ^{3}H-diazepam binding to rat brain membranes, prepared as described in Fig. 1 legend. Muscimol (EC_{50} = 0.6 μM, see Fig. 2 legend), dihydromuscimol (1.9 μM), GABA (3.8 μM), thiomuscimol (4.2 μM), *trans*-aminocrotonic acid (7.4 μM), (*S*)(−)5-methylmuscimol (230 μM), (*R*)(+)4-methyl-GABA (410 μM), (*S*)(−)4-methyl-GABA (550 μM), and (*R*)(+)5-methylmuscimol (2,300 μM) increased specific ^{3}H-diazepam binding to ~200% of the control level[4] (because of limited supply of the latter three compounds the dose–response curves were stopped before maximal effect was achieved). The EC_{50} values for the nine agonists above as well as for 3-hydroxy-GABA and 3-guanidinopropionic acid[5] are closely correlated with inhibitory activity on ^{3}H-GABA binding sites. The EC_{50} values for benzodiazepine receptor activation, however, were 179 ± 32-fold higher than the respective IC_{50} values for ^{3}H-GABA binding[4,6,7] (mean ± s.e.m. for nine GABA agonists).

Thus three lines of evidence indicate that GABA receptors are involved in the increase in ^{3}H-diazepam binding: (1) The GABA antagonist bicuculline selectively and completely abolishes the activation induced by muscimol (Fig. 1*a* and refs 2, 5, 8, 9) and by the eight other agonists described above (data not shown). Bicuculline alone has no effect on this membrane system. (2) The activation of ^{3}H-diazepam binding is stereoselective for GABA agonists (data above) and highly correlated to the affinity of these same agonists for GABA receptors.

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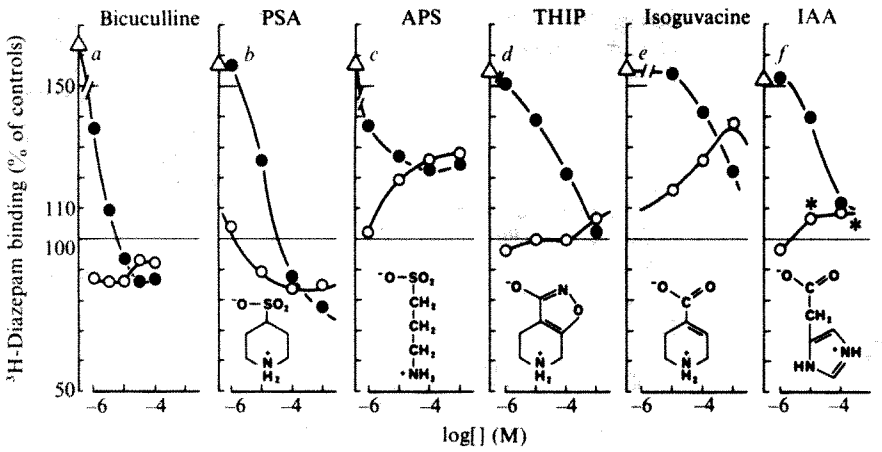


Fig. 1 Effects of bicuculline and some potent GABA-mimetics on ^{3}H-diazepam binding to rat brain membranes. ○, GABA-mimetic alone; ●, GABA-mimetic plus 3×10^{-6} M muscimol; △, muscimol alone, 3×10^{-6} M. Whole rat forebrains were homogenised in 10 vol ice-cold 100 mM Tris-citrate *p*H 7.1, in a precooled Ultra-Turrax homogeniser. The homogenate was centrifuged at 48,000*g* for 10 min at 0–4 °C and the pellet was rehomogenised for a few seconds in another 10 vol of the same ice-cold buffer. Centrifugation and rehomogenisation were performed five times. The final suspension was diluted to 160 vol (vol/weight original tissue) in 100 mM Tris-citrate *p*H 7.1, and aliquots of 2.5 ml were incubated with 0.4 nM ^{3}H-diazepam (14.4 Ci mmol^{-1}, gift from Willy Haefely, Roche) for 20 min at 0 °C in a total of 2.75 ml. GABA antagonists and agonists were added in duplicate in that order just before ^{3}H-diazepam. Samples were diluted to 10 ml in cold buffer after incubation and filtered immediately through GF/C glass-fibre filters and washed with 10 ml buffer. Tritium on filters was assessed by scintillation counting. All binding values were calculated as specific binding, which is total binding minus binding in the presence of 3×10^{-6} M unlabelled diazepam. Control specific binding averaged 677 ± 49 c.p.m. per 15 mg tissue; specific binding in the presence of 3×10^{-6} M muscimol, 1,037 ± 70 c.p.m. per 15 mg tissue; and nonspecific binding 157 ± 15 c.p.m. per 15 mg tissue (mean ± s.e.m. of six experiments). The data shown are from a single experiment which was repeated giving similar results. Bicuculline (Sigma) was freshly prepared in dilute HCl; piperidine-4-sulphonic acid and THIP, H_2O were synthesised by P.K.-L.; APS (sodium salt) was supplied by J. Arnt, Copenhagen; isoguvacine, HCl was synthesised by O. Rosenlund-Hansen, Ferrosan. * Imidazoleacetic acid (10^{-5}–10^{-4} M) increased ^{3}H-diazepam binding (17.6 ± 2.04% in six experiments, $P<0.001$).

Activation of benzodiazepine receptors by GABA is achieved at micromolar concentrations, as are GABA effects on the superior cervical ganglion[10], the cuneate nucleus *in vitro*[11], the crayfish stretch receptor neurone[12] and the dorsal root ganglion neurone[13]. (3) The only non-GABA-agonist (potassium iodide, 30 mM, see also ref. 14) among many tested which increased ^{3}H-diazepam binding in the present membrane preparation acts in an additive way to muscimol, suggesting a different mechanism of action[4].

A second group of potent GABA-mimetics, piperidine-4-sulphonic acid, APS, IAA, THIP and isoguvacine (see below) did not, however, increase ^{3}H-diazepam binding to any great extent (Fig. 1*b–f*), even at concentrations more than 10,000-fold in excess of the respective IC_{50} values[6,7] for ^{3}H-GABA binding (see also refs 2, 15). These compounds surprisingly inhibited muscimol activation to a degree depending on their respective efficacies (Fig. 1*b–f*). Piperidine-4-sulphonic acid also completely inhibited the activation induced by *trans*-aminocrotonic acid and GABA. GABA analogues of restricted conformation (isomuscimol, iso-THIP and homologues of THIP with seven-membered heterocyclic rings[6]) were inactive as inhibitors of ^{3}H-GABA binding, as activators of ^{3}H-diazepam binding and as inhibitors of muscimol activation.

Parallel displacement of dose–response curves for muscimol and linear Schild plots with slopes close to unity (Fig. 2) indicate that piperidine-4-sulphonic acid is a competitive inhibitor of muscimol in a first-order interaction. If piperidine-4-sulphonic acid had been a pure agonist to GABA receptors, an EC_{50} value of $179\times IC_{50} = 7.1$ μM would be expected ($IC_{50} = 40$ nM for ^{3}H-GABA binding, P.K.-L. unpublished). The antagonist affinity, $K_I = 1.5$ μM (Fig. 2*b*), is relatively close to this predicted value, indicating that piperidine-4-sulphonic acid occupies GABA receptors with the expected affinity but without efficacy in the present system. Piperidine-4-sulphonic acid and THIP are antagonists in the present membrane preparation (washed membranes). Partial agonist properties cannot be excluded since both compounds exhibit marginal agonistic effects in Triton-treated membranes[4].

Piperidine-4-sulphonic acid (P.K.-L., D. R. Curtis and E.F. unpublished), APS[16], IAA[6,17], THIP[6,18] and isoguvacine[6,18] are GABA-mimetic in the sense that they inhibit high-affinity GABA receptor binding with IC_{50} values ranging from 6 to 130 nM, and that they are potent bicuculline-sensitive inhibitors of cat spinal cord motoneurones. However, when tested on the coupled GABA-receptor benzodiazepine receptor *in vitro*, these compounds exhibit mixed agonist/antagonist or pure antagonist properties. The physiological relevance of this feature is unclear, but the finding that APS is less active on crayfish stretch receptor neurones than expected from receptor binding studies[19], and the unexpected lack of GABA-like activity of THIP and isoguvacine on E4 cells in the visceral ganglion of *Helix aspersa*[20] may be a consequence of low efficacy.

It is thus conceivable that several classes of GABA receptors exist in the central nervous system; some (those coupled to benzodiazepine receptors) may differentiate a continuum of pure and partial agonists, whereas other classes possess no such selectivity.

The experiments described here introduce a simple technique for studying 'classical' pharmacology in the central nervous system. Other high-affinity binding sites may be coupled, making the principle more generally applicable.

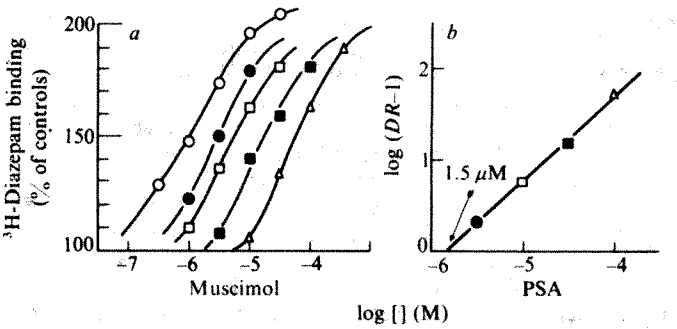


Fig. 2 *a*, Dose–response curves for the effect of muscimol on ^{3}H-diazepam binding to rat brain membranes prepared as described in Fig. 1. Various concentrations of piperidine-4-sulphonic acid were added. ○, No addition; ●, 3×10^{-6} M; □, 10^{-5} M; ■, 3×10^{-5} M; △, 10^{-4} M. *b*, Schild plots constructed from the EC_{50} values for muscimol obtained in *a*. (The dose–response ratio, *DR*, is the ratio of EC_{50} values for muscimol with and without antagonist added.) Slope in *b*, 0.92. The EC_{50} value is that concentration of GABA agonist in μM which increases specific ^{3}H-diazepam binding to half the maximal increase obtainable with optimal concentrations (10^{-5}–3×10^{-5} M) of muscimol. EC_{50} values given are means of at least two values, each obtained by Hill analysis of results obtained by addition of four to ten concentrations of agonist in duplicate assays. The results shown in this figure are from a single experiment which was repeated twice with similar results. Each point in *a* is the mean of two values which differed 2.7 ± 0.5% (25); the control level was 784 ± 15 c.p.m. per 15 mg tissue (9) (mean ± s.e.m. of (*n*) values).

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Solvent accessibility properties of complex proteins

AN important factor in the thermodynamics of protein folding is the partition of amino acid residues between the solvent environment and the protein core. The extent of the interaction between the folded polypeptide and the surrounding medium can be measured by determining the solvent accessible surface area as defined by Lee and Richards[1]. In this communication we present the results of solvent accessibility calculations for the large (195,000 M_r) dimeric enzyme phosphorylase *a* and other multidomain, multisubunit proteins. These results are discussed in the light of the generalisations concerning protein solvent—accessible surface area and shape which have been based on data derived from low molecular weight proteins.

X-ray diffraction studies of proteins have, in many cases, provided atomic coordinates of sufficient accuracy to make accessibility calculations feasible. Using the crystallographically-derived coordinates for a select group of small ($M_r \leq$ 30,000), compact, well determined protein structures, Janin[2] and Teller[3] have demonstrated that the solvent-accessible surface area, A_s, can be expressed as a function of molecular weight:

$$A_s = 11.1\, M^{2/3} \qquad (1)$$

The standard error between calculated and theoretical values of A_s, for structures incorporated in the data base is about 400 Å^2 (ref. 3). This model predicts that protein molecules adopt a globular shape for which the surface area/volume ratio approaches the minimum value characteristic of a sphere. (It is not implied that proteins are necessarily spherical.) Interpenetrating spherical surfaces have been used to model the accessible surface changes in the dimerisation of insulin, the interaction between haemoglobin α and β subunits and of trypsin with its inhibitor[3].

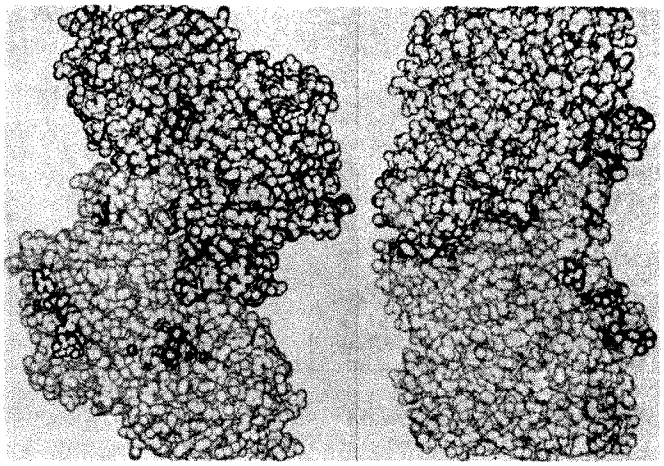


Fig. 1 Two orthogonal views of the Van der Waal's contact surface of the phosphorylase *a* dimer. All nonhydrogen atoms at the surface are shown as well as several ligands which bind to the enzyme: S, maltoheptaose; G, a buried glucose molecule; C, caffeine; plp, the buried coenzyme pyridoxal phosphate; P, the serine-14-phosphate and A, AMP. The atomic positions were determined by X-ray diffraction at 2.5 Å resolution. The two subunits are shown in different shades of grey. Note that although the dimer contact appears to be extensive in these drawings, a large solvent accessible cavity of about 2,500 Å^3 lies at the subunit interface. The left plate shows the face of the dimer that binds to glycogen[11]. This same face is seen edge-on at the right in the orthogonal view.

Using an extended data base, Janin[4] has recently shown that the number of buried ($A_s < 20$ Å^2 per residue) amino acids, n_B, in a protein of n residues is given by

$$n_B = (n^{1/3} - k)^3 \qquad (2)$$

where $k = 1.62$.

As many proteins of biological interest are large, multidomain and oligomeric, it is of interest to determine the applicability of models based on simpler structures to these more complex systems. We have recently determined the atomic coordinate set for rabbit muscle phosphorylase *a* (ref. 5). This protein has about three times more amino acids per polypeptide chain than the largest used to determine the accessibility law. Further, phosphorylase occurs as a dimer in the tetragonal crystal form discussed here, allowing a determination of the protein surface removed from solvent contact by dimerisation. Using a program written by T. J. Richmond, incorporating the algorithm of Lee and Richards[1], we have calculated the solvent-accessible surface areas for monomers and dimers of phosphorylase *a*, chicken triose phosphate isomerase (TIM), horse alcohol dehydrogenase (ADH), human Bence–Jones protein (BJ) and the monomer and tetramer of dogfish apolactate dehydrogenase (LDH). Atomic coordinates were obtained from the AMSOM protein data files[6]. Accessible surface areas and related parameters are summarised and described in Table 1. The data for insulin and the haemoglobin α and β chains given by Chothia and Janin[9], which agree with our calculations for these cases, are included for comparison.

It seems that expression (1) holds well for low molecular weight protein monomers. However, for larger proteins, the model underestimates accessible surface area by a margin of error which increases with the molecular weight of the monomer. This discrepancy has already been noted for oligomeric proteins[3,4]. The model errs most seriously with phosphorylase.

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Table 1 Predicted and observed solvent-accessible surface areas of oligomeric proteins

Protein	Molecular weight	Monomer A_s^{obs} (Å^2)	Monomer A_s^{th}	Monomer F_{as}^{obs}	Monomer n_B	Monomer n_B^{th}	Oligomer A_s^{obs}	Oligomer A_s^{th}	Oligomer F_{as}^{obs}	Interface F_B^{obs}	Interface F_B^{th}
Insulin†	5800	3440	3580	0.59	—	—	5580	5900	0.67	0.17	0.14
Bence-Jones	11700	5590	5730	0.67	33	30	9490	9820	0.72	0.13	0.14
Haemoglobin α†	15100	6940	6800	0.68	—	—	12320	11870	0.72	0.12	0.15
β	16000	7130	7050	0.69	—	—					
TIM	26500	11450	9870	0.70	100	84	19730	16920	0.74	0.14	0.14
LDH	36400	17440	12210	0.66	114	134	47150	34870	0.78	0.32	0.28
ADH	39700	16530	12930	0.71	159	174	29720	22160	0.74	0.10	0.14
Phosphorylase	97400	36400	23530	0.74	357	478	68200	40330	0.76	0.06	0.14

* Derived quantities: A_s^{th}, solvent accessibility calculated from equation (1); for dimers and the tetramer of LDH accessible surface area is given by the expression $2\times(0.86\ A_s^{th})$ and $4\times(0.72\ A_s^{th})$, respectively, where A_s^{th} is the surface area of the monomer[3]. F_{as}, the fraction of potentially accessible surface buried by protein folding is given by $(A_t - A_s^{obs})/A_t$ where $A_t(=1.44\ M)$ is the accessible area of an extended polypeptide chain[7]. n_B^{th} is the number of residues buried by protein folding predicted by equation (2). F_B^{obs} and F_B^{th} are the observed and predicted[3] values for solvent-accessible surface area buried at the dimer or tetramer interface.

† Reproduced from ref. 3.

The expression proposed to predict n_B (equation (2)) is reasonably accurate for most of the cases listed in Table 1 (only monomers are considered), but does not apply for monomers of LDH and phosphorylase. The sphere interpenetration model for dimerisation predicts that approximately 14% and 28% of the monomer surface is buried after the formation of dimers and square planar tetramers, respectively[3]. It is interesting that theoretical values of A_s for the TIM dimer and the LDH tetramer prove to be more accurate predictions than the theoretical values of A_s for the isolated monomers. A minimum surface model seems to fit the oligomers better than their respective monomers. Phosphorylase is again a notable exception. Only 6% of the accessible surface of the monomer (2,300 Å^2) is involved in dimer formation. This analysis does not account for possible conformational changes on oligomerisation (see below).

Although minor deviations in observed A_s and n_B from values predicted by equations (1) and (2) may be attributed to non-spherical shape, this explanation cannot account for the significant underestimation for phosphorylase. For example, one can readily show that a parallelepiped of dimensions sufficient to completely enclose the phosphorylase monomer would have a surface area equal to that of the protein but would contain twice its volume. In fact, the large accessible areas of LDH, ADH and phosphorylase are most probably due to the articulation of each of these molecules into two well-defined functional and structural domains, which gives rise to a complex and invaginated molecular topography. This is illustrated in the space-filling model of the phosphorylase dimer (Fig. 1). Chothia[7] has suggested that in such large multidomain or multisubunit structures, the fraction of potentially accessible surface area buried by protein folding, F_{as}, is essentially constant. Table 1 shows that F_{as} for monomers and multimers becomes asymptotic at 0.75–0.78. This implies that the accessible surface becomes a linear function of M_r for large proteins. A plot of A_s against M_r from Table 1 shows this to be true.

Although only a small fraction of the phosphorylase molecule is involved in dimer formation, the amount of surface area withdrawn from solvent by formation of the complex is quite large, 4,600 Å^2. Loss of accessible surface area is believed to contribute significantly to the free energy of protein association[8,10] which may be expressed (for dissociation) as

$$\Delta H_d + \Delta G_d^l - \Delta G_d^s = \Delta G_d \qquad (3)$$

where ΔG_d^s and ΔG_d^l are free energy terms due, respectively, to the gain in rotational and translational entropy by the subunits upon dissociation and to the entropy lost by solvent as a result of the increase in subunit accessible area. The enthalpy of dissociation, ΔH_d, is assumed to be small[8,9] due to the energetic equivalence between solvent–monomer and monomer–monomer hydrogen bonds and Van der Waals' interactions. The free energy of dissociation, ΔG_d, is $-RT$ ln K_d. These parameters may be evaluated for the dissociation of the phosphorylase dimer.

Using the expression derived from statistical thermodynamics by Janin[10], ΔG_d^s is 33 kcal mol^{-1}. The hydrophobic free energy ΔG_d^l has been given as a direct function of the change in accessible surface area ($\gamma\Delta A_s$, where $\gamma \sim 25$ cal mol^{-1} Å^{-2})[8,9]. Assuming $\Delta A_s \sim 2A_s^{monomer} - A_s^{dimer}$, ΔG_d^l is approximately 155 kcal mol^{-1}. Substituting these values into equation (3), we arrive at the clearly unreasonable estimate of 82 kcal mol^{-1} ($K_d \sim 10^{-60}$) for ΔG_d. If only nonpolar atoms, that is, carbon atoms, are considered in the accessibility calculation, ΔA_s is about 2,600 Å^2, corresponding to ΔG_d of 33 kcal mol^{-1} ($K_d \sim 10^{-24}$). Preliminary kinetic studies (N.B. Madsen, personal communication) indicate ΔG_d for phosphorylase is probably about 11 kcal mol^{-1}.

It is unlikely that errors in the phosphorylase coordinate set can account for these discrepancies. One source of error could be the assumption that the subunit assumes the same conformation within the dimer as it does as a monomer. For example, the free monomer may bury a significant proportion of the accessible surface area which is also observed to be buried in the dimer. If this were indeed the case, we would conclude (assuming $\Delta H_d \sim 0$ and $\Delta G_d \sim 11$ kcal mol^{-1}) from equation (3) that ΔA_d is only 900 Å^2 per monomer. Another source of error could be the assumption of $\Delta H_d \simeq 0$. The enthalpy, if non-zero,

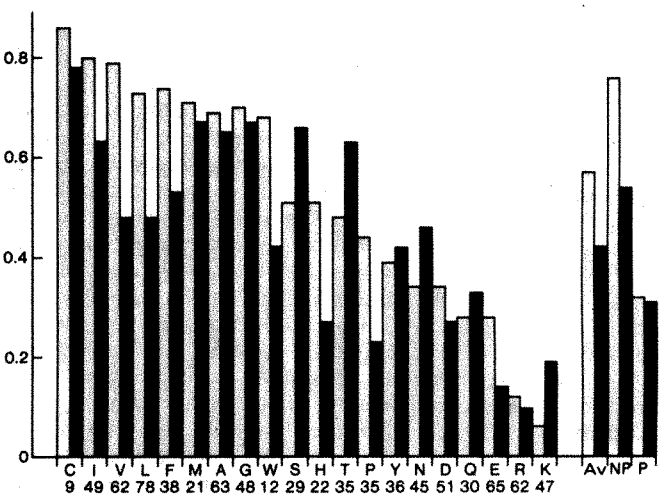


Fig. 2 The ordinate shows the mole-fraction buried for each type of amino acid and the distribution predicted using the partition coefficients determined by Janin[4]. The number of each type of amino acid is shown below the usual one letter code representation. The three additional entries at the right represent respectively the average over all amino acids (Av) the nonpolar amino acids (Ile, Val, Leu, Phe, Met, Ala, Trp, Gly, Pro; NP) and the polar amino acids (P).

could contain contributions from subunit association and/or subunit conformational changes.

As the bulk solvent accessibility properties of phosphorylase differ significantly from those predicted from smaller systems, it is of interest to note the relative accessibility of polar and nonpolar side chains. Chothia[2] has found that the proportion of non-polar surface buried increases with molecular weight, whereas the proportion of polar surface buried is constant. A similar conclusion is drawn from Janin's results[4]. Figure 2 shows the mole fraction buried for each of the amino acids in phosphorylase compared with that predicted for phosphorylase using partition coefficients determined by Janin[4] for each residue, and n_b as observed (357) (see Table 1 and equation (3) in this reference). Generally fewer of the nonpolar residues (Ile, Val, Leu, Phe, Trp and Pro) are buried in phosphorylase than predicted from the data base; the accessibility for the polar residues (Ser, Thr, Asn, Gln and Lys) is lower than predicted. (Note that there is relatively little difficulty with sample size in these statistics since only Trp and Cys appear fewer than 20 times.)

Accounting for these deviations from predicted accessible surface in the case of phosphorylase is difficult. It is unlikely on a biochemical basis that other macromolecules, for example, kinase, phosphatase and glycogen, bury additional surface *in vivo*. The most likely explanation for the discrepancy in accessible surface and the distribution of buried residues is that the data base used for deriving expression (1) rests on a small sample of mainly extracellular proteins of low molecular weight. These proteins are generally required by their environment to be structurally more stable than the large and conformationally labile intracellular regulatory proteins, such as phosphorylase. We feel that the closing statement of Lee and Richards' original paper[1] is still appropriate: "Simple generalisations of any sort are remarkably difficult to see in the summaries so far produced".

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Evidence for non-spliced SV40 RNA in undifferentiated murine teratocarcinoma stem cells

UNDIFFERENTIATED murine teratocarcinoma stem cells do not support simian vacuolating virus 40 (SV40) or polyoma virus (Py) replication or even the expression of the early papovavirus proteins. These undifferentiated cells are also entirely refractory to infection with ecotropic murine C-type viruses. If the stem cells are allowed to differentiate to a variety of somatic cell types, they become susceptible to infection by SV40, Py and ecotopic murine C-type viruses[1–4]. In contrast, adenovirus type 2 can infect and replicate in the undifferentiated stem cells, although not as efficiently as it can in the differentiated mouse cells[5] and certainly much less efficiently than it does in human cells. Studies on the mechanism of the resistance of the undifferentiated teratocarcinoma cells to infection by SV40 has shown that the block is not at the level of virus adsorption, penetration uncoating or transport to the nucleus[6]. We present here evidence indicating that the absence of expression of SV40 genetic information in nondifferentiated teratocarcinoma cells reflects an inability to generate stable viral mRNA. More specifically, the refractoriness of these cells to SV40 seems to be correlated, at least in part, with a deficiency in the splicing of viral mRNA.

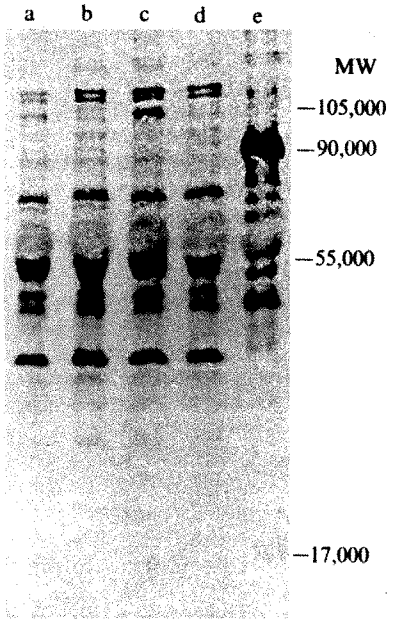


Fig. 1 Analysis of proteins in SV40-infected F-9 cells. Cultures of 1×10^7 F-9 cells uninfected or infected with SV40 (250 p.f.u. per cell) for 17 h, were labelled for 2 h with ^{35}S-methionine (100 μCi ml^{-1}) in methionine-free medium. Proteins were purified and immunoprecipitated with hamster anti-SV40 T-serum as described previously[10]. Samples were electrophoresed in 20% polyacrylamide slab gels (20% acrylamide–0.1% bisacrylamide) for 10 h at 75 V (ref. 11). The autoradiograph shows proteins extracted from uninfected F-9 cells immunoprecipitated with anti-SV40 T-serum (a); or with normal hamster serum (b); proteins extracted from SV40-infected F-9 cells immunoprecipitated with anti-SV40 T-serum (c); or with normal hamster serum (d); control proteins from SV40-infected AGMK cells immunoprecipitated with anti-SV40 T-serum (e).

The F-9 line of mouse teratocarcinoma cells, a nullipotent stem cell line that does not differentiate in normal culture conditions[7–9], was used. Cells were generally inoculated with high multiplicities of infection (MOI) of plaque-purified wild-type SV40 (50–250 plaque-forming units (p.f.u.) per cell). Although infection of differentiated mouse cells (for example primary mouse embryo or 3T3 cells) with SV40 does not lead to the synthesis of viral DNA or the production of late viral polypeptides, substantial amounts of SV40 large T- and small t-antigens and the early SV40 RNA which encodes these proteins can usually be detected. In contrast, others have shown that SV40 infection of undifferentiatiated teratocarcinoma cells does not result in detectable T-antigen synthesis[1], as assayed by immunofluorescence. To confirm this observation we examined the SV40-infected F-9 cells for SV40 antigens using the more sensitive technique of immunoprecipitation and polyacrylamide gel electrophoresis (PAGE). After labelling SV40-infected F-9 cells with ^{35}S-methionine 17 h post-infection (p.i.), proteins in the soluble extracts were immunoprecipitated either with a T-serum (obtained from hamsters bearing SV40 tumours), or with a control normal hamster serum[10]. The immunoprecipitated proteins were analysed on 20% polyacrylamide-SDS

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gels[11]. As shown in Fig. 1c, SV40-infected F-9 cells do not synthesise either of the known early SV40 gene products (90,000-MW T-antigen and 17,000-MW t-antigen[12,13]). However, two distinct polypeptides with apparent molecular weights of 105,000 and 55,000 were immunoprecipitated with anti-SV40 T serum, both from the SV40-infected and from the uninfected F-9 cells (Fig. 1a,c). These two proteins were not seen in the control reactions with normal serum (Fig. 1b,d), suggesting that although anti-T serum has antibodies against the 105,000 and 55,000-MW proteins, these may not be encoded by SV40. Further analysis of these two proteins will be presented elsewhere. It has been previously shown[14,15] that an antiserum from rabbits which had been immunised with cells from the embryoid bodies of mouse teratocarcinomas contain several distinguishable antibody specificities, including an activity against Py- and SV40-transformed mouse fibroblast lines and a variety of mouse tumour cells. Our results suggest that the two immunoprecipitable proteins (105,000 and 55,000 MW) may represent common polypeptides synthesised in undifferentiated and SV40-transformed mouse cells. The significance and potential relationship of these proteins to tumorigenesis are being investigated.

As we were unable to detect SV40 T-antigens in the infected F-9 cells, we sought to determine whether the inoculated SV40 DNA was being transcribed. From previous studies[16] it was clear that the most sensitive assay for detection of viral transcription is the *in vitro* sarkosyl method[17]. This technique relies on the fact that viral transcriptional complexes (VTCs) which are initiated *in vivo* can be extended (but not initiated) *in vitro*; its sensitivity derives from: (1) the ability to incorporate high specific activity precursors into growing RNA chains; (2) removal of potential processing enzymes which could degrade 'unstable' RNA species; and (3) separation of the viral transcriptional activity in the sarkosyl supernatant fraction from the bulk of the cellular transcriptional activity which resides in the chromatin pellet. Ten hours after infection of F-9 cells (MOI = 250) VTCs were isolated and incubated in a standard reaction mixture[18] containing [α ^{32}P]ATP. (In a separate experiment (S.S., E. May and G.K., in preparation), 10 h p.i. was found to correspond to the peak of viral transcriptional activity in mouse cells.) RNA was purified and annealed with the separated strands of two SV40 DNA fragments (representing early and late regions of the viral genome) which had been immobilised on nitrocellulose filters[19] (Fig. 2). The pattern of hybridisation clearly indicates that viral transcription is initiated in SV40-infected F-9 cells. As the transcriptional activity can be detected in the supernatant fraction, this transcription seems to be initiated, at least in part, on free viral DNA molecules[18]. Finally, the ratio of early strand to late strand transcription (5.5 : 4.5), determined by a densitometric tracing of the autoradiograms, indicates that substantial fractions of viral transcription are initiated on both early and late viral DNA strands. Although it is difficult to quantitate the *in vitro* transcriptional activity specifically, similar amounts of virus-specific RNA have been obtained from VTCs of differentiated and undifferentiated mouse cells (S.S. *et al.*, in preparation).

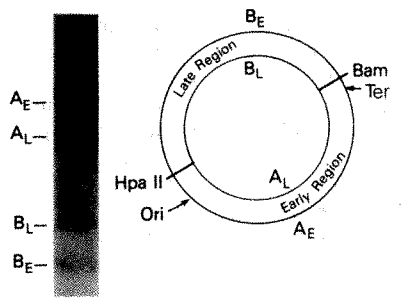


Fig. 2 Analysis of *in vitro*-extended RNA from SV40-infected F-9 cells. Viral transcriptional complexes (VTCs) were prepared using the sarkosyl extraction method[17] from the nuclei of SV40-infected F-9 cells 10 h after infection. After *in vitro* incorporation of [α-^{32}P]ATP (350 Ci mmol^{-1}) into the elongating RNA chains[16], RNA was isolated as previously described[18]. Strand orientation and genomic region of the RNA was determined by hybridisation to the separated strands of *Bam*HI/*Hpa*II-cleaved SV40 DNA immobilised on nitrocellulose filter[19]. Ori, origin of viral DNA replication; Ter, terminus of DNA replication (these sites divide SV40 into the early and late gene regions); A_E = SV40 early coding strand; B_E, early non-coding; B_L, late coding; A_L, late noncoding.

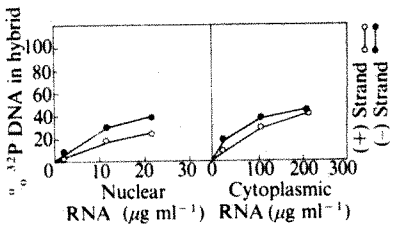


Fig. 3 Analysis of unlabelled *in vivo* viral RNA from SV40-infected F-9 cells. Nuclear and cytoplasmic RNAs were prepared from SV40-infected F-9 cells (250 p.f.u. per cell) 10 h p.i. as described elsewhere[23]. Increasing concentrations of RNA (cytoplasmic and nuclear) were hybridised for 20 h to fragmented ^{32}P-labelled separated single strands of SV40 DNA (specific activity, 1×10^6 c.p.m. μg^{-1}) in conditions previously described[27]; the fraction of SV40 L and E strands in hybrid molecules was determined by hydroxyapatite chromatography[20].

We consequently undertook a second set of experiments in an attempt to detect and characterise viral RNA transcribed *in vivo*. Unlabelled cytoplasmic and nuclear RNAs were purified 10 h after infection of F-9 cells. To determine the proportion of the SV40 genome that is transcribed *in vivo*, increasing concentrations of the cytoplasmic and nuclear RNAs were annealed in solution with separated strands of ^{32}P-labelled single-strand fragments of SV40 DNA. The fraction of each DNA strand in hybrid molecules was determined by hydroxyapatite chromatography[20]. As seen in Fig. 3, the extent of hybridisation between the (−)strand and (+)strand of SV40 DNA and increasing RNA concentrations was almost linear and did not reach a plateau. At the highest RNA concentration used, about 40% of the (−)strand and 20% of the (+)strand fragments appear in duplex molecules. This result supports the VTC study in showing that both early and late strands of SV40 are transcribed in F-9 cells, although the fraction of each strand represented in RNA is not established by this experiment. The paucity of stable SV40 RNAs in F-9 cells prompted a further analysis of these transcripts. As early SV40 RNA seems to be more abundant than late RNA in F-9 cells and only the early transcripts are translated into detectable virus-specific proteins in mouse embryo or 3T3 cells, we focused on an analysis of the early viral RNA. Berk and Sharp have recently introduced an alkaline agarose gel electrophoresis technique which can effectively size and map both co-linear and spliced RNA molecules[21]. ^{32}P-double-stranded DNA probes are annealed with RNA in conditions which favour DNA–RNA annealing. The hybrid species are treated with S_1 nuclease before electrophoresis and the labelled DNA, which can be accurately sized as single-stranded molecules, provides a direct representation of the RNA molecules present. This method has demonstrated the presence of two spliced early SV40 mRNAs which code for T and t antigens[22] (see Fig. 4*a*). We have recently found that the sensitivity of this technique can be amplified if one uses strand-separated DNA probes, thus permitting hybridisation in much less stringent conditions. This modification is crucial to an analysis of transcripts as scarce as those present in SV40-infected F-9 cells.

Both total RNA and cytoplasmic RNA were isolated 10 h after SV40 infection of either differentiated primary mouse embryo (ME) cells or the undifferentiated F-9 teratocarcinoma cells. The cytoplasmic ME and F-9 RNAs were further fractionated into poly(A)-containing (poly(A)$^+$) and non-poly(A)-containing (poly(A)$^-$) fractions. For a probe, ^{32}P-labelled SV40 DNA was cleaved with *Bam*HI and *Hpa*II restriction endonucleases and the early coding single strand of the DNA

fragment mapping from 0.145 to 0.725 (*Bam*HI/*Hp*II A_E) was purified[19] and hybridised with the RNAs described above. After annealing, hybrid molecules were digested with nuclease S_1 and the products were analysed by alkaline agarose gel electrophoresis.

In a control experiment (Fig. 4*a*), the S_1 technique was used to map early SV40 cytoplasmic RNAs from lytically infected monkey kidney (MK) cells. As expected, three bands were observed which measured 0.38, 0.12 and 0.065 SV40 map units. These represent the RNA body coding for T and t antigens, (0.38) and the (0.12) and (0.065) leader segments (Fig. 4*c*).

In contrast, (Fig. 4*b*) the hybridisation of nuclear or cytoplasmic early RNA from SV40-infected F-9 cells produced one band migrating at a position corresponding to 0.52 SV40 units length. This fragment has been positioned at between 0.67 and 0.15 map units and corresponds directly in size and map position with the primary early SV40 transcript found in the nuclei of SV40–infected MK cells (unpublished data). The analysis of RNA from SV40-infected differentiated primary mouse embryo cells is also shown in Fig. 4*b*. Total cellular RNA from SV40-infected ME cells produced two major bands, one corresponding to a non-spliced early SV40 transcript (0.52 SV40 map units) and the second representing the body of the early mRNAs (0.38 SV40 map units). Poly(A)$^-$ RNA contained only the band of the nonspliced RNA while poly(A)$^+$ cytoplasmic RNA contained one band corresponding to the processed early mRNA body. This finding supports previous studies which indicated that polyadenylation of RNA precedes splicing[23,24]. The t (0.12 map units) and T (0.065 map units) leader segments have been run off this gel to obtain a better resolution of the larger bands. The results of this experiment indicate that a spliced early mRNA is synthesised during the infection of differentiated mouse embryo cells by SV40. The detection of the full early transcript in the total cell (nuclear-plus cytoplasmic) RNA as well as the poly(A)$^-$ RNA suggests that the mature cytoplasmic spliced RNAs are derived from this primary transcript. Of particular importance is the detection of the primary transcript alone in the infected F-9 cells. This finding suggests that a major block to SV40 gene expression in F-9 cells is related to the inability to splice the primary transcripts. We cannot exclude, however, the possibility that an additional step in SV40 gene expression, for example the initiation of SV40 transcription, is also depressed in these cells. Nor can we exclude the possibility that other embryonal carcinoma cell lines may have other restrictions to SV40 gene expression. In contrast, spliced early SV40 mRNAs are efficiently produced in differentiated mouse embryo cells. Recent experiments using SV40 deletion mutants[16,25,26] and recombinant DNA molecules (D. Hamer and P. Leder, personal communication) have shown that non-spliced viral transcripts do not accumulate in a stable form. As the undifferentiated cells seem to be unable to splice primary transcripts, this might explain the very low levels of virus-specific RNA in SV40-infected F-9 cultures.

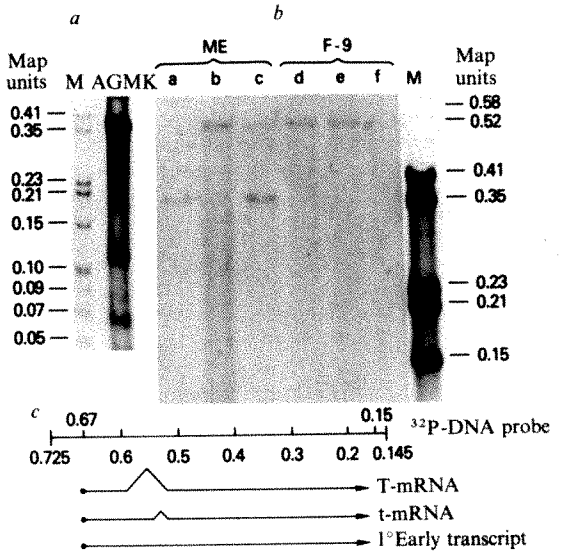


Fig. 4 Sizing of transcripts from SV40 infected mouse embryo (ME) and F-9 embryonal carcinoma cells. Total cellular RNA, poly(A)$^-$ and poly(A)$^+$ cytoplasmic RNAs were extracted from ME and F-9 cells, 10 h after infection with SV40 (250 p.f.u. per cell). These RNAs were separately annealed with a single-stranded ^{32}P-SV40 DNA probe (1.5×10^6 c.p.m. μg^{-1}) extending from map units 0.145 to 0.725 and representing the entire early region of SV40. Preparation of this probe has been described in detail[19]. After annealing for 18 h at 37°C in 50% formamide, 0.4 M NaCl, 0.04 M PIPES buffer (*p*H 6.8), hybrid molecules were digested with an excess of nuclease S_1 (45°C for 30 min) and analysed by alkaline agarose gel electrophoresis[21]. The products of S_1 analysis of hybrids between the early strand probe and poly(A)$^+$ cytoplasmic RNA from SV40-infected permissive MK cells are a control (*a*). Three prominent bands are evident representing the splicons for T- and t-mRNAs (0.065 and 0.12 map units) as well as their 3'-body segment (0.38 map units). *b*, S_1 products of hybrids between the same DNA probe and RNA from SV40-infected ME (a,b,c) or F-9 cells (d,e,f). The RNA preparations included poly(A)$^+$ cytoplasmic RNA (a,d) poly(A)$^-$ total cellular RNA (b,e) and total infected cell RNA (c,f). M, DNA size markers. Map units are given as fractional lengths of the SV40 genome. *c*, The two splices SV40 lytic mRNAs (encoding T and t peptides) and primary early transcript from which they are thought to be processed.

The resistance of F-9 cells to viral infection is not limited to SV40. In addition, polyoma virus[1,6] and ecotopic murine C-type viruses[2,3] are not expressed in undifferentiated, embryonal carcinoma cells. Note that each of these viruses induces spliced mRNAs. A number of other viruses including Sindbis, encephalomyocarditis virus, and vaccinia virus[2] can grow in the undifferentiated embryonal carcinoma cells; but these viruses have not been shown to require splicing for their mRNA maturation. In contrast, the maturation of adenovirus 2 mRNA relies on a splicing mechanism; yet this virus is expressed at low levels in the undifferentiated cells[5]. We therefore suggest that adenovirus may use a distinct processing system in F-9 cells. Such enzymes could be cellular, virus induced or virus coded. The appropriate splicing of SV40 RNA only in the differentiated mouse cells[4] raises the possibility that cell maturation itself is based on the expression of splicing enzymes. It is important to determine if only those mRNAs encoding differentiated functions require splicing, and if the enzymes required for the steps in RNA maturation such as splicing are induced in differentiating cells.

Using the method of Strickland and Mahdavi[8] we have been able to induce the F-9 teratocarcinoma cell line to differentiate. The differentiated cells, after SV40 infection, contain spliced early SV40 mRNAs and synthesise SV40 T-antigen as demonstrated by immunofluorescence and immunoprecipitation (S.S. and G.K., in preparation).

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The relationship of degradative and resistance plasmids of *Pseudomonas* belonging to the same incompatibility group

THE fluorescent pseudomonads include *Pseudomonas* which can cause serious infections in man, and soil bacteria such as *P. putida*. Resistance (R) plasmids have been found in strains of both species and degradative (D) plasmids have been demonstrated in strains of the latter species. Where such plasmids can be transferred by conjugation to a common host strain, it has been possible to establish whether they can stably co-exist. Inability to co-exist (incompatibility) is the basis of their classification[1,2]. It has recently been suggested that both the IncP-2 and IncP-9 groups contain both R and D plasmids[2,3]. In addition the IncP-2-plasmids are both very large[4]. Among R plasmids of enterobacteria, incompatibility is generally an indication of evolutionary relationship in that the DNAs of incompatible plasmids show greater homology with each other than with those of compatible plasmids[5]. However, cases of incompatible plasmids lacking significant homology have also been reported[5,6]. We have examined the extent to which two R plasmids from *P. aeruginosa* and two D plasmids, one from *P. putida* and the other from *P. aeruginosa* (all assigned to the IncP-9 group[3,15,16]) share common sequences. The results suggest that plasmids of the same incompatibility group are not necessarily closely related.

The plasmids used in this study are described in Table 1. Plasmid pMG18 carries the same resistance determinants as R2, plus several additional ones. Plasmids NAH and TOL encode the degradation of naphthalene and toluene respectively. In the course of both degradations, catechol is cleaved and degraded via the *meta* (α-ketoacid) pathway. However, the R plasmids have no known function in common with the D plasmids.

Table 1 Plasmids used in this study

Plasmid	Original host strain	Host strain in this work	Phenotype	Approximate size of plasmid DNA (kilobase-pairs)†	Ref.
R2	*P. aeruginosa*	PU21	CbSmSu	73	7
pMG18	*P. putida*	PU21	CbGmKmSmSuHg	100	8
NAH	*P. putida*	PpG7	Nah	72	9, 10
TOL	*P. putida*	PaW1	Tol	117	11

Abbreviations: Cb, Gm, Km, Sm, Su, Hg: resistance respectively to carbencillin, gentamicin, kanamycin, streptomycin, sulphonamide and Hg^{2+}. Nah, Tol: ability to use naphthalene and ability to utilise toluene and *m*- and *p*-xylenes.

* R2 and pMG18 were transferred from *P. aeruginosa* strain PU21[12], which carries cryptic plasmids[13], to a plasmid-free *P. putida* strain, AC34[14].

† The plasmid sizes were estimated from the sum of the sizes of the fragments derived from *Eco*RI cleavage and resolved on agarose gels. Fragments of λ DNA run on the same gels were used as reference markers.

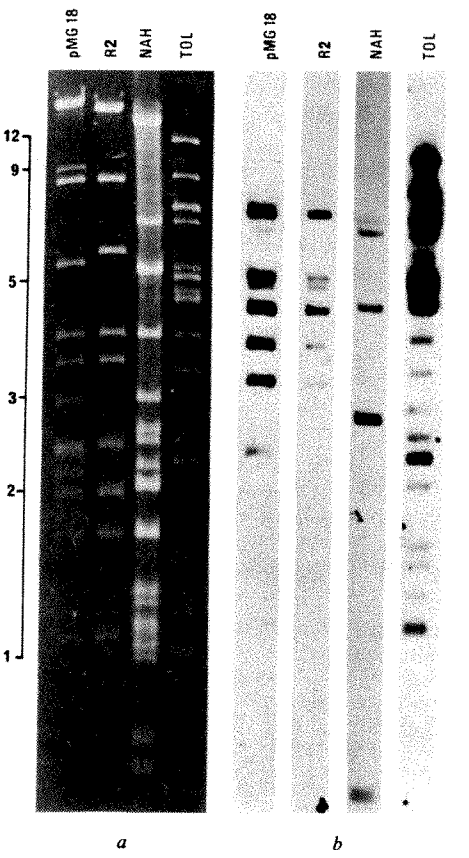


Fig. 1 *a* Banding patterns of *Eco*RI-digested plasmid DNA after electrophoresis on 1% agarose gels and staining with ethidium bromide. *b* Autoradiographs obtained by transferring *Eco*RI-digested fragments of TOL DNA to nitrocellulose filters by the Southern blot technique, and hybridising with the four different ^{32}P-labelled plasmid probes[10]. Probe DNA was labelled by 'nick translation' with [α^{32}P]-dCTP and sheared by sonication to an average length of 500 base-pairs. Hybridisation was performed in Denhardt's solution[20] at 65 °C for 18 h. Autoradiography was carried out for 24 h and then for a further 3 days to reveal weakly hybridising bands. The autoradiographs presented here were exposed for 24 h.

Overall homology was established by DNA : DNA hybridisation on nitrocellulose filters[17]. Plasmid pMG18 contained sequences that were homologous with most, if not all, of R2 (Table 2). The extent of homology in the reciprocal hybridisation (67%) was close to that expected (69%) from this experiment, given that pMG18 is larger than R2 (100 kilobases compared with 73 kilobases).

TOL DNA showed significant homology with both R plasmids (Table 2). Given that the sizes of TOL and R2 are 117 and 73 kilobases respectively, the TOL : R2 hybridisation showed that the two plasmids shared sequences of DNA equivalent to about 11 kilobases (the average of 117×0.11 and 73×0.14). Similarly, we calculated that the DNA sequences common to TOL and pMG18 are equivalent to 20 kilobases (117×0.17 and 100×0.20). However, the R plasmids shared much less homology with NAH; here, the common sequences were only equivalent to about 3 kilobases.

Previous data had suggested that NAH and TOL were related[10]. We found that the extent of this relationship was small. The degree of reassociation of TOL DNA probe with unlabelled NAH DNA was $12 \pm 2\%$, and the reciprocal hybridisation gave $8 \pm 3\%$ reassociation. Thus, we calculated the sequences common to both plasmids as being equivalent to only 9 kilobases (72×0.12 and 117×0.08).

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To localise the related segments of the R and D plasmids and to confirm that the observed homology between NAH and the R plasmids was significant, a series of hybridisations was performed, using radioactively-labelled probe DNAs of the plasmids and Southern blots of endonuclease-generated fragments of unlabelled plasmid DNAs[10].

The close similarity in size between most of the R2 fragments and a series of pMG18 fragments again suggested that R2 was very closely related to pMG18 (Fig. 1*a*). This was borne out by autoradiography, which showed that all R2 bands hybridised with pMG18 probe, and all but three bands in the pMG18 pattern showed homology with R2 probe (data not shown).

In the filter hybridisations described above, pMG18 showed more homology with TOL than did R2. As expected from this result, TOL probe hybridised with more pMG18 bands than R2 (data not shown). These findings were confirmed in reciprocal experiments with pMG18 and R2 probes, which hybridised with the same resolved bands of TOL DNA, plus, in the case of pMG18, several additional bands (Fig. 1*b*). Since these represented small fragments, they were insufficient to account for the difference between 20 and 11 kilobases. However, some of the TOL bands which showed homology with both R plasmid DNAs were composed of more than one fragment, and comparison of the autoradiographs suggested that pMG18 probe was hybridising to a greater degree to some of those bands than did R2 probe.

The results of the NAH hybridisation against TOL DNA essentially confirm those of Heinaru *et al.*[10] for the SAL plasmid, which is very closely related to NAH: that is, homology was largely confirmed to a few bands (Fig. 1*b*). Of these, band 4 in the TOL fragmentation pattern, composed of one fragment species[21], is lost from the Tol$^-$ excision derivative of TOL, pWWO-8 (ref. 22). Only one TOL band showed some homology with all the probes. However, since this band was made up of more than one fragment species, we cannot say if the same fragment hybridised in each case: indeed, the homology could also be in different portions of one fragment.

When each of the R plasmid DNAs was used to probe the NAH digestion pattern, they only hybridised (relatively weakly) with the 5.8-kilobase band. This band was also one of those which hybridised with TOL probe. These findings suggest the existence of a very small 'core sequence' common to all four plasmids. Therefore, for these four plasmids of the IncP-9 group, incompatibility cannot be taken to imply more than a very limited amount of evolutionary relationship.

Table 2 Homology among R and D plasmids of *Pseudomonas* belonging to the IncP-9 incompatibility group

Total unlabelled DNA from strain-carrying plasmid	Percentage of reassociation with labelled plasmid DNA from:			
	TOL	NAH	R2	pMG18
TOL	100±6	12±2	14±3	20±2
NAH	8±3	100±2	5±1	2±1
R2	11±3	4±1	100±3	67±2
pMG18	17±4	6±2	98±3	100±2

Plasmid DNA was isolated by SDS lysis followed by NaCl–SDS precipitation of chromosomal DNA[18] and purified by CsCl-ethidium bromide density ultracentrifugation; it was then labelled *in vitro* with [α^{32}P]dCTP by 'nick translation' (ref. 19), and sheared by sonication to an average size, as determined by agarose gel electrophoresis, of 500 base pairs. The DNA was heat-denatured and used to probe an excess of unlabelled single-stranded whole cell DNA from the same plasmid-carrying strains bound to nitrocellulose filters. The hybridisation was performed at 37 °C for 18 h in 2×SSC/50% (w/v) formamide under paraffin oil[17]. The values shown are the percentages of reassociation (±s.d.) of ^{32}P-labelled plasmid DNA with unlabelled plasmid DNA. Data are of the mean of eight separate determinations, after deduction of the values from control experiments using labelled probe and DNA from the plasmid-free host strains. These control values were about 5% of those for hybridisations with DNA from cells carrying the homologous plasmid.

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Electron microscopic evidence for the circular form of RNA in the cytoplasm of eukaryotic cells

LINEAR single-stranded RNA genomes extracted from several RNA viruses were found to exist in a circular conformation when examined in the electron microscope in partially denaturing conditions[1–4]. The circular conformation of viral RNA presumably arises from base pairing between RNA sequences at the two ends of the RNA molecules. The biological significance of the circular structure of single-stranded RNA is still unknown. Here we report the observation of the circular form of RNA extracted from the cytoplasm of several eukaryotic cells.

Cytoplasmic RNA was extracted from HeLa cells by the procedure of Spradling *et al.*[5]. RNA were dissolved in buffer containing 0.05–0.1 M Tris, 0.005–0.001 M EDTA, *p*H 8.5, and 40–92% formamide, and spread using the basic protein monolayer technique[6]. When examined in the electron microscope, about 1–2% of the molecules could be seen to be in circular form even in highly denaturing conditions (92% formamide, 0.05 M Tris, 0.005 M EDTA, *p*H 8.5). The electron micrographs of some of the circular molecules observed are shown in Fig. 1. RNase treatment completely eliminated these molecules, suggesting that they are RNA molecules. As control, influenza virus RNA and HeLa cell ribosomal RNA were examined. No circular RNA molecules were observed in the electron microscope in the above conditions. 23S and 16S ribosomal RNA extracted from *Escherichia coli* also do not show any circular structure[1]. Therefore, we consider it unlikely that the circular structure observed in HeLa cell cytoplasmic RNA is due to random cross-over of the two ends of the RNA molecules. Furthermore, circular forms were observed even when the molecules were highly stretched by the spreading force.

'Pan-handles' were usually observed in the circular RNA. One of these presumably represents the RNA·RNA duplex formed by the base pairing of the 3'- and 5'-ends of an RNA molecule. Sometimes, the pan-handles exist in the form of 'rabbit ears' (see Fig. 1). That these structures could still be

observed in a highly denaturing condition suggested that these structures have a high degree of sequence homology and a high G·C content. Alternatively, these circular RNAs could be covalently closed as in viroid RNA[7]. The possibility that the circular RNA molecules are due to protein linkage can be ruled out because proteinase K treatment, which we did not use routinely although it is part of the RNA extraction technique[5], did not reduce the number of circles. In circular RNA molecules containing two pan-handles the pan-handles are often at an equal distance from each other.

The length distribution of circular HeLa cell cytoplasmic RNA is shown in Fig. 2. It is interesting that the size distribution seems to be of a multiple integral of 300 nucleotides (~0.22 μm). However, 300 nucleotide-long circles were not observed, probably as they are too small to be seen in the electron microscope.

The circular forms of RNA were also observed in the poly(A)$^+$ fraction of HeLa cell cytoplasmic RNA, although the length distribution is skewed to the shorter range (Fig. 3). Circular RNA can also be seen in RNA extracted from HeLa cell nuclei. However, the amount of circular RNA observed was greatly reduced when the nuclei were washed with detergent and extracted as described by Penman[8]. These results suggest that the majority of circular RNA is probably localised in the cytoplasm and not in the nucleus. Cytoplasmic RNA isolated from CV-1 cells (a monkey kidney cell line) and Chinese hamster ovary cells, and total RNA isolated from *Physarum polycephalum*, were also found to contain similar circular RNA molecules.

The biological significance of circular RNA is unknown. In the case of viral RNA it has been suggested that the pan-handles at the ends of RNA molecules may serve as the initiation site for viral RNA replication[1,2]. The circular RNA that we observed in the cytoplasm may represent cryptic viral RNA or viral RNA in chronic infection. If such were the case, one may be able to detect RNA replication activity in the post-mitochondrial cytoplasmic fraction. On the other hand, circular RNA may represent efficient templates for translation in which the ribosome running off the mRNA template at the 3′-end immediately re-associates with mRNA at the 5′-end to start new initiation of protein synthesis. This model was suggested by Baglioni *et al.*[9] to account for the slow rate of exchange of ribosomal subunits and the stability of mRNA in mammalian cells. Such a model would predict the close association of the cap structure at the 5′-end and the poly(A) sequence at the 3′-end of mRNA molecules. Partial nuclease digestion of mRNA molecules would result in the enrichment of the cap structure in RNA fragments containing the poly(A) sequence. These possibilities require experimental verification.



Fig. 1 Electron micrographs of circular RNA molecules extracted from the cytoplasm of HeLa cells. HeLa cell cytoplasmic RNA extracted according to Spradling *et al.*[5] was precipitated with ethanol at −20 °C and resuspended in 92% formamide, 0.05 M Tris, 0.005 M EDTA, *p*H 8.5, 50 μg ml^{-1} cytochrome *c* and spread on hypophase containing 50% formamide, 5 mM Tris and 0.5 mM EDTA, *p*H 8.5. The RNA molecules were picked up on a pallodron-coated grid and processed for electron microscopy as described elsewhere[10].

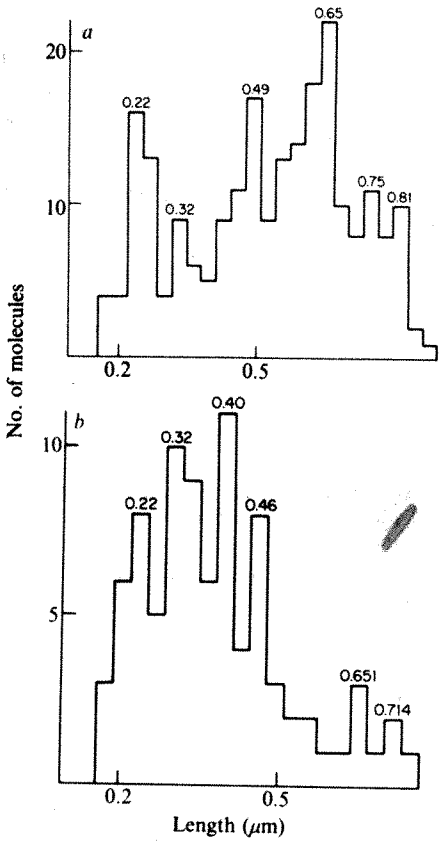


Fig. 2 Histogram of length of HeLa cell total cytoplasmic circular molecules (*a*) and cytoplasmic poly(A)$^+$ circular RNA molecules (*b*).

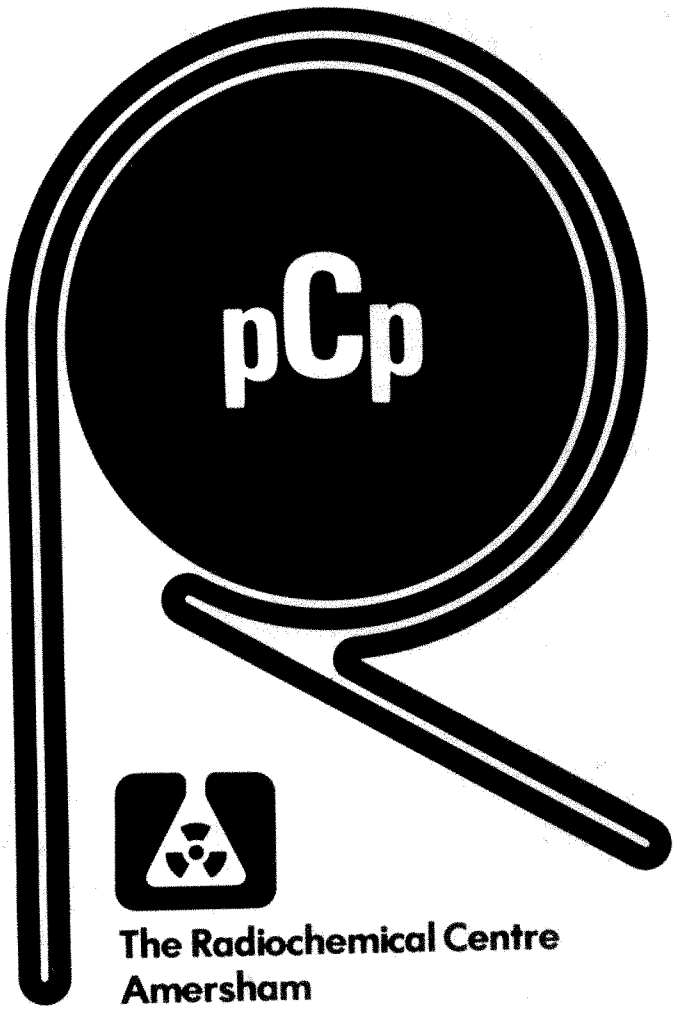
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J. R. Ravetz

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Through four themes, he reviews the great variety of criticisms that have been made of science. The easiest to handle is the first, the claim that scientific explanation is defective as a means of real knowing. The many ambiguities in the concept of "explanation" are sufficient for the fragmentation of any line of criticism. The criticism of the destructive potential of science-based technology merits more consideration. Passmore refuses to allow the shelter of ignorance of consequences claimed by "pure" scientists. For our science is too bound up with technology, both in its present practice and in its basic philosophy as announced by Bacon and Descartes. Although the problem of personal responsibility of the scientist is not easily resolved either way, the collective involvement of science in its applications cannot legitimately be denied.

When he comes to "the scientific spirit", Passmore seems at last to be speaking more from knowledge of man than of books. He coins the useful term "aristoscience" relating to the elite practitioners of the elite fields. For that, the "spirit" requiring analysis is *hubris*. He described very compellingly how this affects the style of scientific work, right down to the self-serving criteria of choice of problems, the setting of inappropriate standards for "immature" sciences, and indeed an indifference to the welfare of ordinary people provided that scientists are well fed.

Finally, he talks of "uniqueness, imagination and objectivity", still rebutting metaphysical attacks but admitting the strength of social criticisms. And this seems to be the verdict, as it emerges towards the end. As a social institution composed of people, science is not immune from any weakness or failing. But as an ideal for knowledge, placing man in a particular relationship to the Universe, science, as the successor to the philosophies of the Renaissance and the Enlightenment, expresses the essential values of our civilisation.

Thus, in a bare hundred pages Passmore has introduced the whole field of criticism of science, including quotes from all the significant authors. Its only defect as an introductory text for students is that the argument flows rather too smoothly from one topic to the next; another edition with sections and break-headlines would much enhance its pedagogical value.

This book was written in response to the crisis of the 1960s; John Passmore might now begin working on the sequel which will be necessary for the 1980s. This epoch has just begun, inaugurated by the nuclear accident at the Harrisburg plant, Three Mile Island, Pennsylvania. In America the Press carried reflective stories with such headlines as "The Nuclear Experts Can't Be Trusted" and "Credibility Crap": "One of the first casualties of the Three Mile Island nuclear accident was scientific credibility . . ." (*Newsweek*, 23 April, 1979). How "scientists" could be involved in the same processes of prevarication, lies and cover-up as the Vietnam or Watergate politicians, is a problem which, I believe, must be considered urgently by those who would understand and defend what is worthwhile in science in the coming period. □

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Hückel theory

Hückel Theory for Organic Chemists. By C. A. Coulson, B. O'Leary and R. B. Mallion. Pp. 182. (Academic: London, New York and San Francisco, 1978.) £9.50; $19.65. *Hückel Molecular Orbital Theory.* By K. Yates. Pp. 371. (Academic: New York, San Francisco and London, 1978.) £20.80; $32.

ORGANIC chemists are beginning to appreciate that an elementary knowledge of molecular orbital theory in its simplest form — Hückel theory — helps greatly in understanding the properties and reactions of organic molecules. These books are not the first to attempt the exposition of Hückel theory for the organic chemist, and they will not be the last; but it is possible that one of them will turn out to be the best and the other the worst.

Charles Coulson was loved by all who knew him, and deeply respected both as a scientist and as a teacher. Hückel's name is given to the theory, but it was Coulson who, first with Rushbrooke and later with Longuet-Higgins, developed it and showed how powerful it could be, for all its simplicity. He lectured on it to organic chemists in Oxford, and in 1971 the lectures were recorded on magnetic tape. Mallion and O'Leary have now transcribed the recorded lectures into print, and although they have been unable to resist the temptation to add some pretty mathematical results of their own, they have confined themselves to appendices and footnotes. The rest is Coulson: precise but not pedantic, simply expressed but not condescending and always honest and good-humoured. One can quibble about a few matters (is the ω technique of any interest now? Is free valence of any use to anybody?) but this is as thorough and readable an account of the principles of Hückel theory as we are likely to see. There are a few misprints, and a few other irritations, such as the use of "less-approximate" to mean "more accurate" and the pervasive hyphenation of adjectival phrases, as in "reasonable (but-not-exactly-correct) supposition", but these are unimportant. The prospective purchaser may be further encouraged by the knowledge that the royalties will go to the Coulson Memorial Fund at Oxford, to support foreign students of theoretical chemistry.

It must be said, however, that this excellent book has limitations in its coverage, and these limitations will be of particular concern to the very people who are expected to read it. The applications of Hückel theory in predicting reactivity are described only briefly, and the more modern ideas which have flourished in the wake of the 'Woodward–Hoffman rules' are not mentioned at all. The book provides an excellent introduction to the basic molecular orbital theory, but it does not completely serve the needs of the modern organic chemist. It is therefore not surprising that the same publisher is responsible for the book by Yates, which seeks to cover much more thoroughly these important new ideas. It is unfortunate, perhaps, that the books arrived for review almost simultaneously, for the virtues of the one emphasise the deficiencies of the other. An example of the difference is given

by the treatment of the Born–Oppenheimer approximation, which is implicit in the Hückel method but need not be made explicit in an elementary treatment. Coulson accordingly does not mention it at all. Yates mentions it twice — once to say that it muust be used, and once to say that it has been used. He does not say what it is. Another example is the treatment of the Coulson–Rushbrooke pairing theorem, which is fundamental to many of the applications of Hückel theory, and important in understanding the unexpectedly wide validity of the theory. Coulson naturally proves the theorem, carefully and precisely, so that there is no doubt about the exact assumptions which are required. Yates has no reference by name to the theorem, either in the index or, as far as I can discover, in the text; and although he could have stated the essence of the theorem, as Coulson does, in a dozen lines, he spreads the statement over several pages, calls it a series of generaliations rather than a theorem, and moreover, by introducing a further weaker generalisation, for which he gives an invalidating counter-example, leaves the reader with the impression that all of these 'generalisations' share the same lack of generality.

Yates claims, with some justice, that organic chemistry students frequently apply important ideas and approaches such as the Woodward–Hoffman rules "without a sufficiently sound understanding of their theoretical basis". Yet he fobs his readers off with phrases like "it can be shown that . . . " or "it turns out that . . . ", even where the result could be derived quite readily. Other arguments are presented in a thoroughly superficial manner. For example, the important class of sigmatropic reactions is dealt with on the basis that "the important molecular orbital of the π framework involved in the migration is considered by Woodward and Hoffman to be the [highest occupied one]". Is the student to take this *ex cathedra* statement as the basis of his sound understanding? There is no discussion of the reasons for the validity of this assumption; nor is there any cross-reference, or index reference, to a later chapter where this class of reaction is studied by another method.

Indeed, there is an irritating lack of cross-references throughout: the reader is continually being told that a certain topic will be treated "later", with no cross-reference to lead him to the right place. The index too, is thoroughly unhelpful: for example, of the four entries under "Woodward–Hoffman rules" one leads the reader to the statement that they will be treated "in the following chapters", and another to the statement that they will be described "later". None of them directs the reader to the full derivation of the rules or even to a statement of what they are.

This lack of consideration for the reader is illustrated in a different way by a sentence in the first chapter, which reads: "For the second molecular orbital . . . a similar treatment would give $E_2 = (\alpha-\beta) / (1-S)$, and since β is generally greater in absolute magnitude then α (and both are negative energy terms) this corresponds to a positive energy level or an antibonding molecular orbital". Now the author has slipped up in his statement (repeated in a diagram) that antibonding orbitals have positive energy. They merely need to have energy greater than α. If the energy had to be positive, and α and β were both negative, then it would certainly be necessary that $|\beta| \gg |\alpha|$. But this is not the case; for with the normal choice of energy scale (adopted implicitly if obscurely on the previous page) α is typically around -15 eV and β around -3 eV. Any attempt to check the relative values of $|\beta|$ and $|\alpha|$ would have revealed this fact, and, eventually, the error about antibonding energies; but the author seems to have been content to state what "must" be true. He has advertised his uncertainty by adding the word "generally", but evidently without contemplating the consequences of $|\beta|$ being less than $|\alpha|$. He also seems to be unaware that it is meaningless in any case to compare values of α and β without specifying the energy datum precisely, as they change in different ways when the datum is changed, and indeed either can be made to vanish by a suitable choice.

Let us then turn back to Coulson, O'Leary and Mallion. They will not take the reader through the important modern ideas in theoretical organic chemistry, but they will provide him with a secure foundation on which to base his study of the more specialised monographs in the field.

A. J. Stone

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Nature-nurture

Intelligence: Heredity and Environment. By P. E. Vernon. Pp. 390. (Freeman: San Francisco and Reading, 1979.) Hardback £10; paperback £5.30.

VERNON sets out to reconcile the conflicting claims regarding intelligence and its transmission and to adopt an eclectic position that gives due weight to all the relevant facts. To this end he presents a critical appraisal of a very large number of studies with admirable lucidity.

Although such eclecticism may sound impersonal, there certainly is no lack of personal involvement on Vernon's part, and it is this as much as anything which gives the book its fascination. In many ways it is an intensely personal statement, from the preface in which he speaks of this book as his last, to the finale in which he mounts an emotive attack on recent trends in childrearing and education and contemplates the breakdown of Western mores and standards.

As the author looks back on the half century during which he himself has made such a distinguished contribution to psychometrics, he clearly perceives that all is not well. To take a specific example, intelligence testing is being banned in some parts of the United States on grounds of cultural bias. Vernon devotes a chapter to effectively countering this accusation. But more generally, psychometrists seem to be an increasingly isolated group, disowned on the one hand by many geneticists and on the other by many developmental psychologists. From the beginning quantification has been the primary goal in psychometrics, and has arguably hindered rather than helped conceptualisation. Vernon argues that "psychometry is quite entitled to use its own brand of operationalism, regardless of philosophical theories of scientific method, provided it works". But does it work?

The bulk of the book is devoted, as the title suggests, to the nature-nurture issue. Vernon makes a plea for scientific objectivity, suggesting that questions of social and political reform are not the business of the psychologist; it is his business simply to supply scientific data. In the context of his argument it is particularly unfortunate that Vernon has been forced to acknowledge (in a prefatory notice) the evidence of systematic fraud having been perpetrated by Burt. Vernon's citations of Burt are exceeded only by those of Jensen, and even given the author's attempts to minimise his dependence on Burt's studies the affair hardly encourages us to accept the impartiality of scientific data.

The book gives detailed coverage of the heritability studies and the criticisms which have been raised against them. Although Vernon offers a stalwart defense of the attempt to estimate heritability, the sheer variety of methodological and statistical doubts raised by critics of the various studies may tend further to undermine the reader's confidence.

Vernon's equivocates on racial differences in intelligence, suggesting that it is highly probable that some genetic differences are involved, but that owing to the confounding of race with environmental differences it does not seem possible to separate their effects. To paraphrase the ancient Zen argument, it may be that the real problem lies not so much in our question being unanswerable, as in our remaining in the state of mind that led us to ask it.

Paul Light

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Advances in metabolism

The Year in Metabolism, 1977. Edited by N. Freinkel. Pp. 448. (Plenum Medical: New York and London, 1978.)

THIS volume is the second in what is proposed to be an annual series, the purpose of which is to review advances in metabolism. The publishers have assembled an impressive list of internationally recognised experts to produce twelve chapters on diverse topics. The appeal of a series like this depends not only on the appropriate choice of subjects;in order to attract the non-specialist, recent advances have to be made intelligible to those without extensive background knowledge. In both respects this volume is largely successful. If there is any unevenness of presentation it is because some of the authors have interpreted their brief literally and dealt only with recent advances, whereas others have written general reviews with extensive references to the earlier literature.

The review by Murad and Aurbach on cyclic GMP metabolism, particularly in relation to disease states, is timely and draws together data which is not readily to be found elsewhere. The chapter by Fajans on diabetes mellitus provides a useful account of genetic aspects and the linking of susceptibility with the HLA system and a possible viral aetiology. Insulin resistance is also discussed together with various aspects of treatment, and there is a good account of possible pathogenic mechanisms in the development of vascular complications.

Another chapter contains an authoritative account of glucagon and somatostatin by Unger. Topics discussed include structure-activity relationships, control of secretion and the bi-hormonal (glucagon and insulin) hypothesis of diabetes mellitus. Inevitably there is some overlap with the chapter by Feligand Koivisto on body fuel metabolism, in which the inter-relationships of glucagon, insulin and somatostatin are considered. Hirsch writes about obesity, discusses what is new and concludes not much. His philosophical discussion of causation and treatment ends on a pessimistic note for the sufferer. Lipids and lipoproteins are discussed by Goodman who selects highlights from a complex subject with care.

Rosenberg's chapter on amino acid discorders also selects a few topics for discussion, including phenylketonuria, the glutamyul cycle and oxoprolinuria. He also gives a fascinating account of the metabolic mechanisms underlying Jamaican vomiting sickness, which is due to the presence of the unusual amino acid, hypoglycin, in unripe ackee fruit.

Seegmiller offers an extensive and detailed account of how human disorders of purine and pyrimidine metabolism are linked to an immunodeficiency syndrome. Possible mechanisms are discussed.

The topic of divalent ion metabolism is covered expertly by Coburn, Hartenbower and Kleeman, who draw attention to how recent advances in vitamin D metabolism are enabling some of the clinical disorders to be better understood. The complex inter-relationships between PTH, vitamin D and calcium, phosphate and magnesium metabolism are summarised. Winick deals with the topic of nutrition, growth and development with particular emphasis on malnutrition, genetic obesity and nutrition in pregnancy. Finally, Williams provides a short but useful review of renal stone disease, and Lieber reviews the metabolism and metabolic actions of ethanol.

The same group of authors have been recruited to contribute to future volumes and this may introduce some difficulties in producing a wide enough variation in subject matter in future editions. It will be interesting to see whether the publishers and authors can maintain the same high standard to be found in this volume.

The book should appeal to a wide audience which should include biochemists concerned with human disease and teaching medical students, as well as endocrinologists and physicians with interests in metabolic disorders.

R.G.G. Russell

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Disorder in crystals

Disorder in Crystals. By N. G. Parsonage and L. A. K. Staveley. Pp. 926. (Oxford University Press/Clarendon: Oxford, 1979.) £28.

IN the hierarchy of matter — gas, liquid, crystal — crystals signify order, pattern; they represent classical discipline as against romantic anarchy. "Damn braces, bless relaxes!" cried that arch-romantic, Blake. The authors of this impressive text might have taken Blake as their mentor; their crystals are for ever relaxing from a state of Roman order into one of Italian disarray; same place, changed ways.

The book is about the various ways in which crystals can lose perfect regularity without ceasing to be crystals: it is a study of local variability superimposed on a basic periodicity. The authors in their Introduction (which neatly summarises the argument of the book) distinguish three types of disorder: disorder of position, orientational disorder and magnetic disorder. These might respectively be exemplified by Cu_3Au, where copper and gold atoms are apt to trespass on each other's appointed lattice sites; NH_4Cl, in which a triad axis of the $(NH_4)^+$ ion can point in different defined directions and the ion itself can execute a click-stop rotation about a triad; and $CsCoCl_3.2H_2O$, containing one-dimensionally ordered antiferromagnetically coupled chains of tilted 'Ising spins'. Any chemist, physicist or metallurgist, however expert he may be in one or other of these very broad categories of disorder, will discover a great variety of parallels to and variants of the particular kind of behaviour he is familiar with. The book is a remarkable achievement of synthesis.

The text begins with a detailed treatment of the thermodynamic and statistical mechanical approaches to phase transformations and degrees of order: it is a mark of the combined rigour and flexibility of the authors that they do not agonise about the propriety of classifying order/disorder transformations as phase transformations. The Ising lattice theory in various dimensions receives a full historical survey, as do the various thermodynamic approaches to the order of transformation; the concept of the λ transformation is frequently cited throughout the book. Even such a technical subtlety as the bond percolation problem (if water be imagined capable of percolating along one type of bond only in a crystal, what proporton of the atoms would be wetted if the crystal were bathed in water?) gets due attention.

There is next a useful chapter on experimental techniques, such as thermochemical measurements, diffraction, NMR, IR and Raman spectroscopy, dielectric permittivity, dispersion and loss measurements, and others. The aim is to explain what kinds of information the various methods can provide, and why; no attempts are made to describe hardware or practical difficulties.

The authors then settle down to particular materials, starting with positional order in alloys. As a metallurgist, the reviewer can confirm that this chapter is reasonably up to date and quite clear in classifying and exemplifying the types of positional order. No attention is devoted here, and little elsewhere in the book, to the kinetics of order–disorder transformations, which is a major topic of current metallurgical concern; and microstructure — for instance, the curious structures resulting from spinodal decomposition — is not considered. Again, some important concepts are not explained but simply used: the notion of 'antiphase domain' is an example. This omission of

definitions happens at intervals in both the theoretical and experimental parts of the book but, given its ambitious scope, this could hardly have been avoided.

The next five chapters (432 pages) constitute the heart of the book. The first, dealing with positional disorder in inorganic compounds, covers in particular the currently important topic of superionic conductors such as an allotrope of AgI, and beta-alumina. (The authors say of AgI that "this singular crystal has often been described as consisting of fluid silver ions in a solid lattice of iodide ions, and even as a 'missing link' between the solid and liquid states".)

A long and illuminating chapter follows on orientational disorder in salts, pride of place going to ammonium salts and including a treatment of ferroelectric salts. This is followed by a clear disssection of the exceedingly involved polymorphs of ice, most of them created by high pressure, and the various descendants of Pauling's statistical mechanics of hydrogen bonds in ice are described.

Two chapters follow on molecular solids, with special emphasis on compounds (such as perfluorocyclohexane) which contain both fixed and labile constituents. Such compounds, called 'rotator phases' or 'plastic crystals', in which some ions exhibit extreme orientational disorder, have remarkable properties such as extreme softness and very rapid diffusion, at appropriate temperatures. Hydrogen and deuterium, various linear and non-linear molecules, and many other groups are analysed, and the chapter finishes with a valiant attempt to systematise the statics and dynamics of these variegated molecular groupings. The concept of a glassy crystal (in which disorder of ions on a sublattice is frozen in on cooling) is introduced. Long-chain monomer molecules are treated quite fully, but degrees of order in polymers are not discussed at all. A special feature in these chapters is the close attention paid throughout to the implications of thermal analysis and, in particular, of the entropy changes associated with order transformations.

A further chapter discusses clathrates (compounds such as β-quinol with large near-spherical structural voids that can be partially filled with 'guest compounds', which behave as independent non-interacting parasites on the host), channel compounds of urea and thiourea, and the metallic intercalates of graphite studied in recent years. An example of what can be deduced from entropy measurements is the authors' conjecture that cyclooctane molecules caught in thiourea channels undergo conformational changes, limited by the channel geometry, at sharply defined temperatures. It would be intriguing to know how polymer chains would behave if constrained within such a host.

The final chapter deals with inorganic magnetic compounds, first in quantum-mechanical generality and then in taxonomic and specific detail. The reader is taken through the luxuriance of phenomena such as ferromagnetism, antiferromagnetism, spin waves, metamagnetism, helical spins, and their hybrids.

After this plethora, it is only proper to outline what the authors have omitted. As already mentioned, polymers are excluded. There is no reference to liquid crystals, that other missing link, which is a pity since, in terms of statistical mechanics, they show a temperature dependence closely similar to that of the Bragg–Williams theory of positional order in alloys or the standard theory of corresponding ferromagnetic states. These omissions are not explicitly justified; other omissions, such as a treatment of the structural implications of non-stoichiometry, or ordering of point, line or planar defects, are made explicit in the Introduction, on the grounds that they have been well covered elswhere.

One can quibble at the margins about omissions and undefined concepts, but the final impression left is of a *tour de force*, a synthesis which has no competitor. The book deserves the close attention of adventurous inorganic and solid-state chemists, solid-state physicists (especially theoreticians) and physical metallurgists. The printing is by facsimile reproduction of typescript, but is quite clear, and in view of its size and scope the book is not overpriced.

R. W. Cahn

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Pollination ecology revived

The Principles of Pollination Ecology. Third edition. By K. Faegri and L. van der Pijl. Pp. 244. (Pergamon: Oxford, 1979.) Hardback £12.50; paperback £7.50.

POLLINATION ECOLOGY is certainly not a new subject, having developed essentially during the eighteenth century and having been given new impetus by the work of Darwin in the nineteenth century. But it is a subject in which interest has lapsed somewhat until relatively recently, perhaps because its study has demanded patient and precise observation rather than more fasionable sophisticated experimentation. It may well be that this very fact is leading to a resurgence of interest in the subject, particularly on the part of the very considerable army of amateur bontanists in Britain. Already the energies of this eager body have been channelled into the detailed mapping of the British flora and now, under the auspices of the Botanical Society of the British Isles, information is being sought regarding the insect visitors to flowers.

In this academic climate, it is apt that Faegri and van der Pijl should launch a third edition of their classic survey of pollination ecology. The last edition was published in 1971 and since that time a great deal of new work has appeared in the literature. In their current bibliography about 30% of references date from this period, and this will indicate the extent of textual revision.

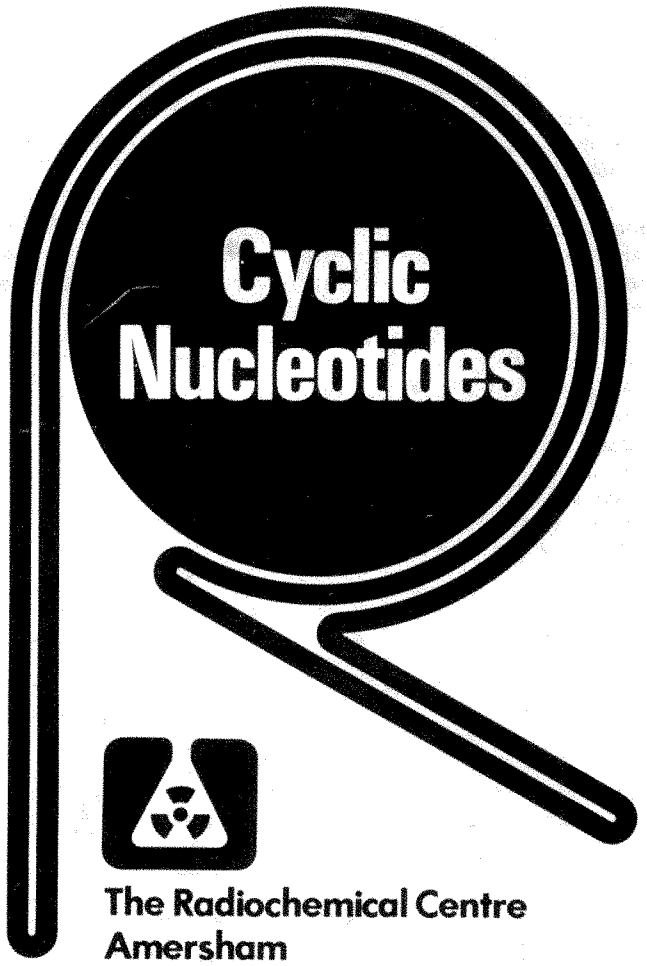
The book has retained its original arrangements, with chapters on general principles of abiotic and biotic pollination, attractants, pollination and speciation, and so on; and then a series of detialed and illustrated case studies of particular plant species. The latter are particularly informative, although the drawings are sometimes so heavily stippled that structural clarity is lost. Simple line drawings would have been preferable.

In general, the text is turgid, probably because it is so heavily packed with examples and references. Although the bibliography is extensive and valuable, perhaps the book could have been improved from the point of view of undergraduate teaching if fewer examples had been dealt with in greater detail. The text generally lacks the diagrams that could have made many points more easy to graps. Frequently an unreasonable familiarity with the literature is assumed and one is referred to the "well-known case of . . ." without further explanation. The full benefit of the text can certainly be obtained only with the backing of a good reference library.

In comparison with Proctor and Yeo's *Pollination of Flowers* (Collins: London, 1973) which, rather surprisingly, is not referred to in the bibliography, this book excels in its crystallisation of fundamental principles from a plethora of facts and observations, but falls short in its coverage of insect adaptations and in the quality of its illustrations. It is, however, a book which should stimulate further interest and research in an area of biology where plants and animals tend to interact to mutual benefit. For this reason alone it is a sadly neglected aspect of ecology which could prove a fruitful subject for practical teaching in undergraduate ecology courses. This book may lead to such developments.

Peter D. Moore

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The papers here deal with all these approaches, and also point out the many gaps remaining in our understanding of peptides. The book thus gives a timely review of the current state of research, and will be invaluable to anyone already involved in the area.

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Fair's Fair.

The Institution of Professional Civil Servants is in dispute with the Civil Service Department over pay – or, rather, over the principles upon which rates of pay are agreed.

The Institution's members in the Professional and Technology grades are being invited to accept a change in criteria which will lead to an effective cut in their relative salaries and a real reduction in their standard of living. This is unfair.

The following information is intended to explain why the Institution, its National Executive Committee, and its members feel it right to resist this unfairness by every legitimate means.

Who is affected?

About 65,000 civil servants whose training, experience and qualifications in work of great value to the nation have, for a number of years, been rewarded on the basis of pay principles of considerable simplicity (as we shall explain below).

They are, for example, architects, chemists, engineers, surveyors, and technologists of every kind. They work at Royal Ordnance Factories, in the Royal Dockyards, at the Clyde Polaris Submarine Base, at Atomic Energy Research Establishments, at Scotland Yard, at Heathrow, at the Royal Aircraft Establishment at Farnborough, at Aldermaston, in the Health and Safety Executive, in the Royal Mint and hundreds of other places where work vital to the nation is carried out. Useful people, you might think. Worth paying properly, too.

What were the principles up to now?

Very simple in essence. Comparison with similar jobs in private industry is the agreed method of settling the pay of most civil servants. So our members' work was compared with that of similar staff in seventy-five organisations outside the Civil Service. From these outside pay rates an agreed "median" salary (i.e. the middle one of the whole range of salaries) can be obtained.

This is conventionally used in most Civil Service pay settlements. However, because of the special nature of our members work it has always been accepted that they deserved "pay somewhat above the average" (to quote the Report of the Royal Commission on the Civil Service). This has been agreed time after time by the Civil Service Department and confirmed by the Civil Service Arbitration Tribunal as is shown in the following table:

1965 pay agreement	**medians + 11%**
1969 pay agreement	**medians + 13%**
1972 pay agreement	**medians + 15%**
1974 arbitration award	**medians + 20%**
1975 pay agreement	**medians + 13%**
Contrast that history with the situation in 1979:	
Civil Service Dept offer:	**medians + NOTHING!**

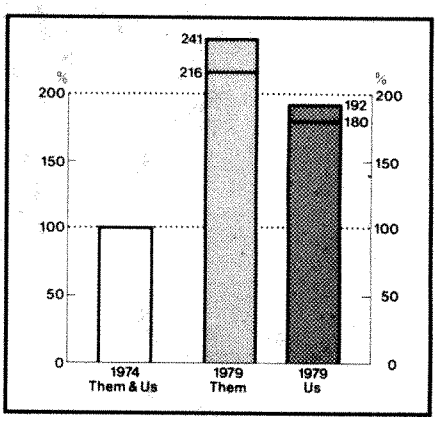
You will immediately conclude that, obviously, there must be a reason. But the Institution of Professional Civil Servants cannot imagine what it can be; nothing has changed since previous settlements. And the Civil Service Department won't tell us. Perhaps they can't imagine what it could be either!

What do the figures really mean?

The easiest way to show why our members are so upset is to compare the 1979 offer with the salaries of those who are used as comparisons outside the Civil Service.

The 1974 salaries of those outside the Civil Service and those inside are represented by the 100% bar in the left hand column of the chart. That is the year of the Civil Service Arbitration Award. In 1979, those outside the Civil Service are being paid between 216% and 241% of their 1974 salaries. That is represented by the lightly dotted bar. (During the same period earnings generally as measured by the official index have moved by 240%.) Those inside – our members in Professional and Technology grades – are being offered between 180% and 192% of the true value of the 1974 award. That is the darker bar in the right hand column.

You see why we describe this as "effectively a cut in salary" and a positive cut in living standards.



Why not arbitrate?

A natural question – especially in view of the fairness of the 1974 award. But arbitration is only right when the processes of negotiation are exhausted. The Civil Service Department will not even start negotiations on the issues in dispute.

What should be done?

There is a simple way to bring the dispute to an end. The Institution is very ready to accept that an independent conciliator should bring the two parties together and assist them towards a solution. Isn't that a responsible attitude and shouldn't the Government respond?

You may now see why our members feel that they have been attacked by their employers and therefore have to defend themselves against what by any standards is unfair treatment. But the Civil Service Department is not really our employer – the Government is! And it represents the whole nation. It represents you.

DoYOU want a fair settlement?

Further information from
The Institution of Professional Civil Servants,
Northumberland Street, London WC2N 5BS.

IPCS

Circle No. 14 on Reader Enquiry Card.

announcements

Awards

Robert Koch Prize in the Medical Sciences has been awarded to **Prof. Ruth Arnon** of the Weizmann Institute's Chemical Immunology Department and to **Prof. Peter Starlinger,** Director of the Institute of Genetics at the University of Cologne.

The Charles Hatchett Award and Medal of The Metals Society has been jointly awarded to **Dr Gilbert R. Speich** and **Donald S. Dabkowski,** both of U S Steel Corporation Research Laboratory.

The Chemical Society Awards for 1978 have been presented to: **Professor G.C. Bond** (Brunel University), **I. Jones** (Sir Hugh Owen School); **Professor C.F. Cullis** (The City University, London); **E.A.V. Brewester** (Davy International, London); **Dr K.U. Ingold** (National Research Council, Canada); **Professor E.A.V. Ebsworth** (University of Edinburgh); **Professor A.R. Battersby** (University of Cambridge); **Professor M.F. Lappert** (University of Sussex); **Professor D.A. King** (University of Liverpool); **Dr. R. Freeman** (University of Oxford).

Dr Trevor Cole CSIRO Division of Radiophysics has been jointly (with **Dr M.G. Clark** of the Clinical Biochemistry Unit, Flinders University Medical School) awarded the Edgeworth David Medal of the Royal Society of New South Wales.

Candidacy to The Harkness Fellowships is open to men and women in any profession or field or study, who are citizens of the UK and have received both secondary schooling and further eduction (or equivalent professional experience in lieu of further education) wholly or mainly in the UK. Candidacy is not open to persons who, between their 19th birthday and 1st September 1980, will have spent more than six consecutive months in the U S. Candidates must be between 21 and 30 yr of age on 1st September 1980, unless qualified in medicine or employed in the Civil Service or the media, in which cases the upper age limit is 33. By that date they must have a degree; *or* an equivalent qualification conferred by a professional body; *or* an outstanding record of achievement in the creative arts, journalism or other comparable career. Within the given age range, preference will be given to younger applicants. Applications: send a s.a.e. carrying 16p postage, and measuring not less than 10 inches by 7 inches, to The Harkness Fellowships (U K), Harkness House, 38 Upper Brook Street, London W1, UK.

The John Eggert-Prize-Foundation will be offered for the fourth time in 1980. Young scientists are encouraged to submit papers from the field of Imaging Science. Imaging Science is meant to embrace a wide field, comprising Optics, Photography with and without silver halides, digital and electronic methods of Image Recording, Analysis and Processing as well as the Physiology and Psychology of Vision. Applications with complete literature references in duplicate should reach Prof. Dr W.F.Berg, at the Dept of Photography, Swiss Federal Institute of Technology, CH-8092 Zürich, no later than 15 October 1979.

Meetings

4-11 August, **STSA/SISCON 1979 Summer Conference,** Coleg Harlech (Chris Prior, Dept of Complematary Studies, The Polytechnic, Huddersfield, UK).

20-25 August, **3rd International Conference on Surface and Colloid Science,** Stockholm (Conseil de l'Europe, F-67006 Strasbourg Cedex, France).

25 August - 9 September, **25th World Vegetarian Congress,** Loughborough (Philip Brown, 53 Marloes Rd, London W8, UK).

12-14 September, **Symposium on the Gunong Mulu National Park Sarawak,** London (A.C. Jermy, c/o Mulu Office, Royal Geographical Society, 1 Kensington Gore, London SW7, UK).

27-28 September, **Vegetables for Feeding People and Livestock,** London (Institute of Biology, 41 Queen's Gate, London SW7, UK).

8-10 October, **6th Biennial Symposium on Turbulence,** Missouri (G.K. Patterson, Chemical Engineering Dept, University of Missouri-Rolla, Missouri 65401).

9-10 October, **Instrumentation in Potentially Explosive Atmospheres,** London (Mrs R.G. Keiller, Sira Institute Ltd, South Hill, Chislehurst, Kent, UK).

11 October, **Personal Protection against Air and Surface Contamination,** London (Professor J.H. Martin, Dept of Medical Biophysics, Blackness Laboratory, University of Dundee, UK).

12-15 October, **5th EMBO Annual Symposium Nucleic Acid - Protein Interactions,** Heidelberg (Dr J. Tooze, EMBO, Postfach 1022.4, 69 Heidelberg 1, FRG).

16-18 October, **30th Annual Fall Meeting of the American Physiological Society,** New Orleans (Steven K. Herlitz, Inc. 850 Third Ave, New York, New York 10022).

18 October, **Process Development Technology Symposium,** London (The Institution of Chemical Engineers, Geo. E. Davis Building. 165-171 Railway Terrace, Rugby, UK).

22-25 October, **RECLAN 79,** Eastbourne (The Conference Secretariat, Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).

18-20 December, **Receptors at Cell Surfaces,** London (Dr D.A. Rees, Unilever Research, Colworth Laboratory, Sharnbrook, Bedford, UK).

13-15 January 1980, **6th Annual Meeting of the International Embryo Transfer Society,** Denver 9 Mrs S. Seidel, IETS, 3101 Arrowhead Rd, LaPorte, Colorado 80535).

21-24 January, **Research on the Environmental Fate and Effects of Drilling Fluids and Cuttings,** Florida (1629 K Street, NW Suite 700, Washington D.C. 20006).

4-6 March, **Petroleum Geology of the Continental Shelf of North-West Europe,** London (The Institute of Petroleum, 61 New Cavendish St, London W1, UK).

17-18 March, **Modelling Dispersion of Transport Pollution,** Cambridge (The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex, UK).

23-26 March, **EURIM 4,** Brussels (Conference Organiser, Aslib, 3 Belgrave Square, London SW1, UK).

24-27 March, **Magnetic Fluids,** Florida (Dr M. Zahn, Dept of Electrical Engineering, University of Florida, Gainesville, Florida 32611).

8 April, **Doping in Sport,** London (Dr D. Simpson, Analysis for Industry, Factories 2/3 Bosworth House, High St, Thorpe-le-Soken, Essex, UK).

8-12 April, **World Biomaterials Congress,** Baden (Mrs E. Maurer, Medical Academy of Vienna, Alser StraBe 4, A-1090 Vienna, Austria).

9-11 April, **Annual Chemical Congress,** Durham (Dr J.F. Gibson, The Chemical Society, Burlington House, London W1, UK).

14-17 April, **Low Energy Ion Beams-2,** Bath (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

15-18 April, **Communications Equipment and Systems,** Birmingham (IEE, Savoy Place, London WC2, UK).

21-25 April, **2nd International Congress on Phosphorous Compounds,** Boston (IMPHOS, 8, rue de Penthievre, 75008, Paris, France).

29 April - 1 May, **Effective Use of Electricity in Buildings,** London (IEE Conference Dept, Savoy Place, London WC2, UK).

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Tetrahedron Reports. No. 55: Some Aspects of Organometallic Chemistry of Nontransition Metals. By Oleg A. Reutov. Pp. 29. No. 56: The Chemical Synthesis of Oligo- and Poly-Nucleotides by the Phosphotriester Approach. By Colin B. Reese. Pp. 37. No. 57: Nucleophilic Addition to Organotransition Metal Cations Containing Unsaturated Hydrocarbon Ligands. A Survey and Interpretation. By Stephen G. Davies, Malcolm L. H. Green and D. Michael P. Mingos. Pp. 31. No. 58: Radicaux Amidyl: Structure et Reactivate. Par P. Mackiewicz et R. Furstoss. Pp. 20. No. 59: From Glucose to Aromatics — Recent Developments in Natural Products of the Skikimic Acid Pathway. By Bruce Ganem. Pp. 31. (Oxford and New York: Pergamon Press, 1979.) £4.95; $10 each. [235

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Alfred P. Sloan Foundation. Report for 1978. Pp. vi + 82. (New York: Alfred P. Sloan Foundation, 630 Fifth Avenue, Rockefeller Center, 1979.) [315

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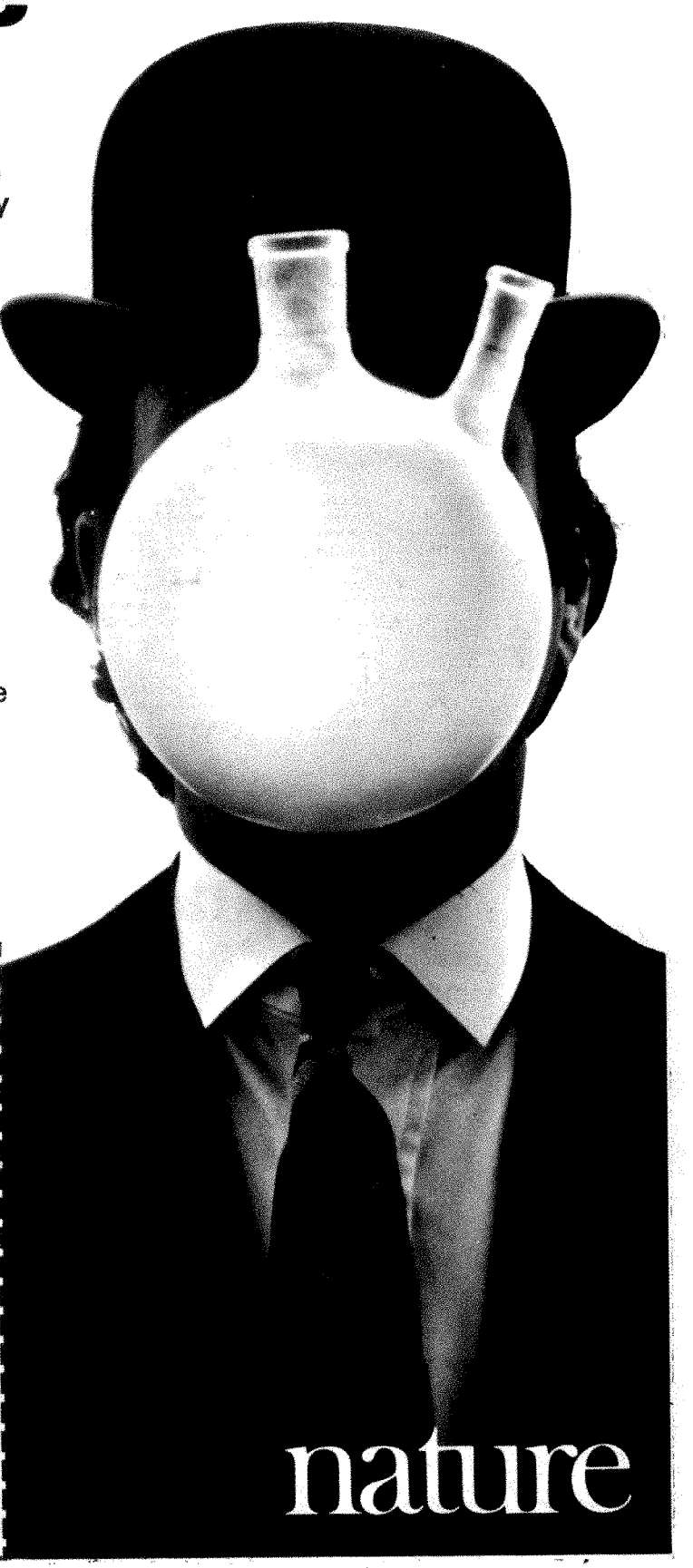
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APPOINTMENTS VACANT

CHURCHILL HOSPITAL RESEARCH INSTITUTE (University of Oxford)

Vacancies exist for two RADIOBIOLOGISTS

in an expanding research group that is examining the biological effects if ionising radiations on a number of normal tissues and organs. These investigations are primarily aimed at improving the treatment of malignant disease by radiotherapy with both conventional photon therapy and fast neutrons. Post suitable for persons with higher degree or equivalent research experience. Responsibilities will depend on the interests and experience of the successful applicants although preference will be given in the case of one vacancy to someone with a qualification in Physics or experience in radiation dosimetry.

Further details can be obtained from Dr J W Hopewell, Churchill Hospital, Research Institute, Headington, Oxford, to whom application should also be sent with the names of two referees by 20th August, 1979. 2670(A)

UNIVERSITY OF WISCONSIN — MADISON

DEPARTMENT OF BACTERIOLOGY

FACULTY POSITION

ASSISTANT PROFESSORSHIP

Available 1980. Applicants with research interests related to host-parasite or cell-cell interactions and a broad background in general microbiology will be considered. Teaching responsibilities could include development of a modern course dealing with interactions between micro-organisms and higher organisms. Individuals with postdoctoral training are invited to send their vitae, most significant reprints, a short statement of projected research plans and the names of three references to Dr J Gregory Zeikus, Department of Bacteriology, University of Wisconsin, 1550 Linden Drive, Madison, WI 53706. An affirmative action/equal opportunity employer. Deadline 1 November 1979. W247(A)

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PATHOLOGY

MICROBIOLOGIST -RESEARCH ASSISTANT.

A vacancy exists for a graduate research assistant in a project to investigate Chlamydia and their role in genital infection. The person appointed will be expected to take a major part in developing immunological methods for detecting chlamydial antigens and antibodies. Work will involve growth of chlamydia in tissue culture, purification, raising antisera and their use in recently developed tests. The project is for 2 years under Professor R.R.A. Coombs. Research experience is essential preferably in virology and/or immunology. Preference will be given to candidates with a PhD. The age range is 24-26 the salary £4,232 to £4,776.

Applications in writing to The Superintendent, Department of Pathology, Tennis Court Road, Cambridge, giving the names and addresses of two referees. 2686(A)

UNIVERSITY OF CALIFORNIA San Francisco

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

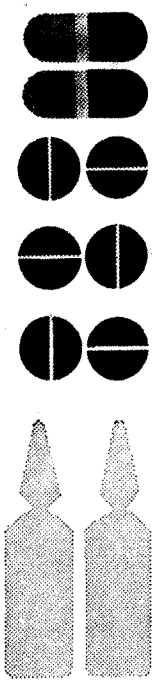
TWO POSTDOCTORAL POSITIONS

(i) A position is available immediately for study of liposomes as *in vivo* drug carriers. Experience in biochemistry, physiology or pharmaceutics desirable.

(ii) A position is available immediately for study of drug interactions at the metabolic level using a perfused liver system. Experience in analytical techniques, medicinal chemistry and/or pharmaceutics desirable.

Salary range: $13,000 to $15,000 for one to two years.

Send résumé, summary of past research and names of two references to Dr C Anthony Hunt, Department of Pharmaceutical Chemistry, S926-S, University of California, San Francisco, CA 94143. W258(A)



Pharmacologists

Following the appointment of Professor D. W. Straughan BSc, PhD, MB, LRCP, MRCS, as Director of Pharmacology, the Greenford Division of Glaxo Group Research Limited is expanding its research programme directed towards the development of new drugs acting on the central nervous system. Several vacancies exist for young and enthusiastic pharmacologists with a good understanding of current concepts relating to the functioning of neuro-transmitter systems and the effects of psycho-active drugs on them.

It is intended that some positions will be filled by new graduates with good degrees who wish to develop their interests in these fields, but there will also be opportunities for people with some previous research experience, particularly of techniques used in neurochemistry, behavioural pharmacology or neurophysiology.

We offer competitive salaries and excellent conditions of employment, including pension and bonus schemes, a subsidised cafeteria and a sports and social club.

Please write, or telephone, for an application form to: Mrs C. A. Carter, Personnel Officer, Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex.
Tel: 01-422 3434, ext. 11, quoting ref: U/273.

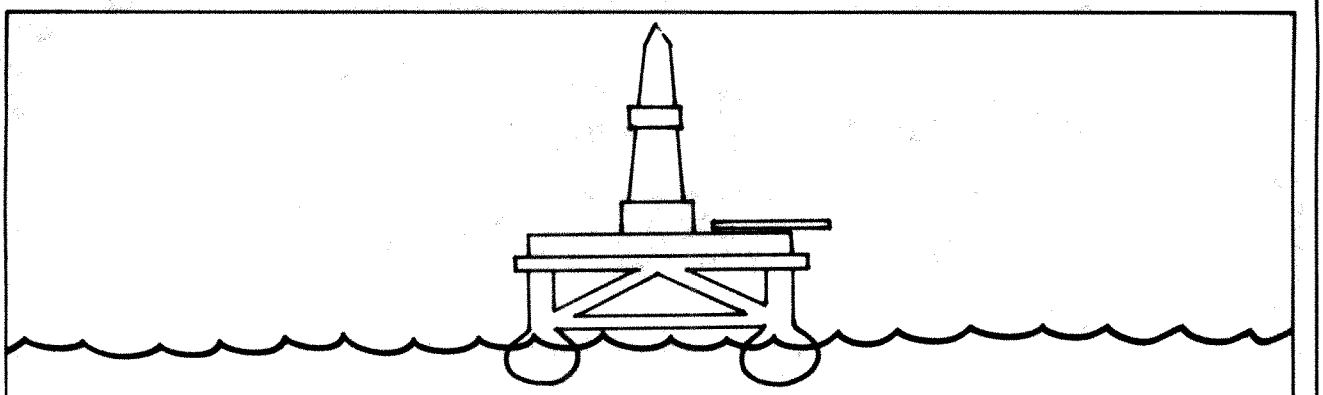
Glaxo Group Research Ltd.

2708(A)

THE UNIVERSITY OF THE SOUTH PACIFIC

Applications are invited for the post of SENIOR LECTURER/ LECTURER IN AGRICULTURAL ECONOMICS (POST 79/75).

This post relates to the area of agricultural macroeconomics, or the economics of agriculture as an industry. Qualification should be a masters degree or higher with specialization in agricultural economics with several years of teaching and research experiences in this general area preferably in developing countries. Salary scales Senior Lecturer: F$11960 to F$14135 pa Lecturer: F$8175 to F$11563 pa (£1 sterling = F$1.81). The British Government may provide salary supplementation in the range £1,842 to £2,796 pa (sterling) for married appointee and £444 to £1,164 pa (sterling) for single appointee (reviewed annually and normally free from tax) and provide childrens' education allowance and holiday visit passages. Family passages; 15% gratuity of basic salary superannuation scheme; appointment allowance; partly furnished accommodation at a maximum rental of 12½% of salary (under review); 3 year contract. Detailed applications (2 copies) with curriculum vitae and naming referees to be sent direct to the Registrar University of the South Pacific, P O Box 1168, Suva, Fiji, by 7 September 1979. Applicants resident in the UK should also send one copy to Inter-University Council 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 2648(A)



Exploration Geologists & Geophysicists

Upgrade your prospects

A further expansion in North Sea Oil exploration is just beginning and our client, a major international oil company, is about to expand on their already substantial interests in this oil province.

Being the expert you undoubtedly are, you possess the specialist knowledge our client seeks for further development of their challenging oil and gas exploration and development programmes. May we assume you are interested? Then let us tell you more.

From the impressive results already achieved, our client is recognised as being at the forefront of North Sea exploration techniques and integrated geological/geophysical sub-surface interpretation. As a major operator with substantial interests both on and offshore, they are now expanding to embrace a number of new projects.

Here is a unique opportunity to apply your own experience and accumulate further knowledge of exploration techniques with one of the foremost exploration groups in the world. Initially, we are talking about working on U.K. acreage, but international careers are available depending upon your own efforts and personal ambition. Opportunities for career advancement into senior management positions both at home and overseas exist for individuals showing outstanding creativity and personal achievement.

To meet our client's immediate recruitment programme, applications are invited for the following London based positions.

OPERATIONS

Geology Graduates with experience as Mud Loggers, Data Engineers, Consultant Wellsite Geologists or with oil company experience that has included wellsite work.

INTERPRETATION

Graduate Geologists and Geophysicists with substantial sub-surface interpretation experience.

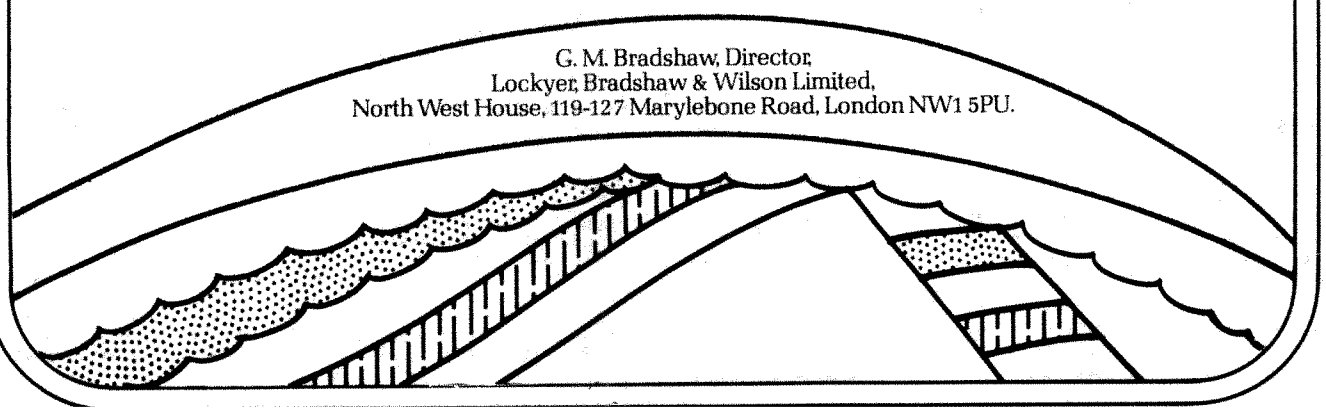
The financial package offered by our client indicates their high regard for active, highly professional exploration specialists.

Please write (quoting ref. CRS 124) with a full C.V. to the address below advising of any companies to whom your application should not be referred. Alternatively an application form can be obtained by telephoning us on 01-402 4525 (between 09.30 and 17.00, Monday to Friday), or Sunday 22nd July ONLY on either 01-527 4720, 01-660 6289 or 01-504 2084.

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2667(A)

Ph D IMMUNOLOGIST/ PHARMACOLOGIST

with a research interest in

RHEUMATOLOGY

We have a vacancy for a PhD Immunologist or Pharmacologist with relevant research experience to work on our rheumatoid arthritis project.

The successful applicant will join a team of biologists investigating cellular and animal models of rheumatoid arthritis.

Fisons offer a competitive salary and assistance with relocation expenses where appropriate.

For an application form please write or telephone

Mr. Bryan Johnson, Personnel Operations Manager, Fisons Limited, Pharmaceutical Division, Bakewell Road, LOUGHBOROUGH, Leicestershire.

Tel: 0509 66361 quoting reference No. RD45/N

This is a re advertisement 2681(A)

FISONS 1978

PHARMACEUTICAL DIVISION

UNIVERSITY OF OXFORD

Sir William Dunn School of Pathology

RESEARCH ASSISTANT

Grade 1A

Salary £4,232 to £7,145

Applications are invited for a Research Assistant supported by the Cancer Research Campaign, to assist with research into the biochemical mechanisms of virus growth. Experience with mammalian tissue culture would be an advantage. Applicants should possess a degree or equivalent qualification.

Applications, in writing, together with the names and addresses of two referees, to the Administrator, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE. 2643(A)

UNIVERSITY OF READING

DEPARTMENT OF SOIL SCIENCE

Applications are invited for a Lecturership in Soil Science with responsibility in soil physics or microbiology or soil biochemistry.

The appointment will be made within the first five points of the Lecturer salary scale (£4,333 to £5,488 per annum).

The person appointed should take up duties on 1 January 1980.

Further information may be obtained from the Registrar (Room 214, Whiteknights House), The University, Whiteknights, Reading RG6 2AH, by whom applications should be received not later than 30 September 1979. 2715(A)

NEWCASTLE AREA HEALTH AUTHORITY (TEACHING)

NEWCASTLE GENERAL HOSPITAL

TWO MEDICAL LABORATORY SCIENTIFIC OFFICERS

required in Muscular Dystrophy Group Laboratories, Regional Neurological Centre —

(1) To assist with development of tissue culture techniques for study of muscle and nervous tissues as part of a research programme which involves several scientific disciplines. Experience of tissue culture and sterile techniques and a knowledge of electron microscopy an advantage.

(2) To be involved in immunological investigation of neuromuscular diseases as part of a multi-disciplinary research programme. Experience of HLA-typing, lymphocyte transformation and RIA techniques would be an advantage.

Candidates must be registered.

Further information from Mr. J. J. Fulthorpe, Senior Chief MLSO, tel. 0632 738811 ext. 573.

Applications, with names and addresses of two professional referees, to Western Sector Administrator, Newcastle General Hospital, Newcastle upon Tyne NE4 6BE, by 17th August. 2661(A)

COMMONWEALTH AGRICULTURAL BUREAUX

Vacancy for

SCIENTIFIC INFORMATION OFFICER

at the

COMMONWEALTH FORESTRY BUREAU

Commonwealth Forestry Institute

South Parks Road, Oxford, OX1 3RD

Duties of post: The scrutiny of technical literature, selection and preparation of abstracts in many subjects related to forestry or forest products (this may involve visits to outside libraries), indexing, preparing for the press and dealing with technical enquiries.

Flexible working hours are based on 37 hours per week. Applicants wishing to work 18½ hours per week, with appropriate adjustments of salary, etc, will be considered.

Qualifications: Applicants should be graduates in forestry or in a biological science or in a relevant branch of physical or engineering science. Strong preference will be given to candidates with a good reading knowledge of French, German or Russian.

Salary: In the range £2,998 to £5,448 (under review) and a compensatory allowance (taxable but not superannuable) of 3.3% to offset personal contribution to Superannuation. Starting salary according to qualifications, experience and age. Promotion to higher scales on merit.

Application forms and full particulars from the Executive Director, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN. Closing date for applications: 31 August 1979. 2694(A)

UNIVERSITY OF KHARTOUM

Sudan

Applications are invited for the post of

CHIEF TECHNICIAN/ SENIOR TECHNICIAN/ TECHNICIAN

in the

HYDROBIOLOGICAL RESEARCH UNIT

Faculty of Science

Salary under review (exclusive of expatriate allowance); Chief Technician £S1,530 to 1,905 pa, Senior Technician £S1,330 to 1,580 pa, Technician £S1,030 to 1,280 pa (£S1 = £1.31 sterling). The British Government may supplement salaries of Senior and Chief Technician in range £4,140 to 4,284 pa (sterling) for married appointee or £2,076 to 2,226 pa (sterling) for single appointee (reviewed annually and normally free from tax) and provide childrens education allowances and holiday visit passages (presently under review). It is unlikely that there will be supplementation for technician grade. Family passages; baggage allowance; accommodation; car loan; superannuation scheme; various allowances.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to the Personnel Secretary, University of Khartoum, P O Box 321, Khartoum, Sudan by 23 August, 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 2647(A)

Behringwerke

We are a wellknown firm of the pharmaceutical industry belonging to the HOECHST-Group with headquarters in the university town of Marburg/Lahn.
Mainly concerned with developing new preparations for therapy and prophylaxis we are looking for a young (male or female)

Scientist/ Virologist

(physician, biochemist, biologist or chemist)

for our virological research department, who should possess a degree preferably in a virological or immunological subject. Relevant experience in this field and general practice in a virological institute are required.
Applicants should be at least 30 years old. Good knowledge of English is essential.
Ability to work in a team is expected.
Financial and social benefits will be offered in accordance with the importance of this position.
Further particulars may preferably be discussed personally.
Please send your application with detailed documents to

Personnel department Behringwerke AG
P.O.B. 1140 D-3550 Marburg 1 Fed. Rep. Germany

BEHRING INSTITUT

W250(A)

OXFORD UNIVERSITY

Sir William Dunn School of Pathology

NUCLEIC ACID BIOCHEMIST

Applications are invited for a post-doctoral research position, funded by the Cancer Research Campaign. The research concerns the preparation and transcription of superhelical templates derived from the nuclear DNA of human cells. Experience in enzyme purification (for the preparation of DNA gyrase and the relaxing enzyme) or the assay of specific transcripts is desirable, but not essential.

The appointment, starting as soon as possible after 1st October 1979, is likely to be for 2 years. The salary will be at an appropriate point on the University 1A scale, £4,232 to £7,145.

Applications, including curriculum vitae and the names and addresses of 2 referees, should be sent as soon as possible to Dr P R Cook, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE. 2625(A)

UNIVERSITY OF NORTH CAROLINA, Chapel Hill

DEPARTMENT OF ZOOLOGY

CHAIRPERSON

The Department of Zoology, University of North Carolina at Chapel Hill, invites applications for the position of

PROFESSOR AND CHAIRPERSON.

The Department's twenty-five faculty members have broad interests and actively participate in four interdepartmental programs. Candidates must have distinguished professional records and must be able to provide dynamic, unifying leadership. Appointment is to the rank of tenured professor; salary is fully negotiable. Nominations and applications including curriculum vitae and the names of at least three references should be sent prior to November 1, 1979 to: Professor C.F. Christman, 106 Rosenau Hall, 201H, University of North Carolina, Chapel Hill, NC 27514. The University of North Carolina is an affirmative action, equal opportunity employer. W252(A)

EUROPEAN JOURNAL OF BIOCHEMISTRY

Applications are invited from graduates in biochemistry, PhD preferably, for the post of

EDITORIAL SECRETARY

at the journal's office in Liège.

Salary will be around £5,800 net per annum, or more according to special experience. Applicants should preferably be nationals of the member states of the European Economic Community.

Applications, which should include the name of at least one referee and complete curriculum vitae, should be sent as soon as possible to Professor Claude Liébecq, 69/054 Boulevard de la Constitution, B-4020 Liège, Belgium. 2533(A)

Junior/basic grade MLSO required to work in routine Histopathology Laboratory. Post entails routine and research work presenting good opportunity for those wishing to take IMLS special examination. Applicants for Junior post should have 5 "0's" including English, Maths and 2 relevant sciences or 2 relevant "A's". Applicant for basic MLSO should have HNC MLS. Salary on MLSO scales.

Apply quoting reference TP1 to The Secretary, Cardiothoracic Institute, Fulham Road, London, SW3 6HP, stating age, experience, qualifications and giving names of 2 referees. Closing date 31/8/79. 2685(A)

ASSISTANT RESEARCH CHEMIST Department of Internal Medicine Martinez VA Hospital, Martinez, CA PhD with minimum of two years' post doctoral experience in the synthesis of pedtides and the operation of an HPLC thorough knowledge of enzyme kinetics and experience in protein purification. An equal employment opportunity/affirmative action employer. Send cv bibliography and references to Corey Largman PhD, Department of Medicine Martinez VA Hospital, 150 Muir Road Martinez, CA 94553. Position to remain open until October 31, 1979. W259(A)

PRINCIPAL SCIENTIST

MICROBIOLOGY AND FERMENTATION

The Brewing Research Foundation, established to carry out co-operative research for the brewing industry in the UK and abroad, wishes to appoint a *Principal Scientist* as head of its *Microbiology and Fermentation Section*.

Candidates, aged between about 30 and 45 should have good academic qualifications and appropriate research experience. The successful candidate, who will be responsible to the Director for the development and organisation of the Section's research programme, will also be expected to carry out his own personal research.

The post offers an excellent opportunity for an alert and ambitious researcher to make a name in this important part of the food and drink industry.

The Foundation provides good conditions of service; the initial salary will be within the range £8,500 to £11,000.

Applications giving details of education, experience, papers etc should be sent to the Assistant Director, Administration and Finance, Brewing Research Foundation, Lyttel Hall, Nutfield, REDHILL, Surrey RH1 4HY. 2671(A)

BIOGEN S.A.

a young company engaged in basic and applied research in the field of molecular biology and microbiology, and with the objective of utilizing the potential of genetic engineering for the production of substances of commercial and medical interest in such areas as health care, agriculture and energy, is inviting applications from

SENIOR AND JUNIOR SCIENTISTS

with experience in molecular biology, nucleic acid biochemistry and microbiology,

for its **Research Laboratory in Geneva,**

Switzerland.

Excellent working conditions, in close contact with leading scientists from Europe and the US.

Salary scale: Group Leaders = SFr 60-85,000, Research Associates = SFr 35-55,000.

Applicants with real interest in both basic research and its applications should send their curriculum vitae (with a description of previous experience and a list of publications) to BIOGEN Laboratory, 3 Route de Troinex, 1227 Geneva, Switzerland. W248(A)

TECHNICIAN

Department of Viral Products

required to work in a laboratory concerned with research into the development and standardisation of vaccine for Poliomyelitis.

Candidates should possess or be studying for an HNC or equivalent in one of the biological sciences. Experience in virology or cell culture would be an advantage although training will be provided.

Salary, depending on qualifications and experience, on the scale £2,892 to £5,034 (pay award pending) including London Weighting, plus superannuation benefits.

Please write or telephone for further details, and an application form, to the Personnel Officer, National Institute for Biological Standards and Control, Holly Hill, London NW3 6RB. Tel: 01-435 2232, ext. 200, quoting Reference VP/103.

NIBSC

National Institute for Biological Standards and Control

2645(A)

UNIVERSITY COLLEGE OF SWAZILAND

Applications are invited for the post of

LECTURER in the DEPARTMENT OF STATISTICS

Applicants should possess a Master's degree or a PhD in Mathematics/Statistics. Experience in Operational Research and/or Quality Control would be an additional recommendation. The appointee will be required to lecture to undergraduates in introductory and theoretical statistics and to be capable of carrying out independent research.

Salary scale: E5,940 to 7,876 pa (£1sterling = L1.86). The British Government may supplement salary in range £1,650 to 2,184pa (sterling) for married appointee or £408 to 960 pa (sterling) for single appointee (reviewed annually and normally free from tax) and provide children's education allowances and holiday visit passages. Family passages; short-term contracts for two or four years for expatriates. If appointment is for a limited period, a 25% gratuity is paid in lieu of superannuation for the first two years of service; 27½% for the second two years. A 10 per cent Inducement Allowance is payable to expatriates not qualifying for supplementation from other sources. Education allowance; medical aid; accommodation; biennial leave.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to the Registrar, University College of Swaziland, Private Bag, Kwaluseni, Swaziland by 27 August 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 2705(A)

THE SOUTH STAFFORDSHIRE WATERWORKS COMPANY

WATER QUALITY DEPARTMENT

CHIEF ASSISTANT CHEMIST

Location: Initially at Lichfield, permanent location at new Central Laboratory in Walsall, West Midlands.

Salary: NJC Water Service Staffs Grade 10, Range £7,776 to £8,352 p.a. (currently under review).

Applications are invited for the above post from professionally qualified chemists having a good honours degree in an appropriate scientific subject and preferably with experience of bacteriological work and procedures. Appropriate administrative experience will be an advantage.

The successful candidate will be required to assist the Chief Chemist and Deputy Chief Chemist in the general operation of the department and initially will be directly responsible for the technical and administrative co-ordination of the Company's laboratory and the establishment of a computerised water quality data bank. Detailed technical competence is required in current methods of water analyses, including advanced instrumental techniques, and the ability to prepare clear and concise technical reports.

The laboratory performs the full range of analytical functions required to satisfy the bacteriological, biological and visible wholesomeness of a large water supply. Modern analytical instrumentation is available for the performance of these functions and the range is to be further extended upon completion of new laboratories presently under construction.

Appropriate transport arrangements will be provided together with the payment of approved relocation expenses where the successful applicant is required to move house.

Applications, giving full details of training and experience, together with the names of two referees should be sent not later than 27th August, 1979 to:-

James Lamont BSc MICE, General Manager, The South Staffordshire Waterworks Company, 50 Sheepcote Street, Birmingham B16 8AR. 2659(A)

SIMON FRASER UNIVERSITY

Burnaby, British Columbia Canada

DEPARTMENT OF BIOLOGICAL SCIENCES

ENTOMOLOGIST

Applications are invited for a tenure-track position as an Assistant Professor in entomology. The successful applicant will be expected to develop a strong research program and to participate in the teaching program at both the undergraduate and graduate level including the Master of Pest Management Program. Candidates must have a PhD degree in entomology or related subject. The position is available immediately, subject to budgetary approval. The initial appointment will be for a period of 2 years, renewable. The salary scale is under revision; the current salary base for the Assistant Professor rank is $18,604 per annum.

Applications should include an up-to-date curriculum vitae, a brief statement of research interests and goals, and reprints of published research. Applicants should request a confidential assessment of their research and teaching ability from three referees, to be forwarded directly to Dr M Mackauer, Chairman, Dept of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada V5A 1S6. Deadline for receipt of applications is 15 September 1979 or when position filled. W240(A)

UNIVERSITY OF ZAMBIA

Applications are invited for the posts of

PROFESSOR/
ASSOCIATE PROFESSOR/
SENIOR LECTURER/
LECTURER

in the following posts in the School of Agricultural Sciences. Soil Science (2 posts). Soil Physics (Irrigation and Drainage) Soil Science (Chemistry and Fertility). Agricultural Engineering (1 post). Agricultural Mechanization, Civil Engineering in developing countries. Rural Economy and Extension Studies (1 post). Agricultural Extension and Rural Sociology, Economics and Psychology.

For all these posts applicants are required to have a PhD and considerable University level teaching experience with research and publications in the areas of their specializations.

Salary Scales: Professor K8,616 to 9,135 pa, Associate Professor K8,232 to 8,616 pa, Senior Lecturer K7,608 to 8,016 pa, Lecturer K5,904 to 7,608 pa (£1 sterling = K1.70). The British Government may supplement salaries in range £2,922 to 5,952 pa (sterling) for married appointees and £930 to 3,366 pa (sterling) for single appointees (reviewed annually and normally free from tax) and provide childrens education allowances and holiday visit passages. Family passages; baggage allowance; superannuation scheme; accommodation; overseas leave; various allowances.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to the Registrar, University of Zambia, PO Box 2379, Lusaka, Zambia by 24 August, 1979. Applicants resident in the UK should also send one copy to Inter University Council, 90/91 Tottenham Court Road, London, W1P 0DT. Further details may be obtained from either address. 2700(A)

UNIVERSITY OF ZAMBIA

Applications are invited for the post of

SENIOR LECTURER/
LECTURER IN BIOLOGY

SCHOOL OF NATURAL SCIENCES

Special reference to Biochemistry required. Research interest desired and preferred in Comparative Plant and Animal Metabolism, Enzymology or Morphogenetic Studies. Applicants should have PhD or equivalent plus university level teaching experience in Enzymology studies. Appointee will be responsible for the teaching of general physiology and biochemistry course as well as conducting an advanced biochemistry course in the final year of the BSc programme. Post tenable as soon as possible. Salary scales: Senior Lecturer K7,608 to 8,016 pa, Lecturer K5,904 to 7,608 pa (£1 sterling = K1.70). The British Government may supplement salary in range £2,922 to 4,350 pa (sterling) for married appointee and £930 to 1,968 (sterling) for single appointee (reviewed annually and normally free from tax) and provide childrens education allowances and holiday visit passages. Family passages; baggage allowance; contract 2-5 years; contributory superannuation scheme; accommodation; various allowances.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to the Registrar, University of Zambia, PO Box 2379, Lusaka, Zambia by 27 August 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 2699(A)

University of Jos
JOS, NIGERIA

Applications are invited from suitably qualified candidates to fill the following vacant posts:—

POSTS

A. Faculty of Medical Sciences

Professor/Reader/Senior Lecturer/Lecturer I & II in the following fields:—
1. Medicine, 2. Paediatrics, 3. Pharmacology & Therapeutics, 4. Psychiatry, 5. Surgery, 6. Obstetrics and Gynaecology, 7. Community Medicine, 8. Anaesthesia, 9. Microbiology, 10. Chemistry Pathology, 11. Pathology, 12. Haematology and Blood Transfusion, 13. Anatomy.

QUALIFICATIONS

PROFESSOR/ READER

The candidate who should have a post-graduate qualification registerable with the Nigeria Medical Council, should be an authority in his field and should have several years' experience in his area of competence.

SENIOR LECTURER/ LECTURER I & II

Candidates for 1, 2, 3 and 4 should possess an MRCP or similar post-graduate qualification. Candidates for 5 should possess an FRCS. Candidates for 6 should possess an MRCOG. Candidates for 7 should possess DPH/MPH and possession of an MRCP or MFCM would be an advantage. Candidates for 8 should possess an FFARCS or DA. Candidates for 9, 10, 11 and 12 should possess an MRC Path, a PhD or equivalent qualification. Candidates for 13 should possess an MS, MSc, or PhD (Anatomy). In the case of Senior Lecturer, candidates should generally possess not less than 5 years' experience in research and teaching in the speciality — 3 years' experience for Anatomist. Possession of a PhD would be an advantage as would training and experience in any sub-speciality in these fields.

B. Faculty of Natural Sciences

Professor in Chemistry and Zoology; Senior Lecturer/Lecturer I & II in the following:—
1. Mathematics with specialisation in Statistics, Differential Equations and Functional Analysis.
2. Botany in the fields of Plant Taxanomy, Plant Anatomy, Microbiology (including Bacteriology), Crytopatic Botany and Hydrobiology.
3. Organic Chemistry, Inorganic Chemistry and Analytical Chemistry.
4. Geology.
5. Zoology.

QUALIFICATIONS

For all posts above, candidates should possess Higher Degrees from recognised Universities with considerable teaching experience. A PhD will be an advantage. For Professors, candidates must have established a reputation in research in their field of specialisation and must have demonstrated capability in initiating and directing research activities at a higher level.

SALARIES

Professors — GL 16 — N11,568 × 526 — N12,720 pa. Reader — GL 15 — N10,296 × 516 — N11,238 pa. Senior Lecturer — GL 13 — N8,064 × 320 — N9,024 pa. Lecturer Grade I — GL 12 — N7,404 × 216 — N8,052 pa. Lecturer Grade II — GL 10 — N5,760 × 162 — N6,732 pa.

CONDITIONS OF SERVICE

Successful candidates will be on probation for the first two years and afterwards confirmed to retiring age if their services are considered satisfactory. Other fringe benefits include pension scheme, part-furnished accommodation or housing allowance in lieu of accommodation. Twenty five per cent contract addition is payable to all expatriates.

METHOD OF APPLICATION

Candidates should send 4 copies of their CV to the Principal Assistant Secretary (Recruitment), Nigerian Universities Office, 180 Tottenham Court Road, London W1P 9LE as soon as possible.

The CV should give the following information: Post desired, full name, date and place of birth, nationality, permanent address, telephone number (if any), current postal address and telephone number (if applicable), marital status, number and ages of children, educational institutions attended with dates, full qualifications plus copies of certificates, previous employment with dates, present employment with dates and salary, list of publications, names and addresses of three distinguished scholars in relevant field as referees, two passport photographs of self. Candidates are advised to request their referees to forward references on them directly to the London Office.

CLOSING DATE: 16th August 1979. 2697(A)

UNIVERSITY OF OXFORD
In association with
St Edmund Hall

UNIVERSITY
LECTURESHIP IN
PHYSIOLOGY

The University proposes to appoint as soon as possible a lecturer in the Laboratory of Physiology. Applications are invited from candidates in all fields of Physiology but the successful candidate will be expected to teach in the fields of either Visual or Renal or Gastro-intestinal Physiology. Salary according to age on the scale £4,232 to £9,000. The post may be held in association with a tutorial fellowship at St Edmund Hall.

Further enquiries concerning both the lectureship and the fellowship should be addressed to the Professor-elect, Dr C B Blakemore, c/o the Administrator, University Laboratory of Physiology, Parks Road, Oxford, to whom applications (8 copies, one in the case of overseas candidates) together with the names of three referees should be sent by 30 September 1979. 2644(A)

THE NATIONAL INSTITUTE FOR HIGHER EDUCATION, LIMERICK

Further expansion of the Institute is taking place at undergraduate and postgraduate levels within the extensive complex of World Bank-financed facilities which have been commissioned as part of the first phase of development of the Riverside campus.

APPLIED MATHEMATICS Lecturer AS4-010

Person appointed will contribute to the development and operation of existing and new courses to degree and postgraduate level. Applicants should have demonstrated expertise in the applications of mathematics and have special competence in the area of numerical analysis. A postgraduate qualification in a relevant area is desirable.

SALARY SCALE: £6,901 to £8,766 p.a. (under review)

Application forms which are available from the Personnel Office, The National Institute for Higher Education, Limerick, Ireland, should be completed and returned *by 24th August, 1979*. 2668(A)

Project Leader

Gas Chromatograph Development

Pye Unicam, part of the multinational Pye/Philips Group of Companies, are leading manufacturers of scientific and analytical instrumentation.

Due to continued expansion, we urgently need an experienced Physicist or Physical Chemist/Engineer. This is a senior position and responsibilities will include contributing to our development programme, carrying out necessary fundamental design studies and obtaining experimental confirmation of their effectiveness.

Candidates should have broad knowledge of the design and use of Gas Chromatographs or related instruments. Several years design experience is essential along with the relevant academic qualifications.

Future prospects are excellent with the successful applicant subsequently becoming responsible for the progress of a new instrument through its development phase, by co-ordinating the design imputs of the other members of the team.

Along with a competitive salary, we offer appreciable fringe benefits, including flexible working hours and group pension scheme.

Please write or telephone for an application form to:
Mrs Jane Wakelin, Personnel Officer. 2691(A)

Pye Unicam Ltd.
York Street, Cambridge, CB1 2PX
Tel: Cambridge (0223) 58866

AGRICULTURAL UNIVERSITY WAGENINGEN

Department of Fish Culture and Inland Fisheries
invites applications for the post of

LECTURER/SENIOR LECTURER

in Pond Fish Culture

The appointee will be expected to make a significa contribution to the undergraduate courses and the supervisi of graduate research assignments in fish culture and fisheri and to pursue research on pond fish culture as practised tropical and temperate regions.

Applicants in possession of a MSc or PhD in Fisheri Agriculture or Biology will be considered on the basis of th experience with biological and technical characteristics of po fish culture and their expertise in the field of aquatic populati dynamics.

Salary on appointment will be up to a maximum Hfl77,200 per annum depending on qualifications a experience (US$1 = Hfl2). Appointment above the maximun also possible.

Particulars can be obtained from Professor Dr E A Huism Department of Fish Culture and Inland Fisheries, P O Box 3 Wageningen, The Netherlands. Applications under nr. 79- are to be forwarded, within one month as of the appearance this announcement, to: Personeelszaken van Landbouwhogeschool, P O Box 9101, 6700 HB Wageninge The Netherlands. W249(A)

UNIVERSITY OF KHARTOUM
Sudan

Applications are invited for the post of

PROFESSOR
in the
HYDROBIOLOGICAL RESEARCH UNIT
Faculty of Science

Salary (exclusive of expatriate allowance); £S6,933 pa (£S1 = £1.31 sterling). The British Government may supplement salary by £5,028 pa (sterling) for married appointee or £3,144 pa (sterling) for single appointee (reviewed annually and normally free from tax) and provide childrens education allowances and holiday visit passages. Family passages; baggage allowance; accommodation; car loan; superannuation scheme; various allowances.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to the Personnel Secretary, University of Khartoum, P O Box 321, Khartoum, Sudan by 23 August, 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 2646(A)

UNIVERSITY OF OXFORD

Departments of Pharmacology and Physical Chemistry

POSTDOCTORAL RESEARCH ASSOCIATE IN HIGH PRESSURE PHYSIOLOGY AND PHARMACOLOGY

A postdoctoral position is available for research related to diving and the effects of high pressure on man and animals, in an interdisciplinary research team conducted by Prof. Sir William Paton and Dr E B Smith. A number of lines of research are being actively pursued, ranging from physical chemistry to human physiology. The successful candidate could work in any of these areas, although preference may be given to those interested in neuropharmacological approaches.

Applications, including statements of research experience and publications, with the names of two referees, should be sent as soon as possible to Prof Sir William Paton, Department of Pharmacology, South Parks Road, Oxford OX1 3QT. It is hoped to make an early appointment to enable the successful candidate to take up the post by 1 October 1979, although this need not rule out applicants wishing to start later. 2672(A)

THE UNIVERSITY OF BIRMINGHAM
FACULTY OF MEDICINE AND DENTISTRY
Department of Anatomy

RESEARCH ASSOCIATE/ FELLOW

Research Associate/Fellow required to join a multidisciplinary team to study the differentiation of skeletal muscle using monoclonal antibodies.

Candidates should be able to offer postgraduate qualifications or experience in Biochemistry or a related discipline. The post is supported for three years by the MRC, with a salary on the scale (to operate from 1st October 1979) £3,775 to £5,488 or £4,333 to £7,521 plus superannuation.

Your application (three copies) should include a curriculum vitae, details of experience and the names of two referees. Send it to the Assistant Registrar, Faculty of Medicine, University of Birmingham, Birmingham B15 2TJ by 24th August 1979, quoting reference RA/ANT/379 or talk to Dr S Salmons on 021-472 1301, ext 2093. 2665(A)

ROYAL POSTGRADUATI MEDICAL SCHOOL
DEPARTMENT OF MEDICINE

POSTDOCTORAL SCIENTIST

required to join a multidisciplinary te investigating the cellular control pituitary hormone secretion using culture techniques. The success candidate will be responsible for component of the project in which nc synthetic analogues of hypothalai hormones are being used to probe pituit receptors and function. Applica preferably have a strong background tissue culture techniques or pharmacolo The post is funded for two years in the f instance.

Salary (1st October) up to £4,910 p £502 London Allowance a year.

Application forms and further det may be obtained from the Person Office, Royal Postgraduate Medi School, 150 Du Cane Road, London W OHS quoting reference number 2/391.

Closing date: 21 days from publicatic 2649(A

SENIOR BIOCHEMIST

This new post will be in the Biology Department of an international industrial firm, leader in cosmetics and hair products.

The applicants should have a PhD degree in Biochemistry and an experience in analysis and separation of proteins. He should have a good knowledge of biochemistry of keratin.

The position requires aptitude to scientific research and ability to lead a team of graduates and technicians.

The successful applicant will work under the direction of the head manager of the Biology Department. The salary will depend on experience.

The research centre is located north of Paris.

Ability to speak and write in English as well as a good working knowledge of French are necessary, other languages being a further asset.

For further information, please send curriculum vitae to L'OREAL — M. JEGO 1, avenue de St Germain — 93601 AULNAY SOUS BOIS — FRANCE.
W261(A)

Royal Observatory, Edinburgh

PROFESSIONAL AND TECHNOLOGY OFFICER

GRADE 1

The Technology Unit at the Royal Observatory has a vacancy for an engineer at PTO I level. The Unit is responsible for research, development and manufacture of a wide range of instrumentation for the National Facilities run by the Observatory, which include the 1.2 metre Schmidt Telescope in Australia, the 3.8 metre Infrared Telescope in Hawaii and the COSMOS Measuring Machine at Edinburgh.

The successful candidate will be responsible for applications of infrared and optical solid-state and vidicon-type detectors to the recording of extremely low levels of radiation. Initially he/she will be involved in the development and construction of a cooled 10 micron array spectrometer, operating with As doped silicon diodes.

The applicant should be a fully qualified engineer; possession of an advanced degree may be an advantage. Applicants will be required to describe successful results with solid-state or vidicon-type systems which they have developed.

Starting salary will be in the range £5,739 to £7,064 depending on age and experience. A non-contributory superannuation scheme is included.

SRC

Further details and application forms from The Personnel Officer, Royal Observatory, Blackford Hill, Edinburgh EH9 3HJ. Tel: 031 667 3321.

CLOSING DATE: 2 AUGUST 1979.

2604(A)

Pharmacologist

Bronchorespiratory Research

Roussel Laboratories is one of the leading pharmaceutical companies in the UK. A vital part of the international Roussel-Uclaf organisation, it researches, manufactures and distributes a range of important ethical pharmaceutical and allied products for use throughout the world.

We are currently developing a number of products within the bronchorespiratory area. We wish to intensify this research programme and need a suitably experienced pharmacologist to play a key role in this work.

Applications are invited from candidates qualifying this year for a PhD, preferably in respiratory pharmacology, but experience of research to PhD level in allied branches of pharmacology will be considered.

The salary is competitive and benefits include free BUPA and life insurance, a contributory pension scheme and generous assistance with relocation expenses where appropriate.

For further information and an application form, please telephone or write to Rosemary Curtis, Assistant Personnel Manager, Roussel Laboratories Limited, Kingfisher Drive, Covingham, Swindon, Wilts SN3 5BZ.
Telephone: Swindon (0793) 24411.

ROUSSEL

2692(A)

UNIVERSITY OF THE SOUTH PACIFIC

Applications are invited for the post of

PROFESSOR IN CHEMISTRY

(Post 79/72) tenable from January 1980. Applicants with a proven record of teaching, research and administrative experience in Chemistry are invited to apply. Preference will be given to candidates with academic interests in the general area of physical/inorganic chemistry, but applications from the organic/biochemical field will be fully considered. Experience in determining tertiary educational priorities in a developing country context would be an advantage. The Head of Chemistry has overall responsibility for the Chemistry component of teaching programmes which includes pre-degree courses, Dip. Ed., BSc., BSc + GCEd., B.Ed and B.Agr.* (*1st year only) degrees and for postgraduate work and regional extension courses. Staff may also be involved in consultancy, research and development projects for associated University Institutes. Salary scale: F$19,008 to F$20,420 p.a. (£1 sterling = F$1.81). The British Government may supplement salary by £2,700 p.a. (sterling) for married appointee or £948 p.a. (sterling) for single appointee (reviewed annually and normally free from tax) and provide childrens' education allowances and holiday visit passages. Family passages; baggage allowance; 15% gratuity; superannuation scheme; accommodation at 12½% of salary (under review); 3 year contract — exceptionally, candidate may be considered for shorter period. Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to the Registrar, University of the South Pacific, Box 1168, Suva, Fiji by 17 August 1979. Applicants resident in the UK should also send one copy to Inter University Council, 90/91 Tottenham Court Road, London, W1P 0DT. Further details may be obtained from either address. 2663(A)

UNIVERSITY OF EDINBURGH

DEPARTMENT OF MICROBIOLOGY

Applications are invited from

CHEMISTS OR BIOCHEMISTS

for two post-doctoral posts to work on a study of the effect of temperature, pH, etc on the physical properties of microbial polysaccharides. This three-year project is sponsored by the Department of Energy. Both positions are on Scale 1A within the range £4,333 to £5,199. For one position, some postdoctoral experience would be helpful, while the second would suit someone with interests in "methodology".

Further details from and applications to: Dr I W Sutherland, Department of Microbiology, Edinburgh University, West Mains Road, Edinburgh EH9 3JG. Please quote reference 5041. 2669(A)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

(University of London)

DEPARTMENT OF IMMUNOLOGY

Personal Research Assistant or Research Medical Laboratory Scientific Officer (grading depending upon qualifications and experience) required to work with Professor I M Roitt. Immunological laboratory experience absolutely essential, preferably in cellular techniques. Opportunity to submit work for higher degree. Starting salary, on appropriate scale according to grading, age, qualifications and experience, up to £5,574 per annum.

Applications in writing, with the names of 2 referees, to Professor I M Roitt, Department of Immunology, The Middlesex Hospital Medical School, Arthur Stanley House, 40-50 Tottenham Street, London W1P 9PG. 2701(A)

UNIVERSITI SAINS MALAYSIA

SCHOOL OF MEDICAL SCIENCES

Applications are invited for the post of

PROFESSOR/ ASSOCIATE PROFESSOR

in any of the following fields: (i) Anatomy; (ii) Physiology; (iii) Pathology.

Qualifications/Experience: Professor should have MBBS/PhD degree with at least 3 years' experience as Associate Professor; Associate Professor should have MBBS/PhD degree with at least 3 years' experience as Lecturer at University level. (Clinical staff will be required to do patient-care duties in the Teaching Hospital. Candidates with post-graduate specialist qualifications and carry out clinical duties will be eligible for a Specialist allowance.)

Salary Range per annum: Professor: from M$35,580 to M$40,380; Associate Professor: from M$30,300 to M$34,380.

Other benefits: Return Passage, Excess baggage assistance, paid annual leave, free medical benefits for staff and family and gratuity at 17½% of total monthly salary will be provided. Car loan facilities are also available.

Tenure: On contract for 3 years, renewable if necessary. Applications containing full personal particulars and current salary to be forwarded to Registrar, Universiti Sains Malaysia, Minden, Penang before 27th August 1979.

W260(A)

KENYATTA UNIVERSITY COLLEGE — KENYA

(a constituent College of the University of Nairobi)

Applications are invited for 2 posts of

PROFESSOR/ ASSOCIATE PROFESSOR

in the

DEPARTMENT OF GEOGRAPHY

Candidates should have a PhD in Geography and considerable teaching experience at university level. They should provide evidence of successful research through publications in internationally known journals. Experience in the training of secondary school teachers will be an advantage. Appointees must be capable of providing academic and administrative leadership in the department. Salary scales: Professor K£4,632 to 5,562 pa, Associate Professor K3,864 to 4,488 pa (K£1 = £1.22 sterling). Family passages; subsidised housing; SSSF or FSSU; non-contributory medical aid scheme.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Registrar, Kenyatta University College, PO Box 43844, Nairobi, Kenya by 28 August 1979. Applicants resident in the UK should also send one copy to the Inter-University Council, 90/91 Tottenham Court Road, London, W1P 0DT. Further details may be obtained from either address. 2704(A)

MAFF: AGRICULTURAL DEVELOPMENT AND ADVISORY SERVICE

Harpenden Laboratory

CHEMIST

The major concern of the Pesticides Registration Department in the Laboratory is the safe and effective use of pesticides, including herbicides in crop protection.

The chemist appointed will provide technical support to the Pesticides Safety Precautions Scheme, and will be responsible for the assessment of applications for the safety clearance of pesticides. The work will include advising and negotiating with agro chemical manufacturers, preparing technical papers for presentation to scientific committees, and liaison with other government departments, local authorities and research organisations.

This post has been exempted from the Government's ban on recruitment.

Candidates should have an honours degree in chemistry, or a relevant discipline, and at least 2 years' postgraduate experience.

Appointment will be as Higher Scientific Officer (£4,725 to £6,210) with starting salary according to qualifications and experience. Salary under review. Promotion prospects. Non-contributory pension scheme.

For further information and an application form (to be returned by 17 August 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote reference SB/4/AL.*

2706(A)

Science Group CIVIL SERVICE

POSTDOCTORAL FELLOW

An immediate opening is available for a recent PhD biochemist with experience in 1) *in vitro* translational systems and/or 2) purification and characterization of phosphatases and phosphokinases. The person chosen will have the opportunity to interact with several interdisciplinary groups and the fellowship will run for two to three years. All interested candidates should send a resumé and three names of referees to:

Dr George Thomas
Friedrich Miescher-Institut
P O Box 273
CH-4002 Basel,
Switzerland W230(A)

UNIVERSITY OF OXFORD

DEPARTMENT OF CLINICAL BIOCHEMISTRY

TECHNICIAN

required for research into regulation of the pyruvate dehydrogenase complex. The post is available initially for two years from October 1. Applicants should have a degree in biochemistry (or a related subject) or possess suitable laboratory experience. Starting salary up to £4,047 per annum.

Applications including the names of two referees to Professor P. J. Randle, Department of Clinical Biochemistry, John Radcliffe Hospital, Headington, Oxford, OX3 9DU. 2578(A)

TECHNICIAN

A Technician is required to assist in a programme of work on neurotoxicity. Training in biochemistry is essential but some experience in physiological techniques would be an advantage. Qualifications: Degree HNC or equivalent. Salary on scale of £3,261 to £4,680 plus London Weighting of £354.

Apply in writing giving curriculum vitae to Mr V. H. Parker, Toxicology Unit, MRC Laboratories, Woodmansterne Road, Carshalton, Surrey.

MRC Medical Research Council

2598(A)

UNIVERSITY OF LEICESTER

DEPARTMENT OF PHYSICS

POSTDOCTORAL RESEARCH ASSISTANT

The University of Leicester is embarking on a project, supported by the Royal Observatory, Edinburgh, to develop focal plane instrumentation for the 3.4 m United Kingdom Infra Red Telescope (UKIRT). A post exists for a research assistant to join the project, firstly to investigate applications of multi-element CID/CCD imaging detectors in infra-red astronomy; secondly to participate in the development of a multi-element photometer and a low resolution spectrometer for UKIRT and thirdly, through the use of one or both of these instruments, to take part in astronomical observations using UKIRT and/or the Tenerife 1.5 m telescope. Research experience with solid state photon detectors, and low noise signal handling methods, would be an advantage, especially if combined with an interest in infra-red astronomical techniques.

The post will be for 3 years. Salary will be within the range £4,232 to £4,776 per annum (£4,333 to £4,910 from October 1 1979) depending upon qualification and experience. Superannuation is under U.S.S. conditions.

Applications, together with the names and addresses of two referees, and a curriculum vitae, should be sent to Professor K.A. Pounds, Department of Physics, University of Leicester, LE1 7RH by August 15th 1979. Further particulars about the project are available on request. 2664(A)

OXFORD UNIVERSITY

PHYSICAL CHEMISTRY LABORATORY

APPOINTMENT OF POST DOCTORAL RESEARCH ASSISTANT

Reactions of atoms and radicals

Applications are invited from chemists or physicists to work on a laser flash photolysis method for measuring the rates of reactions between atoms and radicals, using absorption and resonance fluoresence techniques.

The appointment is for 2 years in Research support grade 1A with membership of U.S.S. Starting salary is up to £4,776.

Applications with curriculum vitae and the names of two referees should be sent as soon as possible to Dr M.J. Pilling, Physical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ. 2677(A)

CAMBRIDGESHIRE COLLEGE OF ARTS AND TECHNOLOGY

LECTURER IN BIOLOGY

required from 1 October 1979 or as soon as possible thereafter to teach general biology at GCE 'A' level and TEC Certificate level. The ability to teach a specialist biological subject at HTEC/degree level would be an advantage. Applicants should be graduates, and the successful candidate will be expected to become involved in the research programme of the Science Department.

Salary: £3,480 to £5,992 starting point depends on qualifications and experience.

Forms and further details from: Head of Science Department, CCAT, Cambridge, CB1 2AJ. Forms to be returned by 17 August 1979. 2719(A)

UNIVERSITY OF NEWCASTLE UPON TYNE

DEMONSTRATOR IN THE DEPARTMENT OF ENGINEERING MATHEMATICS

Applications are invited for a post of

DEMONSTRATOR

in the Department of Engineering Mathematics for a period of 3 years. The person appointed will be expected to have a good mathematics degree and preferably a higher degree, but applicants with a degree in other disciplines and good mathematical experience will be considered. It would be an advantage if the applicant were to have an interest in some aspect of engineering. The person appointed will be required to undertake tutorial work in mathematics, including statistics, for engineering students and there will be an opportunity to participate in some of the lecturing duties undertaken by the Department.

Salary will be at an appropriate point on the Grade 1B (Bar) scale: £3,689 to £5,321 per annum (£3,775 to £5,488 per annum from 1st October, 1979), according to age, qualifications and experience. Membership of the appropriate University superannuation scheme will be required.

Further particulars may be obtained from the Senior Assistant Registrar (F.P.) The University, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU with whom applications (2 copies), together with the names and addresses of 3 referees, should be lodged not later than 10th August, 1979. Please quote reference N. 2651(A)

KING'S COLLEGE LONDON

DEPARTMENT OF BIOPHYSICS

RESEARCH TECHNICIAN

Applications are invited for the post of Technician to work on Wellcome Trust-supported research on the mechanism and control of chromatin transcription. Experience in sub-cellular fractionation techniques, protein and nucleic acid biochemistry and restriction endonuclease mapping would provide useful background.

The appointment would be for one year in the first instance starting 1 September (or sooner). Salary on scale £3,455 pa to £3,860 pa or £3,998 pa to £4,580 pa (inclusive) according to qualifications and experience.

Applications with curriculum vitae and the names of two referees should be sent to Dr J Allen (Ref: 217825/N), Department of Biophysics, King's College London, 26-29 Drury Lane, London WC2B 5RL. 2666(A)

JOHN INNES INSTITUTE

SCIENTIFIC OFFICER

required in the Department of Applied Genetics to work on problems associated with the micropropagation of potato. This will involve the use of tissue culture techniques. The appointment is for three years and is funded by the Potato Marketing Board.

Applicants should hold an Honours degree in botany, agriculture or a related field. Salary £3,289 to £5,085. Starting salary depending on qualifications and experience. Non-contributory superannuation.

Applications, quoting reference AG/44 giving details of qualifications and experience and the names of two referees should be sent to the Secretary, John Innes Institute, Colney Lane, Norwich, NR4 7UH, not later than 20th August, 1979. 2674(A)

ROBERTSON RESEARCH INTERNATIONAL LIMITED

SEDIMENTARY GEOLOGIST

Robertson Research International Limited has a vacancy in their head office in North Wales for a sedimentary geologist. Applicants should have a PhD in geology and be familiar with the full range of sedimentological techniques relevant to carbonates and/or clastics. The position is challenging with career prospects, and will require the successful candidate to work both on individual assignments or in a team of geologists. Work is concerned with the study and intepretation of rocks from oil prospective areas. Preparation of technical reports is an integral part of the duties and experience in this field is required. Successful applicants must be willing to travel and work both within the British Isles and abroad. Salary is competitive and is negotiable depending on age, experience and qualifications, and the position is permanent and pensionable.

For application forms please apply to:

Janet Hedges, Robertson Research International Limited, 'Ty'n-y-Coed', Llanrhos, Llandudno, Gwynedd LL30 1SA, North Wales. 2657(A)

BIOCHEMICAL VIROLOGY

A junior or senior postdoctoral position is available to study biochemical aspects of influenza virus replication and antigenic variation in influenza viruses. Successful candidates must have strong backgrounds in biochemistry. Send curriculum vitae and a statement of career goals to Dr R G Webster, Division of Virology, St Jude Children's Research Hospital, P O Box 318, Memphis, Tn. 38101. An Equal Opportunity/Affirmative Action Employer. W236(A)

The CAMPDEN FOOD PRESERVATION RESEARCH ASSOCIATION

Chipping Campden, Glos., GL55 6LD., requires a

FOOD SCIENTIST, FOOD TECHNOLOGIST, or HOME ECONOMIST

to head the quality appraisal team in its Department of Agriculture and Quality. The successful candidate will be enthusiastic, have qualities of leadership and get on well with people. Salary in the Research Officer grade £3,048 to £5,316.

Futher details and application form from the Secretary-General at the above address. 2675(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL

DEPARTMENT OF HISTOPATHOLOGY

GRADUATE NEUROSCIENTIST

required to take part in research programme on effects of alcohol on brain development. Biochemical and/or morphological expertise desirable.

Appointment will be for three years, funded by a research grant from the Medical Research Council. Salary up to £4,914, dependent on qualifications and experience.

Application forms and further particulars may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS quoting reference number 6/205/N. 2678(A)

NATIONAL UNIVERSITY OF LESOTHO

Applications are invited for the

CHAIR OF BIOLOGY

tenable from January 1980. Applicants must have a PhD or Masters degree in Biology. They are expected to have documented research ability as well as teaching experience. Lesotho offers considerable scope for research in ecology, palaeontology, and agricultural botany/zoology and applicants with these specialisms would be particularly welcome. Salary scale: R8,580 to 9,000 pa (£1 sterling = R1.86). The British Government may supplement salary by £3,870 pa (sterling) for married appointee or £2,766 pa (sterling) for single appointee (reviewed annually and normally free from tax) and provide childrens education allowances and holiday visit passages. Family passages; baggage allowance; superannuation for those on contract terms is 25% gratuity for the first 2 years rising to 27½% and 30% for each subsequent period of service; 15% inducement for those not in receipt of supplementation unless married to another supplemented member of staff; education allowance; study leave; overseas leave.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Assistant Registrar (Appointments) NUL, Roma, Lesotho by 27 August 1979. Applicants resident in the UK should also send one copy to Inter University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 2698(A)

UNIVERSITY OF ABERDEEN

SENIOR LECTURESHIP IN ANATOMY

Applications are invited for the above newly created post. Candidates should have a strong background in morphological science, and ability to teach both gross and microscopic anatomy is essential. No field of research interest is excluded, but applications from those interested in microscopy, cell biology, neurobiology or endocrinology will be especially welcome. While candidates with a medical qualification would be preferred, non-medical applicants are by no means excluded.

Salary on scale £8,698 to £10,775 per annum, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 18 August 1979. 2658(A)

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL

(University of London)

POST-DOCTORAL RESEARCH FELLOW IN ELECTROPHYSIOLOGY

required to investigate the actions of peptides on cells of the peripheral nervous system in collaboration with Dr T.B. Bolton on an MRC project. Salary in the usual scale (University 1A).

Appointment for 3 years from October 1979 or soon as possible thereafter. Applicants should be committed Electrophysiologists, preferably not more than 28 years old, with experience of intracellular recording techniques. Curriculum vitae, with names of 2 referees, to Dr Bolton (from whom further details can be obtained — 01 672 1255 Ext. 4722), Pharmacology Department, St. George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE. Closing date 31st August. 2717(A)

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university college of swansea

Technicians

Applications are invited for the following vacancies of Technician in the Department of Zoology.

Trainee Technician: Applicants should be under 21 years of age and have four GCE 'O' level passes or Grade 1 CSE, including mathematics and a science subject. The successful candidate will work in an instrument laboratory and/or teaching laboratories.

Technician Grade 3: Applicants should be over 21 years of age and have an ONC or equivalent. The successful candidate will work in an instrument laboratory and/or teaching laboratories.

Technician Grade 5: The successful candidate will be required to give training in histological techniques for light and/or electron-microscopy.

The salaries will be as follows:

Trainee Technician: up to £2,397 per annum

Technician Grade 3: £2,931 to £3,336 per annum

Technician Grade 5: £3,474 to £4,056 per annum

Application forms may be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea SA2 8PP, to whom they should be returned by *Tuesday, 7 August, 1979.* 2689(A)

UNIVERSITY COLLEGE LONDON

Applications are invited for the post of

RESEARCH ASSISTANT

from recent graduates in Biochemistry. Starting date after August 1 by mutual agreement. Salary in range £4,219 to £5,307 inclusive of London Allowance, according to age and experience. The project, under the direction of Professor B. R. Rabin, is concerned with the biosynthesis of membrane proteins and is supported by the Cancer Research Campaign.

Applications including full curriculum vitae and names of two academic referees should be sent as soon as possible to Professor Rabin, Dept of Biochemistry, University College London, Gower Street, London WC1E 6BT. 2703(A)

CHARING CROSS HOSPITAL MEDICAL SCHOOL

(University of London)

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a post of temporary Research Assistant. Duties attached to the post include assistance in the chemical synthesis of heterocyclic compounds and in the study of their metabolism. Interest in experimental work and some experience in organic chemical synthesis are the prime requirements. Subject to a probationary period the appointment will be for two years at a salary of £3,000 per annum. Enquiries to Mr W O Sykes, telephone 01-748 2040, ext. 2610.

A vacancy also exists for a Technician or Junior Technician to assist with the technical work of the Department. Salary on scale £3,261 to £4,680 (Technician) or within range £1,905 to £3,015 (Junior Technician according to age and experience). A London Weighting Allowance of £354 is payable in addition and the salary scales are currently under review. Further information from Mr D J Strutt, telephone 01-748 2040, ext. 2043.

Applications for both appointments to be submitted on forms obtainable from the Secretary, Charing Cross Hospital Medical School, The Reynolds Building, St Dunstan's Road, London W6 8RP, by 3rd September. (Ref: 032/3). 2696(A)

SCIENTIFIC OFFICER

BLOOD GROUP REFERENCE LABORATORY

A graduate is sought for research and development of new techniques relating to the standardisation of reagents (including albumin and enzymes) used in blood group serology. Some experience in protein chemistry or immunochemistry desirable.

Applications in writing with details of experience and the names of two referees to Miss K. Lomas, Blood Group Reference Laboratory, Gatliff Road, off Ebury Bridge Road, London SW1W 8QJ 2714(A)

THE OPEN UNIVERSITY

Earth Sciences Department

Technician £3,474-£4,056

A vacancy exists in the new Mass Spectrometry Laboratory for a Technician. The successful applicant will be responsible for the day to day running of the ultra-clean laboratory area. Duties will include distillation and calibration of ultra-clean reagents, preparation of samples for the solid-source mass spectrometer, and stock control of chemicals and laboratory equipment. There will be considerable opportunity to work closely with academic staff in the development of new techniques and their implementation in a wide variety of projects in the geological sciences.

Applicants should possess a degree in Chemistry with at least four years relevant experience.

Application forms and further particulars are available from The Recruitment Office, (JD3790/1), The Open University, PO Box 75, Walton Hall, Milton Keynes, MK7 6AL or telephone Milton Keynes 63404: there is a 24 hour answering service on 63868.

Closing date for applications is 10th August. 2679(A)

OXFORD UNIVERSITY
PHYSICAL CHEMISTRY LABORATORY

APPOINTMENT OF POST DOCTORAL RESEARCH ASSISTANT

Applications are invited from chemists or physicists to work on the development of lasers for isotope separation. Applicants should have some experience of working with lasers.

The appointment is for 2 years in Research support grade 1A with membership of U.S.S. Starting salary is up to £4,776.

Applications with curriculum vitae and the names of two referees should be sent as soon as possible to the Administrator, Physical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ. 2676(A)

UNIVERSITY OF DURHAM
DEPARTMENT OF ZOOLOGY

POST-DOCTORAL RESEARCH ASSISTANT

required from 1st October 1979, for a three-year project on control of lipogenesis and membrane biogenesis during hormone-induced cell differentiation in avian liver. Research experience in biochemistry or a related field is essential and experience of cell culture/immunochemistry would be an advantage.

Initial salary in the range £4,333 to £4,910 per annum within range 1A, plus superannuation.

Applications (3 copies) including curriculum vitae and naming three referees should be sent by 10 August 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham, DH1 3LE, from whom further particulars may be obtained. 2655(A)

Postdoctoral position open for

YOUNG ELECTRON MICROSCOPIST

interested in supramolecular, biological structures and who is prepared to use various specimen preparation techniques, for which instruction is provided. Some proficiency in electron microscopy in any biological field is required. Well equipped laboratory (including STEM); will work in close collaboration on many biological research projects.

Send curriculum vitae and names of three references to:
Prof. Edward Kellenberger, Department of Microbiology, Biozentrum, University of Basel, Klingelbergstrasse 70, 4056 Basel, Switzerland. W255(A)

BIOCHEMIST
Topic: Parathormone dependent calcium influence on lymphocyte functions

Application from suitable qualified candidates are invited for a research fellowship. Considerable experience in hormone-receptor interactions, ion transport and cellular biology is required. All modern technical equipment is available. Payment BAT II a. The appointment will be made for 3 years. Applications with curriculum vitae, recent publications and the names of two referees should be sent to:

H. Meyer zu Schwabedissen, Calcium Metabolism Research Group, Inst. Dr R.D. Hesch, Dept Endocrinology, Medizinische Hochschule Hannover, Karl-Wiechert Allee 9, Germany. W256(A)

MEDICAL RESEARCH COUNCIL
LABORATORY OF MOLECULAR BIOLOGY

RESEARCH ASSISTANT

required to take part in studies on the biochemistry of transcriptional regulation in prokaryotes and eukaryotes.

Qualifications: Degree or Higher National Certificate or equivalent. Salary: Scales under review. Present starting salary for candidates just qualified £3,261 pa, rising by annual increments to maximum of £4,902 pa. Prospects of promotion to higher grade.

Please apply to Miss F M Taylor, MRC Laboratory of Molecular Biology, The Medical School, Hills Road, Cambridge CB2 2QH. 2693(A)

MRC MAMMALIAN GENOME UNIT

The Medical Research Council invite applications for the post of

DIRECTOR

of their Mammalian Genome Unit, which is housed in the University of Edinburgh.

Candidates should be persons of high scientific standing and proven ability to lead a research team. The salary will be within the range for the Council's Special Appointments Grade, which is equivalent to the Universities' professorial range.

Further particulars may be obtained from Dr K I Gibson, Medical Research Council, 20 Park Crescent, London W1N 4AL to whom applications should be addressed. The final date for receipt of applications is 7 September, 1979. 2707(A)

INSTITUTE OF CANCER RESEARCH: DIVISION OF CHEMISTRY: A POST-DOCTORAL ORGANIC CHEMIST will be required from October 1st to work as a member of a multi-disciplinary project group concerned with the development and application of new reagents for the detection of steroid hormone receptors in human tumours. The post, for three years, will be funded by the MRC and the work will be carried out in the Institute's Haddow Laboratories in Sutton, Surrey. Starting salary will be in scale £4,261 to £4,805 p.a. (MRC non-clinical scientist Grade II) plus London Allowance of £502 p.a. Applications with CV and details of research experience (in duplicate) and with the names of two referees should be sent to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 300/G/11. 2710(A)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF PURE MATHEMATICS

TEMPORARY LECTURESHIP

Qualified Mathematicians are invited to apply for the above post, which will involve normal lecturing duties in the department. The appointment will run from 1 October 1979 to 30 September 1980.

Salary will be on the scale £4,333 to £8,992 (to operate from 1.10.79) plus superannuation.

Starting salary will depend on age, experience and qualifications.

Applications (three copies) including curriculum vitae and the names of three referees should be sent by 17th August 1979 to the Assistant Registrar, Science and Engineering, University of Birmingham, PO Box 363, Birmingham B15 2TT, from whom further particulars can be obtained. Please quote ref: NA1. 2684(A)

UNIVERSITY OF BRISTOL
SCHOOL OF CHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Postdoctoral Research Assistant to join a group investigating the metabolism of gibberellins in higher plants. Some experience in the synthesis of isotopically-labelled compounds would be advantageous.

The appointment, funded by the SRC, is for a maximum of two years. Salary in the range £4,232 to £4,776 p.a. (scale subject to revision from 1st October, 1979).

Applications, enclosing a curriculum vitae and the names of two referees should be sent to Professor J. MacMillan by 31st August, 1979. 2650(A)

INSTITUTE OF CANCER RESEARCH: DIVISION OF CHEMISTRY: A JUNIOR TECHNICIAN is required to work in the Institute's Haddow Laboratories in Sutton, Surrey as part of a research team investigating steroid hormone receptors in human tumours. Applicants should have two 'A' levels in Maths, Physics or Chemistry. Starting salary will be £2,169 p.a. (under review) plus London Allowance of £354 p.a. Applications in duplicate with the names of two referees should be sent to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 301/B/82. 2709(A)

FELLOWSHIPS

FELLOWSHIP AT THE FRIEDRICH MIESCHER-INSTITUT, Basel, Switzerland

In the Friedrich Miescher-Institut, a fellowship (2-3 years) is available for a PhD or MD in a research group examining the role of endogenous C-type viruses in leukemogenesis and lymphocyte function. Salary in accordance with the Swiss National Foundation. Travel costs will be covered.

Candidates, preferably with experience in immunology, virology or nucleic acid chemistry, please write to:
Dr Christoph Moroni, Friedrich Miescher-Institut, P.O. Box 273, CH-4002 Basel, Switzerland. W253(E)

THE CITY UNIVERSITY
DEPARTMENT OF PHYSICS

POST-DOCTORAL FELLOWSHIP IN GEOPHYSICS

A post-doctoral fellowship financed by the Natural Environment Research Council is available for a study of the inter-action of elastic-waves with sub-surface features in solid media. (With special reference to shallow seismic surveys.)

The programme involves numerical modelling together with experimental verification on both laboratory and field scales. A geophysicist, physicist, engineer or applied mathematician would be eligible, the primary qualification being an interest in computer modelling. The appointment will be for 2 years and the commencing salary is expected to be £5,007 including London Weighting with superannuation U.S.S.

Further information can be obtained from Professor A F Brown, Department of Physics, The City University, Northampton Square, London ECIV 0HB, tel. 01 253 4399 ext. 391 or 215. 2720(E)

UNIVERSITY OF DUBLIN
Trinity College

POST-DOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a post-doctoral research fellowship supported by the National Board for Science and Technology to study the structural control of mineralisation across central Ireland, using detailed structural methods and remote sensing. The project will involve three years of collaborative research with a small group of structural geologists and computer scientists.

Salary scale: £4,173 to £4,516, plus field expenses.

Further details can be obtained from Dr W.E.A. Phillips, Department of Geology, Trinity College, Dublin 2, telephone (01) 772941, Ext. 1074, Telex No. 5442 TCD EI, to whom applications with curriculum vitae and the names of two referees should be addressed.

Closing date for receipt of applications will be August 31st 1979. 2718(E)

UNIVERSITY OF WARWICK
RESEARCH FELLOWSHIPS IN MICROBIOLOGY

Several postdoctoral positions are available in the Microbiology group in the Department of Biological Sciences, to work on the biochemistry and physiology of methane-oxidizing bacteria with Dr H. Dalton and the control and regulation of cellular differentiation in prokaryotic model systems with Dr C.S. Dow. Candidates with interests in either of these topics should contact the above named lecturers from whom further details may be obtained.

The appointments will be for three years in the salary range £4,333 to £5,488 p.a. Application forms from the Academic Registrar, University of Warwick, Coventry CV4 7AL quoting Ref.No:51/A/79 2711(E)

QUEEN MARY COLLEGE (University of London)
ZOOLOGY AND COMPARATIVE PHYSIOLOGY DEPARTMENT

Applications are invited for a Postdoctoral

RESEARCH FELLOWSHIP

to work with Dr L.J. Goodman on sensory mechanisms controlling orientation during locomotion, including flight, in the honeybee. Applicants should be experienced in electrophysiological recording techniques and preferably familiar with intracellular recording and staining methods. Appointment for 3 years from 1 October.

Initial salary in range (under review) £4,835 to £5,412 p.a. (including London Allowance). Please apply as soon as possible, giving age, qualifications, experience and names of 2 referees, to The Registrar, (N) Queen Mary College, Mile End Road, London E1 4NS. 2687(E)

INSTITUTE OF CANCER RESEARCH
A three year post-doctoral Fellowship is offered for a Protein Chemist in the Division of Molecular Biology to work on the isolation and characterisation of chromosomal proteins. Experience of protein sequencing techniques would be an advantage. Salary in range £4,261 to £4,805 per annum plus London Allowance of £502 per annum.

Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place London SW7 3NU, quoting ref 300/G/10 2654(E)

FELLOWSHIPS and STUDENTSHIPS continued

EMBO

European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY AUTUMN 1979 AWARDS

Next deadline: August 31, 1979

EMBO long term fellowships are initially awarded for one year. Applications for a renewal for a second year and subsequently **in cases of exceptional scientific merit for a third year** are considered.

To be eligible a candidate must hold a doctors degree. Preference will be given to European and Israeli applicants wishing to work within Europe or Israel. EMBO long term fellowships are not, however, awarded for exchanges between laboratories within any one country. Applications for fellowships to be held outside Europe and Israel are considered but they have a lower priority, as do applications from non-European scientists wishing to work in Europe or Israel.

Successful applicants will be notified of their awards on October 29, 1979.

Further details and application forms may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F. R. Germany.

W251(E)

MAX-PLANCK-INSTITUT FÜR MOLEKULARE GENETIK, BERLIN

offers

RESEARCH FELLOWSHIP

and seeks

BIOCHEMICAL TECHNICIAN

A postdoctoral research fellowship is available for the study of function, structure and assembly of ribosomes. Experience in either electron microscopy (negative staining and shadowing of biomolecules), or immunochemistry, protein chemistry, or in the field of protein synthesis, while not essential, would be an advantage. The fellowship is available immediately for up to 2½ years with probable extension (DM40,000 to 50,000 pa). Applications should include a curriculum vitae, a list of publications, and the names of at least three referees.

Applications are invited from **Technicians/Research Officers** for work involving electron microscopy and/or immunochemistry. Applicants should have at least two years relevant laboratory experience and should be able to work on research projects without day-to-day supervision. (Position available immediately, for up to three years; salary scale depending on experience and age: DM25,000 to 30,000.)

All applications should be sent to Professor Georg Stöffler, Max-Planck-Institut für Molekulare Genetik, Ihnestrasse 63/73, D1000-Berlin 33. W257(E)

UNIVERSITY COLLEGE, DUBLIN and DUBLIN INSTITUTE for ADVANCED STUDIES

POST-DOCTORAL FELLOWSHIPS in MATHEMATICAL PHYSICS

pplications are invited for two positions upported by the National Board for cience and Technology to work on wave ropagation in elastic materials with plictions to seismological problems. The ellowships are available from 1st October 79, for one year initially with extensions ssible for a further two years, at a salary the range £3,837 to £3,994. to £4,153 to ,311. to £4,476. (at present under view).

Intending applicants should, without lay, contact either Professor Michael ayes, Department of Mathematical aysics, University College, Dublin, 4, elephone 693244, or Professor John ewis, School of Theoretical Physics, ublin Institute for Advanced Studies. 10 rlington Road, Dublin, 4, Telephone 0748. 2716(E)

Southampton THE UNIVERSITY

SOLAR CELL THEORY
Post-doctoral SRC Fellowship

Applications are invited for the above post-doctoral post which is available from 1 September 1979, and is suitable for appropriately interested mathematicians, physicists or engineers. Salary up to £4,505.

Applications giving the names of two referees should be sent to Professor P T Landsberg, Department of Mathematics, University of Southampton, Highfield, Southampton SO9 5NH, before 9 August 1979. Please quote Ref: N. 2662(E)

QUEEN MARY COLLEGE (University of London)

LASER MEASUREMENT OF BOVINE SPERMATOZOA MOTILITY

Applications are invited for a 2-year Postdoctoral

RESEARCH FELLOWSHIP,

from 1 October, to work with Dr D.A. Ross, in conjunction with the Ministry of Agriculture Cattle Breeding Centre, Reading, to verify the viability of routine assessment of samples of frozen semen, using the new fibre optic Doppler anemometer, and to correlate motility measurements with fertility trials. Candidates with a PhD in Physics or Electrical Engineering and interest in or direct experience with laser anemometry or photon correlation preferred. Initial salary in range (under review) £4,835 to £5,412 p.a. (including London Allowance). Please apply by 15 August, giving age, qualifications, experience and names of 2 referees, to The Registrar, (N), Queen Mary College, Mile End Road, London E1 4NS. 2688(E)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF BOTANY

SRC/CASE RESEARCH STUDENTSHIP WITH ENGLISH CHINA CLAYS LTD

(Amelioration of Derelict Land Materials for Vegetation establishment)

This work will examine the effects of organic and inorganic amendment to raw china clay waste, to improve situations for land reclamation. It should provide interesting work for someone with practical and theoretical interests.

Applications with the names of two referees should be received not later than 9th August, 1979, by The Registrar, The University, P.O. Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/706/N. 2660(F)

UNIVERSITY OF YORK

DEPARTMENT OF BIOLOGY

ARC POST-DOCTORAL RESEARCH FELLOWSHIP IN CHLOROPLAST BIOCHEMISTRY

Applications are invited for a post-doctoral Research Fellowship to investigate the characteristics and control of chloroplast division in wheat leaves under the direction of Professor Rachel Leech. The project will involve the study of the role of the nuclear genome in the regulation of chloroplast division. Preference will be given to applicants with experience in both plant biochemistry and plant ultrastructure.

The appointment, which will be on Grade 1A of the national salary scales for research staff, will be for three years.

Informal enquiries and further particulars from Professor R. M. Leech, Department of Biology. Two copies of applications, giving a curriculum vitae and the names of two referees, should be sent by Friday 1 September 1979 to the Registrar, University of York, Heslington, York, YO1 5DD. Please quote reference number 11/6105(a). 2653(E)

PHYSICAL BIOCHEMISTRY

Applications are invited for a post-doctoral research fellowship to work on the spectroscopy and kinetics of ribosomal systems in the Max-Planck-Institut für Molekulare Genetik in West Berlin.

Candidates are expected to have a PhD in chemistry or physical biochemistry and some experience of spectroscopy. Research experience in biochemistry and some knowledge of German would be helpful but not essential.

Applications (which must include a full academic curriculum vitae and a short statement of research experience) should be sent to Dr P. Woolley, Max-Planck-Institut für Molekulare Genetik, Ihnestrasse 63-73, 1000 Berlin 33, W. Germany. W254(E)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF CANCER STUDIES

PhD STUDENTSHIP

A good honours degree graduate (Microbiology, Biological Science, or Virology) is required to join a project which has a three year Cancer Research Campaign grant to study the interaction of human wart viruses with human epithelial cells in tissue culture.

Applications giving a full curriculum vitae and the names of two academic referees should be sent to Dr P H Gallimore, Department of Cancer Studies, University of Birmingham. 2695(F)

STUDENTSHIPS

UNIVERSITY OF BRITISH COLUMBIA Vancouver, Canada

DEPARTMENT OF PHYSIOLOGY

Applications are invited for a research studentship available from September 1st, 1979. The successful candidate will be expected to work in the field of the reflex control of the cardiovascular and respiratory systems and to register for a higher degree. Applicants should have a First Class or Upper Second Class Honours degree in a biomedical subject.

The studentship will be valued at $6,500 per annum and may be supplemented by additional teaching duties.

Applications including a curriculum vitae and the names of two referees should be sent as soon as possible to Dr J R Ledsome, Department of Physiology, UBC, Vancouver, BC, Canada V6T 1W5. W243(F)

FOR SALE and WANTED

ALMOST ANY SCIENTIFIC and learned periodicals, proceedings, transactions etc. wanted in long and complete runs/sets. H. Pordes Ltd., 529B Finchley Road, NW3 7BH. Tel: 01-435 9878 after 2 p.m. 2673(L)

ASSISTANTSHIPS

UNIVERSITY COLLEGE LONDON
CHEMISTRY DEPARTMENT
POST-DOCTORAL RESEARCH ASSISTANTSHIPS

Applications are invited for SRC post-doctoral research assistantships in the following fields:
Dr A J Bloodworth: A new approach to the synthesis of crown ethers and related macroheterocycles.
Dr T M Cresp: The total synthesis of some biologically active sesquiterpenes.
Professor A G Davies: Homolytic reactions of organic and organometallic compounds, and the study of the radical intermediates by e.s.r. spectroscopy.
Dr M D Johnson: Rearrangements of organic ligands in organotransition metal compounds.
Dr B P Roberts: E.s.r. studies of transient organic and organometallic radicals and radical ions, generated by electrochemical, photochemical, and non-acqueous flow techniques; some organic synthesis will also be involved.

Appointments will be for one year in the first instance, with the possibility of renewal, and the salary will be within the range £4,763 to £5,576 plus USS.

Applications, with a curriculum vitae and names and addresses of two referees, should be sent as soon as possible to the appropriate staff member at the Chemistry Department, University College London, 20 Gordon St, London WC1H 0AJ, UK. (Tel: 01-387 7050) 2702(P)

THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF CHEMISTRY

Applications are invited from men and women for an
S.R.C. POSTDOCTORAL RESEARCH ASSISTANTSHIP
to study the photochemistry of carbanions and, in particular the cis-trans photo-isomerisation of diphenyl-allyl and related anions. Experience of vacuum techniques for handling reactive compounds and/or photo-chemistry or photophysics (flash photolysis, measurement of fluorescence lifetimes and spectra) needed.

Tenable three years from 1 October 1979. Initial salary up to £4,910 a year on Range IA.

Applications, naming two referees to Drs B. Brocklehurst and R.N. Young, Chemistry Department, the University, Sheffield S3 7HF, from whom further information can be obtained. Quote ref: R 343/G. 2680(P)

UNIVERSITY OF MANCHESTER
DEPARTMENT OF MEDICAL BIOPHYSICS
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a post-doctoral research assistantship, tenable for one year in the first instance, to study molecular organization in collagen fibrils. Experience in electron microscopy and in computing essential.

Applications, with names of two referees, should be sent immediately to Dr J.A. Chapman, Dept. of Medical Biophysics, Medical School, Oxford Road, Manchester, M13 9PT, from whom further particulars can be obtained. Please mark envelopes "PDRA application". 2713(P)

UNIVERSITY OF OXFORD
LABORATORY OF MOLECULAR BIOPHYSICS
POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for an SRC post-doctoral research assistantship to carry out research on the refinement of the crystal structure of glycogen phosphorylase b at 2A resolution. The refinement is a challenging problem and should lead to a detailed understanding of the conformation, control and catalytic properties of this key control enzyme. An ICL 2980 computer in the University of Oxford and a Cray 1 backed by an IBM 360/165 at the SRC Computing Laboratory at Daresbury are available for this work. Applicants should have experience in crystallographic computing. The position is for 3 years with salary according to age and experience in the range Grade 1A (£5,048 at age 27). Applications with the names of two referees should be sent by September 1st to Laboratory of Molecular Biophysics, South Parks Road, Oxford OX1 3PS. 2721(P)

UNIVERSITY OF LONDON
ST BARTHOLOMEW'S HOSPITAL MEDICAL COLLEGE
and the
INSTITUTE OF NEUROLOGY

Applications are invited from good honours graduates in Biochemistry or related subjects for a
RESEARCH ASSISTANTSHIP
to undertake investigations into the metabolism of muscle mitochondria in both normal and diseased states. The successful candidate would be expected to register for a PhD degree.

The appointment is for three years from 1st October at an initial salary (under review) of £4,191 per annum including London weighting.

Applications, with c.v. and names of three referees, should be sent by 20th August to Dr J B Clark, Biochemistry Department, St Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ (Tel: 01-253 0661, ext 22). 2628(P)

UNIVERSITY OF EAST ANGLIA
Norwich

Applications are invited for one
SRC GRADUATE RESEARCH ASSISTANTSHIP
in the School of Chemical Sciences (salary 1B scale) to work on the magnetic circular dichroism spectra of metalloproteins and their inorganic models. The post will involve liaison with groups of biochemists at various Universities and is suitable either for a chemist or a biochemist with physio-chemical interests. The award is tenable for three years. If appropriately qualified the successful candidate will be permitted to register for the degree of PhD. Candidates should have or expect to obtain soon a good honours degree. Application, including the names of two referees, as soon as possible, to Dr A. J. Thomson, School of Chemical Sciences, University of East Anglia, Norwich NR4 7TJ, from whom further details may be obtained. Salary from £3,689 rising by increments of £271. 2652(P)

ST PATRICK'S COLLEGE
Maynooth, Co Kildare
Ireland
DEPARTMENTS OF BIOLOGY AND CHEMISTRY

Applications are invited for a post-doctoral research assistantship supported by the NBST to study fundamental biological and chemical aspects of the ensilage process, in association with An Foras Talúntais (The Agricultural Institute). The project involves the development of a novel model silo in which the fundamental biochemistry, chemistry and microbiology of the process of silage making will be studied in an interactive manner. (Salary scale: £4,173 to £4,516, under review.)

Further details can be obtained from either Professor C M Quinn, Department of Chemistry, or Professor P A Whittaker, Department of Biology, St Patrick's College, Maynooth, telephone (01) 286262, telex 31493, to whom applications with curriculum vitae and the names of two referees should be addressed. 2690(P)

COURSES

BIRBECK COLLEGE (University of London)
DEPARTMENT OF ZOOLOGY
M.Sc. IN ENTOMOLOGY

This two-year, part-time evening course is designed primarily for entomologists but also allows teachers and others to extend their training in a scientific discipline and to become conversant with the tools of current research. Topics covered include: sensory and neuromuscular physiology, respiratory physiology, osmotic and ionic regulation, insect genetics, taxonomy and economic entomology.

For further details contact the Departmental Secretary, Department of Zoology, Birkbeck College, Malet Street, London, WC1E 7HX. 2683(D)

UNIVERSITY OF LEICESTER
DEPARTMENT OF ASTRONOMY
SRC RESEARCH ASSISTANTSHIP IN THEORETICAL ASTROPHYSICS

Applications are invited for a research assistantship for three years from 1 October 1979. Salary scales at present are £4,232 to £5,850 with agreed scales at £4,333 to £6,066 and subject to further review expected to be effective from 1 October 1979.

The current theoretical research interests of the Department are in the areas of (a) cataclysmic variables and (b) active galaxies which are carried on in collaboration with the X-ray and infrared astronomy groups. For further details and application form write to The Secretary, Department of Astronomy, The University, Leicester LE1 7RH. The closing date for applications is 20 July 1979. 2712(P)

BIRBECK COLLEGE (University of London)
DEPARTMENT OF ZOOLOGY
M.Sc IN COMPARATIVE PHYSIOLOGY

A two-year part-time evening course suitable for zoology graduates wishing to specialise in the comparative aspects of the adaptations of animals to their environment. Topics covered include: osmotic and ionic regulation; respiratory & circulatory physiology; endocrinology; neurophysiology. Students are introduced to a wide range of modern physiological techniques.

For further details contact the Departmental Secretary, (N) Department of Zoology, Birkbeck College, Malet Street, London, WC1E 7HX. 2682(D)

ASSOCIATESHIPS

HERIOT-WATT UNIVERSITY
DEPARTMENT OF CHEMISTRY
RESEARCH ASSOCIATES

Applications are invited for one SRC Postgraduate and two SRC Postdoctoral Research Associateships to commence on 1 October 1979.

(a) Quantitative Studies in Silaethylene Chemistry
The successful applicant will collaborate with Dr. P. John and Professor B. G. Gowenlock.
A physical chemist is required with experience in gas phase kinetics to continue studies of reactive intermediates in silicon chemistry.

(b) Reactions of Elemental Phosphorus with Transition Metal Complexes
The appointed person will work in conjunction with Dr. W. E. Lindsell. Previous experience in synthetic organometallic/inorganic chemistry would be advantageous.

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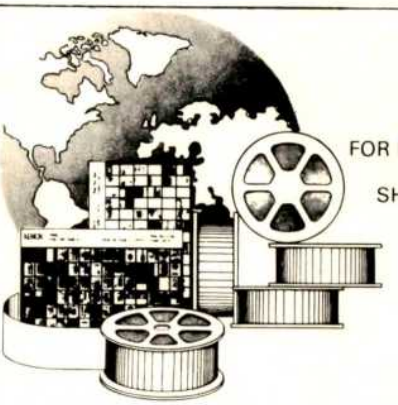
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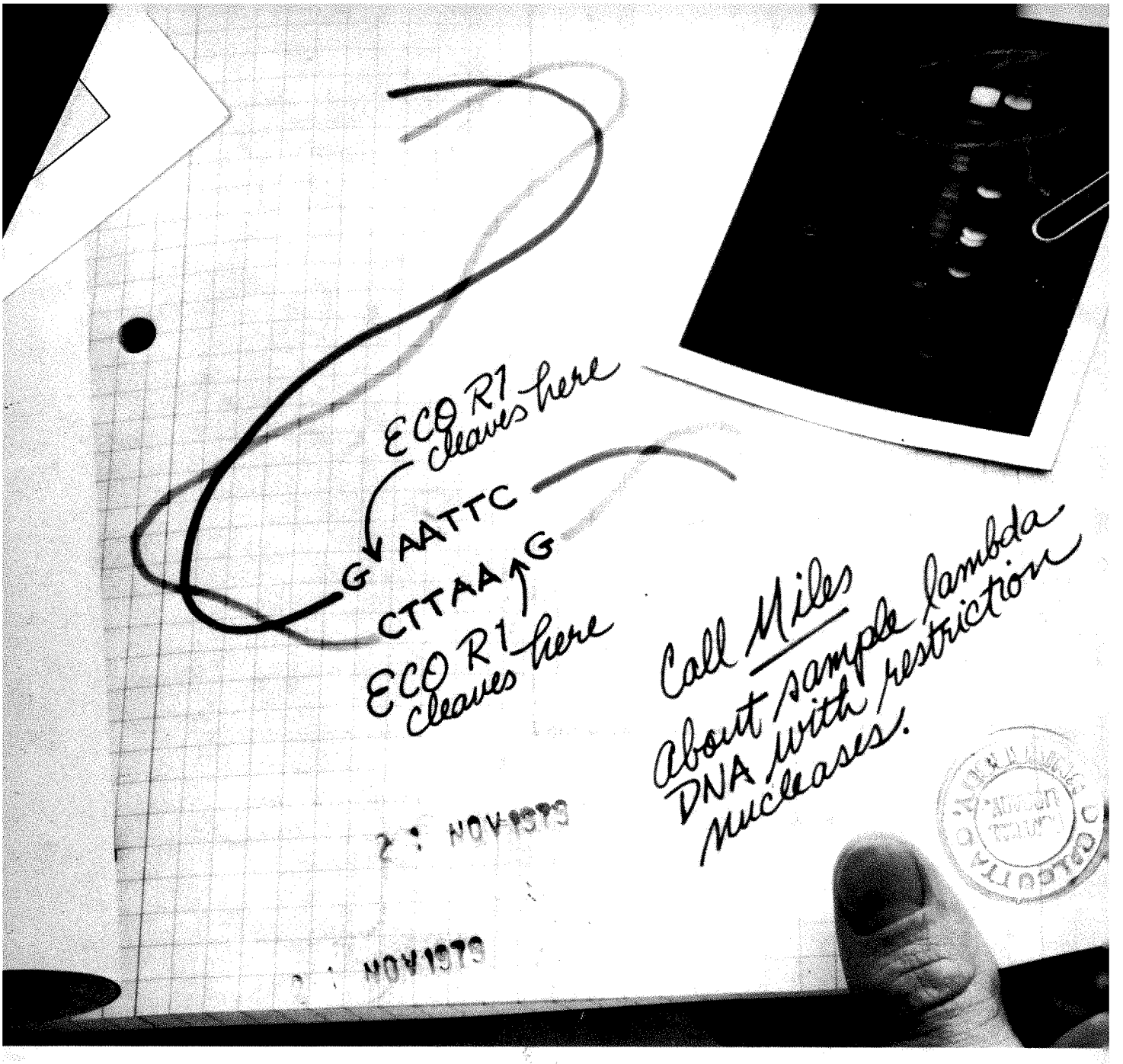


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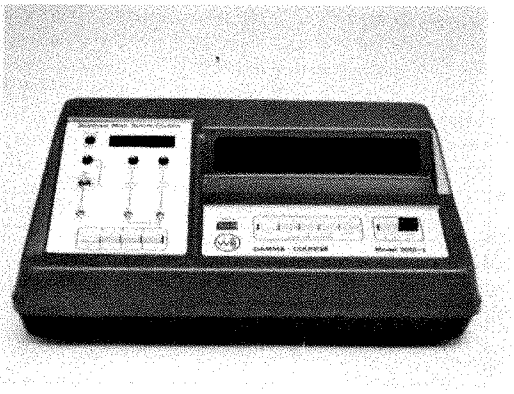
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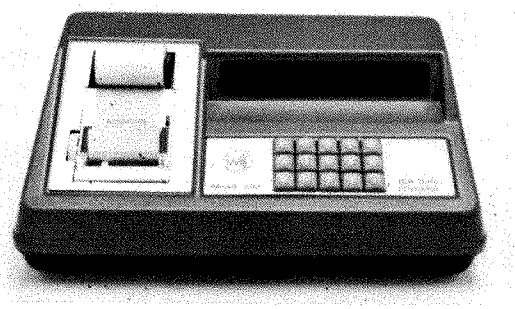
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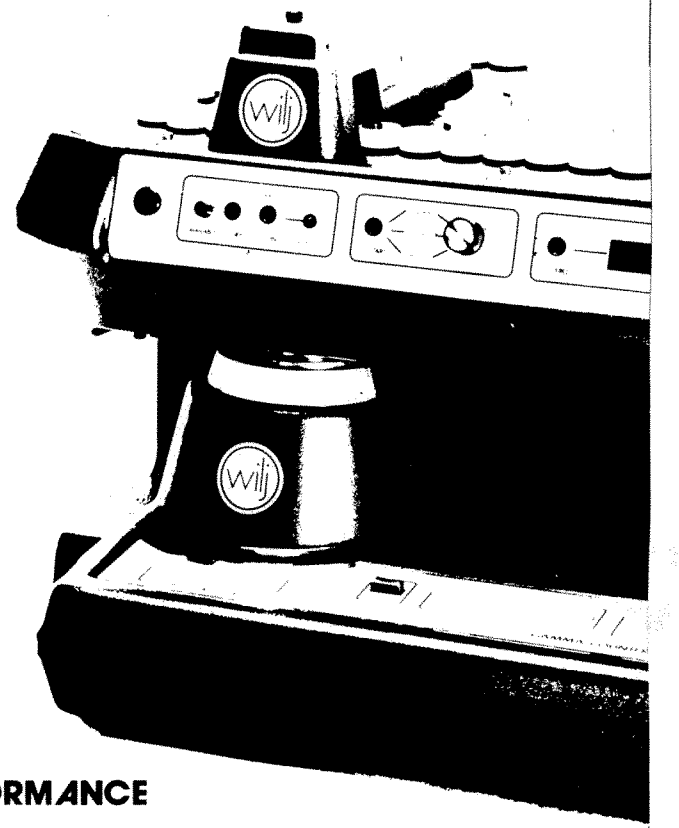
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Cover picture

Parasites and populations: the role of disease in regulating population growth is reviewed on page 361. The cover shows three-spined sticklebacks (*Gasterosteus aculeatus*) from the Japanese pond garden at Imperial College Field Station, Silwood Park, infected with microsporidian parasites (*Glugea*.sp).

Photo: F. W. Wright and H. A. Devitt.

Vol.280 No.5721 2 August 1979

nature

Volume 280 2 August 1979

Hunting the quangos 345
SRC apprenticeship scheme/Max-Planck venture fails 347
Brazil: scientists protest; rainforests destroyed 347
In brief 349
Egypt and Israel . . . scientific cooperation? 350
Hungary steers a course without UN aid 351
Fine-tuning for British technology 352
Correspondence 354

NEWS & VIEWS

Decline of the Ca hypothesis of visual transcution/ New wave of superfluid ^{4}He/Measuring weak absorption spectra/Eukaryotic transcription *in vitro*/ Implications of immunological heterogenity of tumours/Is NGF an enzyme?/Stellar rotation in Erice 355

REVIEW ARTICLE

Population biology of infectious diseases: Part I — *R.M. Anderson and R.M. May* 361

ARTICLES

Possible optical observation of the companion star to the binary pulsar PSR1913+16 — *P. Crane, J.E. Nelson and J.A. Tyson* 367

A κ-immunoglobulin gene is formed by site-specific recombination without further somatic mutation — *J.G. Seidman, E.E. Max and P. Leder* 370

LETTERS

Persistent emission from X-ray burst sources and the nature of galactic bulge X-ray sources — *J. van Paradijs, P.C. Joss, L. Cominsky and W.H.G. Lewin* 375

The ring systems of Jupiter, Saturn and Uranus — *R. Smoluchowski* 377

Cyclic reaction mechanism in the electrodeposition of aluminium — *T.E.G. Daenen* 378

Effects of acetylene and soil water content on emission of nitrous oxide from soils — *J.M. Bremner and A.M. Blackmer* 380

Non-uniform vertical distribution of fine sediment in the Amazon River — *W.F. Curtis, R.H. Meade, C.F. Nordin, Jr, N.B. Price and E.R. Sholkovitz* 381

Palaeomagnetism of stalagmite deposits — *A.G. Latham, H.P. Schwarcz, D.C. Ford and G.W. Pearce* 383

Growth rate of a clam from the Galapagos Rise hot spring field using natural radionuclide ratios — *K.K. Turekian, J.K. Cochran and Y. Nozaki* 385

Eucalyptus fruits in stratigraphic context in Australia — *G.J. Ambrose, R.A. Callen, R.B. Flint and R.T. Lange* 387

Long-term recognition of father's song by female zebra finches — *D.B. Miller* 389

Vertebrate and invertebrate predation intensity on freshwater zooplankton communities — *P.A. Lane* 391

Autoradiographic localisation of benzodiazepine receptors in the brains of humans and animals — *W.S. Young, III and and M.J. Kuhar* 395

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Central remyelination restores secure conduction	*K.J. Smith, W.F. Blakemore and M.J. Kuhar*	395
The optical mechanism of the eye of *Limulus*	*M.F. Land*	396
GTP hydrolysis in intact rod outer segments and the transmitter cycle in visual excitation	*W.E. Robinson and W.A. Hagins*	398
Potentiation of Ca^{2+}-dependent K^+ activation by theophylline is independent of cyclic nucleotide elevation	*P.A. Smith, F.F. Weight and R.A. Lehne*	400
Whitefly have the highest contraction frequencies yet recorded in non-fibrillar flight muscles	*R.J. Wooton and D.J.S. Newman*	402
Antiserum specific for motor nerve terminals in skeletal muscle	*J.R. Sanes, S.S. Carlson, R.J. von Wedel and R.B. Kelly*	403
T-cell-derived helper factor allows Lyt 123 thymocytes to differentiate into cytotoxic T lymphocytes	*H. Wagner, M. Röllinghoff, R. Schawaller, C. Hardt and K. Pfizenmaier*	405
Lymphomas with cytotoxic activity	*T.W. Chang and H.N. Eisen*	406
The role of glucocorticoid receptor and gene expression in the anti-inflammatory action of dexamethasone	*S. Tsurufuji, K. Sugio and F. Takemasa*	408
A new proposal regarding the subunit composition of $(Na^+ + K^+)$ATPase	*O. Hansen, J. Jensen, J.G. Norby and P. Ottolenghi*	410
Semi-synthesis of human insulin by trypsin-catalysed replacement of Ala-B30 by Thr in porcine insulin	*K. Morihara, T. Oka and H. Tsuzuki*	412
Chlorophyllin–apomyoglobin complexes	*R.C. Davis and R.M. Pearlstein*	413
An inhibitor protein of nuclear protein kinases	*F. Farron-Furstenthal*	415
A chiasma–hormonal hypothesis relating Down's syndrome and maternal age	*P.H. Crowley, D.K. Gulati, T.L. Hayden, P. Lopez and R. Dyer*	417
Specific DNA methylation sites in the vicinity of the chicken β-globin genes	*J.D. McGhee and G.D. Ginder*	419
Long regions of single-stranded DNA in human cells	*G. Bjursell, E. Gussander and T. Lindahl*	420
Codon–anticodon interaction at the ribosomal peptidyl-site	*R. Lührmann, H. Eckhardt and G. Stöffler*	423
Atmospheric lead pollution of grass grown in a background area in Denmark	*J.C. Tjell, M.F. Hovmand and H. Mosbaek*	425

BOOK REVIEWS

Sociobiology: Sense or Nonsense? (Michael Ruse)	*Richard Dawkins*	427
The Interpretation of Visual Motion (Shimon Ullman)	*K. Prazdny*	428
Inhibitors of Protein Biosynthesis (David Vazquez)	*Eric Cundliffe*	428
Strategies of Information Processing (G. Underwood, editor)	*Patrick Rabbitt*	429
Mechanics and Energetics of Biological Transport (E. Heinz)	*S.R. Caplan*	429
Problems of Linear Electron (Polaron) Transport Theory in Semiconductors (M.I. Klinger)	*J.C. Inkson*	430
Science of Materials (W. Brostow)	*E.H. Andrews*	430

OBITUARY

Dennis Gabor	*Eric A. Ash*	431
Airey Neave	*Arthur Palmer*	433

(appeal for the Airey Neave Memorial Trust on page 354)

NEWLY ON THE MARKET vi

nature

2 August 1979

Hunting the quangos

Britain's quangos should open themselves up more; putting them on the defensive will hardly help.

THE councils, committees, boards, institutes and so on, listed in the box, are all quangos. There exists no unique definition of a quango (originally a quasi-non-governmental organisation), but it may be taken that it performs governmental or public functions, or spends public money. It is not part of a government department, but it does have a dependent relationship with a department or with a minister for authority and/or for funds.

A quango-hunt is on at the moment; it is said that government ministers have been encouraged to see how many can be disbanded or trimmed. So it is valuable to have a report, *What's wrong with quangos?*, published by the Outer Circle Policy Unit (£2.00), which gives some background to the incredible diversity of quango that now exists. There are certainly serious questions to be asked of quangos — the report identifies issues of financial and political accountability, adequate provision of information concerning their activities and the secretive nature of much of the ministerial patronage that surrounds top appointments. In other words, quangos should be more open to scrutiny and accessible to the general public than they often are.

The question of who gets the plum jobs is a case in point. There is at present no provision whatsoever for most of the chairmanships of quangos to be advertised, nor (as the report points out) is there anything like adequate provision for an incompetent chairman to be brought to book. Furthermore many quangos have prestigious and influential councils where, again, the selection process is veiled from public view. And the council member's duties are most unlikely to contain any explicit instructions to consult or inform a particular constituency.

Many of the ministerial appointments to quangos draw from the very British concept of a pool of 'the good and the great' — a list of worthy people said to reside in Civil Service Department filing cabinets (the list, that is, not the people). But this list is not comprised of those who at various times have responded to advertisements or who have expressed a wish to involve themselves in policy matters — indeed, anyone who did so would probably be regarded as too pushy for appointment to anything. Rather, it is the product of a grapevine, and so unlikely to contain an adequate representation of those with radical views, those whose profession keeps them remote from well-trodden paths, or those not yet forty.

Many (but not all) of the scientific quangos listed fulfil very necessary functions which would still have to be done (by civil servants?) if there were wholesale trimming, 'rationalisation' or cuts. To this extent they are probably fireproof. But quangos also have the merit of exposing outsiders to insiders and *vice versa*, and so should fulfil a vital role, in highly compartmentalised Britain, of providing at least a modicum of cross-fertilisation across the yawning chasms which exist between communities — academics, bureaucrats, industrialists, politicians. For this reason, there should be the greatest resistance to any move to replace quangos, particularly by civil service operations. However, there is still scope for quangos to improve their relationship with the outside world, both in terms of recruitment and in terms of the provision of adequate information on their mode of working. A wholesale quango-hunt will cause just the opposite to happen.

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Nature Vol. 280 2 August 1979

Universities accused of inefficient book-keeping

David Dickson reports on continued skirmishing between US politicians, administrators and scientists over use of research funds

Government auditors have accused Harvard University of claiming over $2 million in "inappropriate" costs charged to federal research projects carried out by the university's School of Public Health between 1975 and 1977. They further state that, on the basis of sample studies of cost transfers, "we cannot attest to the proprietary of about $15 million charged to federal grants" during this period, out of a total of $37.1 million audited.

These conclusions have emerged from an audit carried out by the Department of Health, Education and Welfare following allegations that the university was violating agreed procedures for claiming research costs from the federal government. The university has already announced its intention to challenge the draft audit, which has not yet been publicly released; but the incident has fuelled further skirmishing between politicians, administrators and scientists over how universities should account for their use of public research funds.

The politicians insist that, since the funds come from the public purse and their expenditure has been decided by Congress, then it should be demonstrated that they are spent in the way that Congress intended. But the universities claim that too much time spent accounting for the use of funds can hamper the research scientist and may ultimately undercut the value of the research itself.

Allegations about Harvard's misuse of funds were first made by Dr Phin Cohen, previously a research worker in the School of Public Health, in 1975. He repeated the allegations — and provided details of DHEW's draft audit — at a hearing held recently by the intergovernmental relations and human resources subcommittee of the House committee on government operations.

A particular focus of the hearing was a report just completed by the General Accounting Office claiming that many audits of university research expenditures were "not as effective as they could be". In some cases, inadequate university accounting systems meant federal auditors were unable to give an opinion on the allowability of costs charged to federal grants and contracts.

The GAO points out, for example, that of $1.2 billion in research grants and contracts audited by DHEW in 1977, over a third was not properly documented. In one university, $53.7 million of personal service costs, out of $111 million charged to federal grants and contracts over a three year period, could not be adequately supported, since they were based on budget estimates rather than actual certified costs.

The GAO was also critical of some of the DHEW's audit operations. Department officials, however, while accepting some weaknesses in auditing procedures, placed considerable blame on the universities which, they said, frequently failed to keep accounts in a form which meets federal standards. Mr Edward Stepnick, assistant inspector general for auditing at DHEW said compliance with auditing requirements was non-existent or inadequate at about 70% of the major colleges and universities audited.

Universities, in turn, continue to charge that the accounting requirements placed on them are often unreasonable and unrealistic. Their accounting is in fact, much better than they are being given credit for, according to Dr Max Binkley, vice-president for finance at Colorado State University. Publicity alleging scandal and malfeasance gave the public the wrong impression of issues which principally involved questions of accounting precision and documentation, he said. "A scientist is deeply engrossed in the pursuit of new knowledge in his or her field of specialisation, with the result that efforts towards precision in costing are disruptive and burdensome and constitute an impediment to the accomplishment of the ultimate objective."

All sides hope that the situation will be improved by the recent revision of Circular A-21, a document published by the Office of Management and Budget laying down guidelines for charging the direct and indirect costs of research. Considerable differences still remain over costing principles, — particularly the way that research workers should account for the time spent on various projects — "the toughest and longest standing problem revealed by our audits" according to Mr Stepnick.



Harvard: sitting tight and challenging the audit

Under existing accounting rules, scientists receiving federal grant money have to report regularly on the way they have spent the previous month or two weeks. Under the revised A-21, a university can opt for a monitored workload system, in which the distribution of time is agreed before the project begins. Changes in the agreed workload have to be reported, and "significant" changes entered in the accounts. Mr Bowman Cutter, director of the budget in OMB, said this was a reasonable compromise, reached after considerable negotiation, between the demands of accountability and the flexibility needed by universities to administer research in an effective way.

University representatives, however, disagreed. They had been pressing that only *significant* changes in workload need be reported. "Insignificant changes to workload occur daily, perhaps even hourly," Dr Brinkley said. "Having to note all minor variations in activity was virtually impossible." He told the subcommittee that OMB's decision meant most institutions would not now adopt the monitored workload system, but opt instead for the personnel activity reporting, with which neither the government nor the universities were satisfied. "The overzealous attempt by the government to refine the system has evidently brought its defeat", he said.

Dr Thomas A. Bartlett, president of the Association of American Universities, told the subcommittee that his association wanted a number of changes in federal accounting rules. These included adoption of a monitored workload system "as a fully acceptable means for documenting effort", and grouping related grants for the purposes of administration. On the first, he echoed Dr Binkley's complaints that restrictions demanded by OMB in the revised Circular A-21 will probably deter widespread use of the system.

Dr Cohen suggested some more radical changes. He first described to the subcommittee how concern at the way university administrators had controlled the use of his grant money had led him to report the matter to the National Institutes of Health; following an investigation, the university had agreed to repay $132,350. Dr Cohen suggested the "re-enfranchisement" of principal investigators, who would sign all time and effort reports, salary certifications and reports of expenditure, rather than delegating these functions to university officials. □

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SRC tries apprenticeship scheme

DETAILED plans for two new experimental schemes aimed at improving the performance of graduates in industry are now being finalised by Britain's Science Research Council. The programmes, to be based on part-time studies, represent an unusual step by the normally academically-orientated SRC and indicate a strong feeling that British industry in general makes poor use of its scientific and engineering intake.

Although many of the UK's larger companies have adequate training schemes for developing the industrial potential of new graduates, many smaller firms make practically no arrangements for this. The two SRC projects aim to remedy the bias.

The first scheme, known as graduate initial education, will take the form of an apprenticeship package which will follow newly qualified students into industry. Through systems of tutorials, demonstrations and company-based projects over a two- or three-year period it will tailor their performance to the specific needs of their firms. A first experimental package, to cost about £100,000, is to be ready for student intake in October 1980.

The second programme will consist of technological topping-up education for graduates of about 10-year standing who require up-dated instruction on new industrial practices. This will include the many managers whose educations cover only transistor electronics and not modern microelectronics. The scheme will aim at familiarity with a basic working knowledge.

In both systems, universities will probably undertake instruction, and may even award degrees if it is thought the schemes would otherwise affect staff-student ratios. However, in the case of the technological top-up programme, it is possible that the Open University, with its experience of popular presentation, may be introduced to instruct industrialists who might be alienated by a dry academic approach.

At the moment, both schemes are still at a rudimentary stage of planning and although they have been given the full go-ahead by the central council of the SRC, approval still has to be given by the Department of Education and Science as part-time higher education is not at present considered to be part of the council's remit.

Fine-tuning for technology, page 352.

Robin McKie

Max Planck production venture fails

"WE will concentrate future activities on our original function, which is to commercialise research results of the Max Planck Institute by licensing only," according to a spokesman of Garching Instrumente, a 100% commercial subsidiary of Max Planck Gesellschaft. The young enterprise has run into considerable difficulties, and finally has been forced to close down activities connected with the development and production of highly sophisiticated instruments and pilot equipment — activities that accounted for nearly three-quarters of Garching Instrumente's turnover and employment.

MPG established Garching Instrumente in 1970 as a limited liability company whose aim was to commercialise technically useful research results of the Max Planck Institute in the industrial field. Stimulated by successful American ventures of a similar kind (from which the un-German name "Instrumente" was borrowed) Garching Instrumente was not only to deal with licensing (especially of Max Planck Institute patents), but also with the development, production and distribution of high technology hardware.

From its beginning, the new company raised considerable hopes and expectations, for it was considered to be the prototype of the young, small high technology venture needed in West Germany. The importance attached to it can be deduced from the remarkable efforts of the Federal Ministry of Research and Technology in the early 1970s to establish a risk financing corporation specifically to assist risky high technology ventures.

In the first years of operation, at least, Garching Instrumente seemed to fulfil the high expectations. Turnover increased to DM4 million with around 24 staff; among its clients were big companies like Siemens AG, as well as small enterprises, research institutes and universities. About 75% of the licence agreements were contracted with the instrument, electronics and pharmaceutical industries.

As time passed, however, it became increasingly obvious that research work on the one hand and manufacturing business on the other were very different, and that switching from one to the other is not easy. The activities of the manufacturing division of Garching Instrumente seemed to be less and less in line with the expectations of the Max Planck Gesellschaft, and there were rumours not only of management deficiencies but also of losses higher than could be tolerated even in a newly founded venture. Now, as a consequence, MPG has closed down all development and manufacturing activities, dismissed the management and reduced the staff to a mere handful. Activities are now restricted to licensing.

The new management looks optimistically to the future: "The actual turnover out of licence agreements already exceeds DM1 million. That brings us near the break-even point of our present activities."

Klaus Höpfner

Maurice Bazin reports from Brazil on the return of the exiles for the country's major scientific conference

Scientists protest at US tycoon's Amazonian project

OVER 5000 scientists and social scientists congregated in the Brazilian north-east town of Fortaleza in mid-July for the 31st meeting of the SBPC, the Brazilian Society for the Progress of Science. The meeting's central theme was 'Dilemmas of Scientific Production in Brazil', reflecting the participants' preoccupation with the ways in which General President Figueiredo's Brazil is helping — or hindering — science. The problem of external dependency also lurked in the background as an ever-present spectre.

"The developed countries control science and technology, but they charge for letting us use it. And they charge dearly . . . . It is enough to see the chronic deficit of the Brazilian balance of payments. Part of this deficit comes from paying royalties and patent rights; these are, in the last analysis, the price we pay for scientific and technological dependence" read an official note distributed by the general secretariat of the SBPC on the opening day. This point came up at a round-table on 'National Scientific Policy' which involved three representatives of agencies financing scientific research in Brazil. Physics Professor José Leite Lopes, visiting his country after ten years of exile, declared: "It is useless to discuss a science policy without discussing the forces which oppose its formulation. Shall we discuss scientific policy without discussing economic policies?" He then added that nothing could be done as long as the economic forces representing the interests of multinational companies determine technological and scientific production in Brazil.

The president of the SBPC reminded everyone that the Brazilian scientific community was never consulted in the past about national projects, and it became evident during the meeting that academics were ready to take advantge of the recently granted freedom of expression to criticise strongly the government's options. The relaxed atmosphere of the meeting (where mornings were left free to allow participants to enjoy the town's beaches) was interrupted, however, when local police arrested five student participants for distributing "subversive material"; a federal security agent declared later that "in addition, they all had taken drugs".

The hottest discussions centred on two huge ongoing projects: the Jari agro-industrial project in the Amazonian jungle and the nuclear agreement with West

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Germany to build eight 1000 Megawatt nuclear plants in Brazil. Daniel Ludwig, an American citizen, owns a land area the size of Switzerland at the confluence of the river Jari and the Amazon. Investments in homogeneous forest plantation, a cellulose factory, cattle raising and rice growing on this site amount to $700 million, and will reach $1.5 billion in a few years when a paper factory is completed.

Banners reading "Amazonia is ours" were spread around an overflowing amphitheatre as Charles Briscoe, the American technician in charge of the Jari Project, explained that "Project Jari covers more than one million hectares in the State of Pará and the Federal Territory of Amapá . . . There are no boats loaded with gold or other riches leaving the country." But critics pointed out that all of Jari's activities are geared exclusively for export, and that the appropriateness of their technology is never considered. Agricultural production, for example, is fully industrilized: rice is sown by plane and tree planting and cutting are fully mechanized. Parts of Briscoe's presentation met with boos from the participants, several of whom denounced foreign control and the elimination of the indigenous Indian population.

West German nuclear deal heavily criticised

The government's nuclear programme was strongly criticised by Brazilian physicists on economic and political grounds; the technical and environmental arguments which dominate the nuclear debate in industrialised countries were not brought up. Professor Luis Pinguelli Rosa of the Federal University of Rio de Janeiro proposed a drastic revision of the contract with West Germany. He claimed that Brazil is in a position of strength: "Germany presently does not have other clients for its reactors. The only reason we did not succeed in imposing our conditions is because we owe $40 billion to the industrialised world, and that forces us to follow a policy which is perfect for our creditors but not for ourselves." He also indicated that an official report by the Ministry of Planning recognises that the cost of electrical energy produced by nuclear reactors is three times that from hydroelectric schemes.

A longer-term view was presented by Professor Mario Schemberg, who trained some of the best known Brazilian physicists before he was forcibly retired from the University of Sao Paulo by the military government in 1969. He made an appeal for "freeing ourselves from the useless weight of the nuclear agreement with Germany, and cancelling the pledges which the military dictatorship made without consulting the people." He proposed an energy programme for Brazil for the next fifty years which would emphasise research in solar energy and nuclear fusion. □



Where have all the flowers gone?

THE savage destruction of the Brazilian rain forests has been dictated, to a large extent, by economic pressures over which the country's government can exercise little control. Yet this rapidly dwindling heritage of exotic plants and animals kindled the imagination of early explorers and continues to contribute to our understanding of our natural environment. In Brazil today the cultural and economic influence of the West is very apparent, and so it is strange to find little native interest in natural history reflected on the shelves of the big bookshops of cities such as Rio de Janeiro, Brasilia and Belo Horizonte. Despite a good deal of enquiry the only books I could find dealing with Brazilian wildlife at all were translations of glossy foreign 'coffee table' volumes.

Long searches through the second-hand markets uncovered only four more: a series published in São Paulo in the 1950s dealing with insects, birds and mammals; and a magnificent translation of an illustrated French book on Amazonian primates, published at the turn of the century — whose value was, unfortunately, well appreciated by the dealer. The Brazilian flora suffer a similar neglect, even though, at certain times of the year, one would be well advised to take some sort of field guide along when visiting the local fruit and vegetable markets.

Being able to name and classify species of plant and animal is an essential cornerstone of biological science and education. Once these basic elements can be identified, and their interactions understood, one may begin to appreciate the fragility of eco-systems such as the tropical rain forest.

It is disturbing, therefore, to find that wouldbe medical students, when asked to give the names of all the animal species they knew, could list, on average, only half a dozen — invariably creatures of medical importance such as the mosquito, the cockroach and the 'barbeiro', the bug vector of Chagas disease. Although this survey carried out by Professor Angelo Machado of the Federal University of Minas Gerais, did not include questions on plant life, my own experiences suggested that here the situation is even worse. Indeed, one graduate biologist I spoke to was surprised to discover that natural selection occurs outside the animal kingdom!

Machado is a well respected morphologist and neuroanatomist, whose spare time pursuits include some most elegant studies on the ecology and taxonomy of neo-tropical dragonflies. His wide interests include conservation — he is a founder member of one of the few environmental pressure groups in the country — and, more recently, an attempt to understand the lack of interest in wildlife which is apparent in the country's development of its natural resources. Brazil's politicians and administrators, he feels, are 'frightened by nature', and so he has turned to studying the development of the concept of the 'forest' in school children throughout the country.

This survey is still in its early stages, but Machado has already found that the child's concept of the forest, and its association with fear, begins to form early in life. It seems to be based on nursery rhymes and children's stories imported from Europe and North America, which emphasise the dangers of going into the woods.

In the younger child this ignorance can be easily counteracted. Drawings and paintings of the 'forest' made by 7-10 year olds commonly included aeroplanes spraying the undergrowth with bullets and napalm; but after the same children had been taken by Machado on a trip to the real forest these were replaced by birds and butterflies, and the children paid far more artistic attention to leaf forms and flowers.

At the present rate of development Brazil's forests may only survive for another 30 years, but if Machado's children can be so easily convinced of its value there is some hope that this new generation may allow it to stand for longer. The final decision may perhaps lie with the teachers rather than the politicians.

David Bousfield

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news in brief

Third World space science institute proposed: An International Institute for Space Science and Electronics, where scientists from developing countries can carry out front line research in radio science, electronics and space applications, may soon be set up in Kenya. Establishment of the institute was strongly recommended by a recent workshop in Ooty, India attended by scientists from ten developing nations and organised by the Tata Institute of Fundamental Research's Radio Astronomy Centre. The new institute's first project will be the construction of a $20 million Giant Equatorial Radio Telescope (GERT). Nobel Laureate Anthony Hewish of the UK Cavendish Laboratories, consultant to the workshop, said he was strongly in favour of the project which he thought was "good and sensible". GERT will be the world's earliest warning system for geomagnetic storms and will also carry out pulsar research, make rapid surveys and give information on the structure of radio galaxies. According to Hewish, GERT's research is unlikely to be repeated in other countries because it will use wavelengths of around 300 MHz whereas most other research in this area tends to use shorter wavelengths. Developing nations should be able to finance the project themselves using technology within their capabilities. India, Kenya, and Nigeria have expressed strong initial interest in the project.

US trade unionists oppose expansion of nuclear power: Twenty US trade unionists, including the presidents of two international unions, have issued a statement calling for an end to the development of nuclear power. They accuse the nuclear industry of using "scare tactics" in threatening that such a move would cause widespread unemployment. Attitudes towards nuclear power within the US trade union movement are sharply divided, largely because the construction and operation of nuclear power stations provides many jobs for the members of a number of powerful unions. However the recent letter, represents a growing awareness among trade union members of the hazards as well as the advantages of nuclear power.

Of the 20 signatories of the statement, five are union members who live and work near the Three Mile Island nuclear plant. In addition to ending the development of nuclear power, the statement demands: guaranteed new, safe jobs at full union wages, with paid retraining to all now employed in nuclear or nuclear-related industries; a substantial shift of investment resources and workpower to conservation, clean and safe coal, and solar energy; and local, democratic control of energy sources, production and distribution.

Computer scientists continue strike: Computer specialists in the telecommunications section of the UK Postal Service voted overwhelmingly last week to continue their selective strike in the telephone billing department. They also voted to censure the leadership of their union, the Society of Civil and Public Servants, for recommending acceptance of management pay offer of 18½%. The union has been on strike since March in support of a pay claim for parity with professional engineers in the Post Office and with administrative grades in the Civil Service Department. The strike has been joined by the Civil and Public Service Association representing 34,000 clerical grades including computer operators. A total of 100 people are on strike and are being supported by a strike levy on the 40,000 members of both unions.

Academy panel calls for new "microstructure science" research programmes: A panel of the US National Academy of Sciences has warned that the development of micro-electronic technology appears to be outstripping the basic scientific knowledge on which such technology depends. It recommends the setting up of a number of new research programmes, primarily in universities, in fields that it calls "microstructure science and engineering". The academy panel also encouraged greater collaboration between university and industry laboratories, as well as an increase in programmes of non-proprietary research and development in industry. *Microstructure science, engineering and technology.* Solid State Sciences Committee of the National Research Council.

Getting steamed up over waste heat: Combined heat and power (CHP) could account for a third of domestic heating next century, according to a Department of Energy report published last week. The report estimtes that CHP saves between 2% and 9% of all primary energy consumption when it is fully operative by utilising waste heat from modern power stations. Extracting this heat at high enough temperatures provides space heating and hot water for domestic, commercial and public buildings. CHP is likely to be a viable economic option for district heating in areas with a net heat load density above 15 megawatts per square kilometre and so can be used for 80% of the heat load in Greater London, West Midlands, Greater Manchester, Merseyside and Glasgow. CHP's potential role in industry is limited since 70% of electricity generated is already associated with heat recovery. For CHP to be successful by 2000, investment must begin immediately, and it will have to compete with other fuels, particularly gas, for funding during the next 10-20 years. *Combined heat and electric power generation in the UK.* HMSO £3.75

French agronomists protest over potential redundancies: Scientists at the Institut National de la Recherche Agronomique, an organisation which supports most of French agronomy research and employs 7,000 people, recently locked up their director-general for several hours in an angry protest against a change in the institute's status that could lead to redundancies. The French government intends to change INRA into a group of specialised subsidiaries to exploit commercially certain results of agronomy research. The government is concerned about the increasing penetration of the French agricultural and food processing industries by British and Dutch interests. The trade unions involved see two dangers in the proposal: a reduction in fundamental research in favour of more development oriented research and less security of employment. INRA's researchers currently have the status of *fonctionnaires* (civil servants) and under the modified statutes they would lose the guaranteed employment that they now have.

What changed Sir Richard's mind? The government appears to have found a respectable volunteer to fly a kite for its plans, already mentioned in the press, to eliminate some of the less prestigious universities and polytechnics. Sir Richard Way, the Crown-appointed principal of King's College, London, who previously ran London Transport, made an astonishing *volte-face* in his speech at the recent exhibition to celebrate 150 years of the college. A press release prepared before the opening ceremony contained the unsurprising claim that new cuts in university expenditure would "probably make it impossible for the British university system to maintain the high standards at which it had always aimed."

However, when Sir Richard arrived in the company of the Education Secretary, Mark Carlisle, to open the exhibition, he announced that he did not in fact consider the universities as a whole to be short of money (12 July, page 97). Sir Richard went on to hark back to his days at the War Office when a shortage of money led to a painful reduction in the number of battalions by a process of amalgamation. Sir Richard made it clear that he considered a similar solution appropriate to higher education so that centres of excellence should not suffer.

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The Israel-Egypt peace treaty has opened up the possibility of scientific links. Here, 'Nature' correspondents describe the views from Cairo and Tel Aviv

Egypt: divided and uncertain

THE Egyptian scientific community is divided on the issue of collaboration with Israel. A small number of high ranking scientists see many advantages in undertaking joint projects; they argue that the Israelis have certain expertise in research areas that are important to Egypt, and Egypt should take advantage of the Israeli offer to collaborate in these areas.

There is, however, considerable opposition to this view from the rank-and-file scientists. Their argument is that the essential need of Egypt is not expertise but funding. If Israel can fund Egyptian research ventures, all well and good; but that's where collaboration ends. The Americans and Europeans already occupy positions of prestige in Egyptian scientific establishments as advisers and consultants, and many Egyptian scientists fear that Israelis will be added to the list. And they consider this not so much collaboration but more a form of domination.

Over the last few years, the Egyptian scientific community has been looking more and more towards the Arab OPEC countries as a major source of funding. A number of Egyptian projects in solar energy, desalination, oceanography, pollution studies and agriculture are already being financed by some of these countries, while Egyptian scientists and technicians form the backbone of industry and academia in Saudi Arabia, Kuwait, Bahrain and the United Arab Emirates. Despite the Arab boycott of Egypt, these scientists continue to work in Arab OPEC countries, many joint projects still continue, and these countries continue to recruit scientists and technicians from Egypt. The expectation of further collaboration with Arab OPEC countries is, therefore, much stronger than the possibility of co-operation with Israel. Many Egyptian scientists believe that co-operation with Israel will seriously damage the possibility of further collaboration with the Arab OPEC countries.

Dr Fahamy Ramadan, Secretary General of the National Research Centre of Cairo, sums up the feeling of many Egyptian scientists when he says: "All Arabs are brothers. Brothers fight; but they do not stay divided for long. They eventually make up. Our Arab brothers will make up with us, and much sooner than most people think". It seems that most Egyptian scientists would rather wait for their "Arab brothers" to make up with them then actively collaborate with Israel.

Ziauddin Sardar



Sinai 1967: can the battlefront now become a symbol of peace and co-operation?

Israel: a one-sided love affair

DISCUSSIONS about scientific and technological co-operation between Israel and Egypt have so far been very much like a one-sided love affair; while dozens of Israeli professors and politicians have spoken about the potential benefits of such co-operation little response has been forthcoming from their counterparts in Egypt. To be sure, there have been unofficial meetings between top Egyptian and Israeli scientists in third countries like the US and Germany usually on the initiative of well-meaning intermediaries. But so far the Egyptians have avoided public statements on the subject.

Yet the Israelis go on hoping and talking. The most dramatic presentation of a co-operation scheme was made some two months ago during Sadat's visit to Beersheba by Joseph Tekoah, former Israel ambassador to the UN and new president of Ben-Gurion University of the Negev. Speaking at an internationally televised campus ceremony, with Begin and Sadat on either side, Tekoah announced that the university had decided to establish a $100m fund for desert research of potential benefit to both Israel and Egypt. Egyptian scientists, he added, were invited to participate in the research projects and in the fund's administration. Sadat was all smiles, but that — so far — has been the only Egyptian response.

Slightly more guarded was the recent statement by Haifa Technion president Amos Horev, who expressed the Technion's willingness to enter into bilaterial relations with Egyptian institutions. As a first step he expected that Egyptian technological and science university graduates might study for higher degrees at the Technion, and that Egypt might wish to utilize the Technion's research laboratories and testing facilities. But under no circumstances, Horev warned, should Israelis delude themselves into thinking that they were goiong to 'save Egypt'.

The most logical place for co-operation to begin would be the Sinai peninsula, where for the last 12 years Israeli scientists have done extensive research, the results of which are now being turned over to the returning Egyptians. This is by no means a new idea, as a number of American Presidents, beginning with Eisenhower, have suggested the establishment in Sinai of joint Israeli-Egyptian power stations and desalination plants. Before this scheme was finally shelved, scientists working at the Oak Ridge National Laboratory under Dr Alvin Weinberg had produced a detailed report on how it could be carried out. They envisaged the installation of two reactors with a combined output of 1000 MW, which would provide power for new industries producing chemicals, fertilizers, plastics and aluminium, as well as for desalinating enough water to irrigate 300,000 acres of land in Egypt and the Negev desert.

These plans still exist, as do new ones engendered by the euphoria of peace. Yet the Egyptians remain reticent, as is evident in the experiences of an Israeli scientist who has just returned from a visit to Egypt. He travelled there on a second, non-Israeli passport, but his hosts, top figures in the Egyptian scientific establishment, know that he lives in Israel, as does anyone who reads his papers in the journals. Behind closed doors the Egyptian scientists spoke enthusiastically about the possibility of co-operation with Israel in spheres like agriculture, medicine, solar energy and desalination. Yet when someone came into the room, such discussions stopped and the guest was introduced as being from Europe.

In order to overcome this apparent Egyptian hesitance about bi-national co-operation, leading Israeli scientists have suggested that international organizations should sponsor large scientific conferences in Israel and in Egypt, where it is hoped there would be massive participation of Israeli scientists when the meeting took place in Egypt, and of Egyptian scientists when it was in Israel. Moreover, people here place great stress on the need to choose topics where the Egyptians excel and can teach the Israelis, as well as fields where the opposite situation exists.

Nechemia Meyers

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Hungary steers a course without UN aid

Hungary's Academy of Sciences is now working out its Five Year Plan for 1981 — 1985, the first in which it will be entirely without the support of the United Nations Development Programme. For although by 1975 the Hungarian economy had passed the formal criterion for a 'fairly well-developed country', careful management of the UNDP funding ensured a certain carry-over beyond the official termination date. Furthermore, careful negotiation with the UNDP administrators cut down the expenditure on administrative overheads, so that an unusually high proportion of the funds available actually went on the equipping of Hungarian science, rather than on the expenses and infrastructure associated with the presence of international 'experts'.

A leading figure in these negotiations was Dr Bruno Straub. A biochemist of some renown, since the mid 1960s he has had an impressive second career as an administrator for scientific projects. In 1969, he was appointed a Vice-Chairman of the International Atomic Energy Agency committee for working out a nuclear safeguard agreement, and after the withdrawal of the chairman, Kurt Waldheim, from active participation, he took over as *de facto* chairman. In 1971, he was elected to the executive board of the International Council of Scientific Unions, serving on its Environmental Commission. And in his native country, Dr Straub applied his skill and renown as an organiser of science to a problem which, as a biochemist, he felt to be a major priority — the sad state of Hungarian biology.

Since the Hungarian economy is considerably dependent on agriculture, the development of the country's biological expertise seemed to him a matter of pressing concern. Unfortunately, in the mid-1960s, Hungarian biology was firmly in the hands of what Dr Straub described as "the old people". These, he explained, were "biologists and zoologists, only interested in systematics and taxonomy". Such an attitude, Dr Straub explained, found a certain support among the administrators. Not for ideological reasons — Lysenkoism never took hold in Hungary and the principle of a free inflow of information always held good.

The problem was simply one of finance. "You can do taxonomy cheaply, but experimental biology means instrumentation. You can get simple things here in Hungary or else from Poland — but sophisticated work means dollars; and that means a special decision of the government."

By tapping the UNDP aid to Hungary (about $1 million per year over five years), it became possible for the Academy of Sciences to construct and equip a modern biological research centre at Szeged with four institutes — biochemistry, biophysics, plant physiology and genetics, with a fifth institute, enzymology, sited in Budapest. Between 1973 and 1978, the Szeged Centre received some $1.2 million from UNDP, more than 50% of which was spent on equipment, with the participation of foreign experts reduced to "just those who could really help us".

In return for this special arrangement, Hungary had to undertake to give scholarships at Szeged to trainees from developing countries, to whose problems Hungary is still fairly close. Dr Straub, who had accepted the post of General Director of Szeged in 1973, on the understanding that it should be a rotating leadership between the five sections (he resigned in 1978), was able to persuade the Academy of Sciences to support this scholarship scheme. "While the UNDP money was still coming in, they were quite keen about it", he explained, "now they are a little less happy, but still, the scholarships continue". Luckily for intending applicants, the working language at Szeged is English, and the third-world trainees at present include representatives of Vietnam, Korea, a number of African countries, and (so goes the Szeged joke), a citizen of Lancashire.

Research at Szeged varies from basic investigations of immunological reactions in blue-green algae, through studies of the genetic basis of nitrogen fixation (with relation to lucerne, Hungary's most important legume), to contract research funded by agricultural concern. According to Dr Farkas, Director of the Institute of Plant Physiology, such contract work can raise the salary of those involved by up to 10 to 50% — though not, unfortunately, his own, since Directors and Vice Directors of Academy Institutes are not allowed to accept contract payments. The government, he explained, is trying to bring in material incentives (ie bonus payments) in a number of aspects of scientific life, both for economic reasons ("it works out cheaper in the long run") and also to stimulate creativity. He himself was not entirely happy about the contract scheme, he said, pointing out that many people would prefer to see a regular increase in basic salaries. Work for the agricultural cooperatives, such as the current project on the technology of vegetative propagation of vine-stocks, was "a burden, but beneficial". Nevertheless, he intimated that he would not be sorry if the Academy were to reverse its present policy on bonus payments, by setting a limit as to how far an individual scientist can augment his salary. Otherwise, there might be a real danger that scientists would be distracted from their main line of research.

Whatever the temptation at the laboratory-floor level, Hungarian science does not intend to abandon fundamental research. According to Dr Istvan Lang, Deputy General Secretary of the Academy of Sciences, fundamental research accounts for some 14% of Hungary's total R & D costs. "We think we must keep it at this level, and the state budget gives it a long-term guarantee."

There is no question of a cut-back; the main problem is what sort of fundamental research should be carried out. Hungary, he said, with about 0.9% of the total world capacity for research, clearly must be selective, and has tended to concentrate on fields in which Hungarian scientists had already made a name — mathematics, physics, and biochemistry. Within these fields, there is a certain amount of "target orientation". For example, Hungary wants to develop the pharmaceutical industry. "Hence we hope that a young biologist will choose his work with this in mind, rather than getting attracted to space biology, say!" (Though Hungary does, in fact, have a space biology programme, as well as a five year project at Szeged on the asymmetry of living matter, now being phased out in the words of its director, "until we find another living system or make life artificially, to provide material for comparison".)

Responsibility for science planning is divided between the Academy of Sciences and the National Committee for Technical Development, both of which are subordinated to the Government Science Policy Committee, headed by Gyorgy Aczel, one of the Deputy Prime Ministers. Policy decisions taken by the Science Policy Committee are binding on the Academy, Secretary Lang explained, "But what you have to understand is how our planning system works. We are a centrally planned economy, but we don't plan everything centrally."

As an example of academic freedom, Hungarian style, he cited his recent work for a special council to decide on future development of the Lake Balaton area. "When I was elected head of the Council, I chose as our first task the evaluation of all existing data, and the preparation of recommendations to the Government. These were discussed and approved by the Council for the Environment and Nature Conservation. Next a regional development plan was elaborated on the basis of these recommendations".

"Our forecasts shocked public opinion," he continued "We had to point out that if nothing changed, if the input level remained the same and the rate of control the same, then in 15 years' time, Balaton would be green with eutrophication and unacceptable as for leisure use".

It is, perhaps, not a matter of great surprise that the new plan was accepted on June 10.

Vera Rich

Fine-tuning for British technology — or will political pressures put a spanner in the works?

The Finniston report on engineering and education could be crucial for the UK's industrial revival, but its proposals may run counter to Conservative policy. **Robin McKie** reports



BRITAIN'S recently-elected Conservative government is facing a dilemma that could expose basic contradictions between its much-publicised antipathy towards public spending and its equally well-proclaimed desire to boost the country's industrial growth. The predicament will come in the form of the Finniston report, a major policy document now being completed by leading industrialists and educationalists, which will recommend a package of proposals for regenerating scientific and technological impetus within the UK's manufacturing industry.

At the centre of these plans lies the proposed formation of a British Engineering Authority which would take over engineering education, training and research throughout the country. Such a body would also be responsible for the registration of engineers, in the same way as the General Medical Council handles the registration of all UK doctors, and may even take on the role of granting licences for engineers wishing to act as industrial consultants.

However, given the new administration's recent moves to scrap a thousand similar 'quasi-autonomous non-governmental organisations' (or quangos as they are affectionately known), it is hard to envisage them allowing any increased public control of the private sector — particularly in view of the Conservatives' election pledge to leave industry alone "to get on with the job".

Corrective measures are badly needed, nevertheless, for it is now widely accepted that Britain's manufacturing industry is not as competent, efficient or productive as our foreign competitors. In particular, companies are accused of very poor intakes of scientists and engineers into management — a tendency which discourages further gifted and ambitious students from such a career route. This means a resulting intake of poorer engineering and technolgical students, with even worse prospects of reaching management — and so the spiralling problem becomes more critical.

Indeed, it is a crisis recognised by the new Minister of Science, Mr Neil Macfarlane, who believes that UK industry has shown scant regard for scientific and engineering talent in the past. "I think it is greatly to be regretted that the engineer and the scientist are generally relegated to a secondary level in the hierarchy of any industrial organisation in the UK. We have been taken over by financiers and lawyers", he said in a recent interview with *Nature*.

With such difficulties in mind, the previous Labour government set up the committee of inquiry into the engineering profession, led by Sir Monty Finniston, head of Sears Engineering and former chairman of the British Steel Corporation. Its task was to review:

- British industry's requirements for supplies of engineers;
- The role of engineering institutions in controlling standards of qualifications;
- The advantages and disadvantages of registration and licensing of engineers;
- Comparable arrangements in other countries, particularly in Europe.

But Sir Monty and his committee deliberately chose to interpret this remit in the widest possible terms and have set about preparing an entire blueprint for ensuring industrial regeneration in Britain. This is not to be done on the macro-level of gross economic distortions (as in recent budgets which have attempted major inflations of markets and profits), but by tinkering at a micro-level to fine-tune the country's industrial engine and prepare it for improved performance.

A central part will be played by the proposed British Engineering Authority which will take over the role of registration of engineers. At present this task is performed by the Council of Engineering Institutions, a central body made up of representatives of the country's many engineering institutions (such as the Institution of Electrical Engineers and the Institution of Mechanical Engineers) which specify, approve and enforce the minimum standards of professional conduct. Many now consider the CEI to be a weak, ineffectual body which merely awards chartered engineering status to anyone with a relevant degree at ordinary pass level. Instead, the new authority, which would probably include lay representation, would operate as a strong independent agency that would restore public confidence in the profession.

The new system would control the awards of titles — 'qualified engineer', 'qualified engineering technician' and a specially-created elite called 'diploma engineer' — only to those with sufficient training, experience and education. This would allow tight control over engineering practice by ensuring that those who fell below standard would lose their titles, and would make it an offence for them to continue to use these titles.

The diploma designation would represent a move towards creating an elite similar to the German 'superengineer', although this is more an attempt to boost the status of UK engineers than a bid to create a particularly practical type of engineer.

A further controversial plan being considered by the Finniston committee is a proposal that all university and polytechnic engineering courses should be closely scrutinised by the new authority — and that institutions failing to meet basic standards should be stripped of financial support. At present such an accreditation body operates in the United States, but it has no financial influence over university departments and only recommends to potential students and future employers those courses it believes are satisfactory. In considering this new, and far more radical proposal, the committee will be suggesting that the University Grants Committee and the Science Research Council be stripped of their present control of funding for engineering education and research — a move that would no doubt be strongly contested.

Indeed the proposal has already been

rejected by the main committee, but the sub-group on registration and licensing has since insisted that it be re-considered, stressing that a bold measure is necessary to swing education behind the interests of manufacturing industry. "We think that there are forces of inertia in higher education that will stand in the way of a reform of engineering education unless leverage is exerted", stated a recent report from the sub-group to the main committee.

A further consideration that may influence the Finniston group is the likely constraint on future education spending. As increased resources are especially vital for engineering, the committee may recommend that the education sector is no longer a suitable outlet for funding and suggest a more fundamental alternative. This argument could find particular favour with the Conservatives, as it proposes moving money away from traditional public spending areas and closer to the interests of private industry.

The proposed British Engineering Authority would also monitor training schemes for graduates entering industry. At present, it is well-known that many smaller firms offer inadequate and inferior training programmes and such students would be at an immediate disadvantage upon the introduction of a strict registration system. This has been one important motivation behind the UK Science Research Council's launching of an experimental programme — to be known as graduate initial education — which will next year offer part-time postgraduate university-based instruction in industrial subjects for science and engineering graduates.

Thus it is envisaged that the BEA will maintain a strong hold over the flow of engineers into industry. Basic education would be monitored through an accreditation system; training would be controlled by close scrutiny of companies; and the actual quality of manpower would be governed through a tight registration system.

At the top end of the market, quality would be supervised by operating a licensing system for consultant engineers who offer special expertise in particular industrial areas. This would ensure the quality of those engineering experts who advise companies and public bodies and would act as a further qualification on top of registration.

These moves all reflect recognition that British industry is flagging not just because of poor investment in modern technology but also because it fails properly to utilise available talent and intellect. And even when this problem is overcome, systems will still have to be devised for professional, practising engineers to keep abreast of current technological developments. For example, many UK managers have an education which covers only transistor electronics; their lack of knowledge of modern solid state physics can lead to fears and prejudices about working with complex microelectronic technologies. The solution probably lies in various forms of continuing education courses, to be taken every five or ten years.

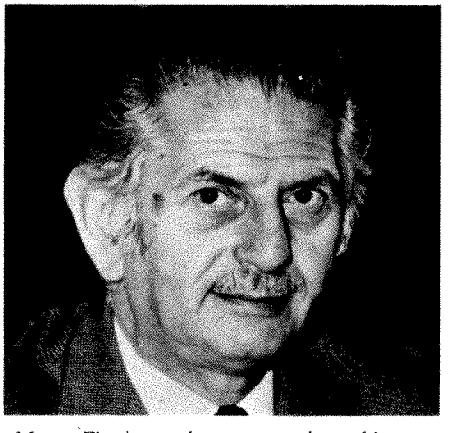
The extent of this problem has already motivated the Science Research Council to arrange technological topping-up courses — based at universities — for graduates of about ten years standing, and these are soon to be introduced into the UK. The Finniston committee is likely to favour a similar scheme which would also include grants for fares, fees and books.

Other proposals to boost the quality of personnel in industry will include methods for helping already-trained women engineers to get back into industry, and ways of improving attitudes towards industry in school classrooms. In this latter area, a particularly favoured plan is for interchanges of staff between schools and major manufacturing companies.

But such a scheme would not mean simply recruiting teachers from former industrial personnel — they may have left the commercial sector because they failed to operate adequately there. Instead a system of rotation of roles is seriously being considered, although it is acknowledged that this could cause difficulties with the trade unions involved.

In outlining these various proposals, the Finniston committee has recognised that one major problem has been the vast expansion of the British university sector in the early 1960s. This has led to colleges being elevated to university status, producing a more academic approach and forcing them away from local industries with which they previously had strong ties.

Now the Finniston committee is considering setting up regional engineering centres — to be run by the BEA — which would provide a mixing ground for academics and industrialists. The centres would be based at universities but would have the explicit roles of co-ordinating and generating engineering research, applications and training in relation to the particular industrial needs of a region. Such institutions could be set up at Imperial College (London), Aston (Birmingham), Leeds-Bradford, and Glasgow-Strathclyde universities.



Monty Finniston: due to report later this year

Another problem caused by university expansion has been the diverting of most engineers into degree courses meant for chartered engineers — those concerned with the innovation and creation of new technologies. The old college and part-time routes which provided the country's many technician engineers (those concerned with maintaining existing technology) are now virtually extinct.

As a result students who have received chartered engineering education have ended up totally unprepared as technician engineers. Finniston may consider introducing a new technician engineering degree course at universities to provide the most suitable education for those aspiring to the proposed 'qualified engineering technician' status.

At the moment, there is still strong agreement over the various issues between the 18 members of the committee and it is unlikely that a minority report will accompany the final publication of the full Finniston document. Indeed, one member, although slightly critical of the over-representation of academics and "fringe people" not directly concerned with industry, considered the committee had shown "a remarkable working chemistry" over the past 18 months.

The strength of this chemistry is likely to be tested in the new few weeks as work on the report is completed — for the committee is now under strong pressure to water down its recommendations, particularly from some engineering institutions who view a strong independent authority as a threat to their power and independence.

And within the committee, there are some signs of resolve weakening. In particular, there is now talk of finding a role within the BEA for the much-vilified CEI (because it has had past experience of controlling standards of education and training for engineers), although it had previously been agreed there would be practically no room for the CEI in the new authority.

This is only a small indication, but it could spell a warning that Finniston, in the hope of preventing undue reaction and prejudice to the report, may fudge the available options. In turn this could give the Government an excuse to further water down the proposals, eventually rendering them totally ineffectual.

Such a dilution of Finniston's proposals could have tragic consequences for Britain. The country's industrial slump can be blamed squarely on the failure to take up technological innovations and to make use of scientific and engineering talent. No amount of tinkering with sterling revaluations or credit markets will alter this, and strong remedial action is urgently required. The Finniston report — to be published some time between September and December — represents the Government's chance to change Britain's future technological development for the better.□

correspondence

Airey Neave Memorial Trust

SIR, — Many people have suggested to us that they would like to commemorate the life of Airey Neave, assassinated this year by a bomb in the Palace of Westminster (for obituary see page 433). Some have sent money now banked in a special account.

Since Airey Neave believed in the supreme importance of freedom under the law, we consider that we can achieve something that he would have held dear by creating a Trust to provide for research into the extent of personal freedom under national laws. This may include the provision of travelling scholarships. Candidates will be chosen after they have submitted a short synopsis of the scope of the paper they intend to write. The papers, embodying their research, will be published.

In promoting these studies, priority will be given to applications by candidates from fields in which Airey Neave had a particular interest; former pupils of Radley College or Abingdon School and graduates of Merton College, Oxford; holders of degrees in science or law; members of the legal profession; former prisoners of war; authors; Members or former Members of either House of Parliament.

The value and number of such scholarships will depend upon the amount of money received. Application has been made for the Trust to be registered as a Charity.

Those who wish to be associated with this appeal should send their contributions to The Airey Neave Memorial Trust, c/o The House of Commons, London SW1. Covenant forms will be available on application to the Trust.

Yours faithfully,

JOHN TILNEY (Chairman)
and the committee of the Trust.

London SW1, UK.

Costs of toxicity testing

SIR, — That ICI Ltd is spending £6 million per annum on its toxicity testing programme seems to underline the fears of smaller companies that the tests they are asked to undertake are too expensive. This is even more the case if we concede that these tests can be but a crude guide to the possible hazards to man. Still, the bankruptcy of a few firms and a reduction in innovation might be a small price to pay for the sensation of safety we all crave; in any case, as the new legislation applies to new chemicals, only the larger more affluent companies with new product developments are likely to be much affected.

Or are they? Mr Langley has suggested (5 July, page 9) that industry ought to be doing more, anyway, under the 1974 Act, and the new notification scheme merely records that defined work has been completed successfully. Is there an implication here that existing chemicals will be subject to the same testing arrangements? The logic of the Act suggests this ought to be so — and recent experience with monomers has thrown doubt on 'long manufacturing experience' as a safeguard on health, making it more likely that some experimental results will be needed.

In this case, the economic impact could be substantial and, because it is happening at a time when another world recession is expected, it could have even more profound effects than usual. It is time to establish some priorities. Let the Health and Safety Executive produce guidelines, not on how to test chemicals, but on how to select chemicals which require to be tested — unless they intend to deal only with new chemicals. Let us concentrate our resources on developing test procedures which effectively screen chemicals for the properties we want to avoid at all costs; and leave lesser hazards until we can afford to attack them. Above all, let us recognise that life is a hazardous and invariably fatal process which will surely survive twentieth century industrialism, if we keep our scientific 'cool'.

Yours faithfully,

D. M. CONNING

British Industrial Biological Research Association, Surrey, UK.

Attack on paranormal is unscientific

SIR, — It is surely profoundly unscientific to claim that "neither electro-magnetism nor any other scientific theory can explain" ESP phenomena as Taylor and Balanovski do (14 June, page 631). The most anyone can ever claim is that existing theories do not explain a phenomenon so far as they can see — someone else may be clever enough to find an explanation within existing theories, or to discover a new explanation. The latter possibility is not "ruled out" by the "successful reductionist approach of science," but is probably the main attraction of science to its practitioners. To go on to deduce that an argument of this sort casts any light on whether the phenomenon exists is even more bizarre — would they have argued against the heat of the Sun before relativity and the theory of fusion? Or do they now assume there is doubt that the mu-meson is heavier than the electron because no-one has yet suggested an explanation?

It might be instructive for these authors to study the history of the famous argument over the age of the Earth at the end of the last century. Kelvin had calculated the age from the time taken for cooling; the result was far too short to allow species to have evolved. Biologists suggested Kelvin might have ignored some unknown source of energy, and were roundly mocked by the physicists for being "unscientific". The fact that Kelvin's proponents were both confident and arrogant did not prevent them being wrong — as the subsequent discovery of radio-activity proved.

I might add that I do not believe there is much evidence for ESP, but this is based on the absence of unambiguous experiments rather than on anyone's inability to think of an explanation.

Yours faithfully,

M. WHIPPMAN

London SW20, UK.

West Germany does not pursue Berufsverbote

SIR, — Your note "German psychologists issue study of Berufsverbote" (31 May, page 363) is utterly misleading. The West German government does not pursue a policy of Berufsverbote. Nobody is banned here by the government to enter any profession of his choice. The federal and state governments reserve the right to select those who apply for employment as civil servants, a status that provides life-long job security. I cannot see anything illiberal when the federal and state governments reject civil service status to a handful of totalitarian radicals. To proceed differently would endanger the liberal system in any western democracy. In my opinion, the slogan Berufsverbote is an infamous lie, cleverly invented by leftist totalitarians to discriminate against the most liberal political system that has ever evolved in the rather sad course of German history.

Yours faithfully,

F. MILLER

University of Munich, West Germany.

Degree-day values

SIR, — In a recent issue of *Nature* (14 June, page 565) the potential value of the 'degree-days' measure was indicated. Readers who wish to obtain degree-day values for the UK may be interested to know that such information is available in *Energy Management*, published by the Department of Energy; and is also reproduced in *Energy Digest*, published by Allens (Clerkenwell) Ltd.

Degree-day users requiring early figures may obtain these from the EQAS service (Freefone 3140, or 8305 in Scotland). Detailed information is available in the free booklet *Degree Days*, available from: Library, Department of Energy, Thames House South, Millbank, London SW1P 4QJ.

Yours faithfully,

P. A. WOOD

Newport Pagnell, Buckinghamshire, UK.

The caddish salamander

SIR, — The vocabulary available to sociobiologists includes altruism, nepotism, selfishness and spite, but is still incomplete. Arnold (*Z. Tierpsychol.* **42**, 247; 1976) describes how a male salamander (*Amblystoma*) may intrude between the members of a mating pair, imitate the female, and induce the rival male to release a spermatophore which is then covered with the intruder's own sperm; the latter achieve fertilisation. For this I propose the term *caddish* behaviour.

Yours faithfully,

S.A. BARNETT

Australian National University, Canberra, Australia.

Erratum

In the issue of *Nature* of 21/28 December, page 754, in a letter by Drs Karlson and Dixon on biochemical nomenclature, the word 'bisphosphate' was twice misprinted as biphosphate in the last two lines of the third paragraph. The authors were pointing out the distinction between 'diphosphate', indicating a single diphosphate group in ADP, and 'bisphosphate', indicating two separate substitutions — as in fructose 1,6-bisphosphate.

news and views

Decline of the calcium hypothesis of visual transduction

from P. Fatt

IN a recent issue of *Nature* (Miller & Nicol **280**, 64; 1979) and in this issue (Robinson & Hagins, page 398) papers appear which are concerned with the involvement of guanine nucleotides in the action of light on the photoreceptor. In both the authors seem somewhat reluctant to discuss how their findings might fit into an overall mechanism of visual transduction. This reticence is a recently acquired characteristic of workers in the field, for which readers may wish some explanation.

What has happened is that over the period 1972–78 thinking about visual transduction in the vertebrate has been dominated by the 'calcium-as-the-intracellular-transmitter' hypothesis, proposed by W.A. Hagins (*A. Rev. Biophys. Bioeng.* **1**, 131; 1972) and strongly supported with experimental evidence by him, (see refs in Robinson & Hagins (this issue); Miller & Nicol *Nature op. cit.*) his coworkers and others (see *Vertebrate photoreceptors* (eds Barlow & Fatt) Academic Press, London, 1977). In the case of the retinal rod outer segment, which contains a stacked array of flattened sac-like disks, enveloped by but separated from the surface (or plasma) membrane of the cell, the hypothesis is made up of the following elements. (1) There exists a capacity for pumping Ca ions from the cytoplasmic space (that is, the space internal to the surface membrane but external to the disk) across the disk membrane to the intradiskal space where they are accumulated. This pumping is not directly dependent on the state of the visual pigment (rhodopsin), but is regulated by the concentration of Ca^{2+} in the cytoplasmic space. Operation of the pump requires metabolic energy (which Robinson and Hagins now suggest is provided by hydrolysis of guanosine triphosphate (GTP) to the diphosphate (GDP). (2) At some stage in the thermal conversion of the visual pigment molecule that follows photoisomerisation of its chromophore (that is, on bleaching), a pore is created in the disk membrane through which Ca^{2+} can penetrate, so that there occurs a nett passge of these ions along their electrochemical gradient, from the intradiskal to the cytoplasmic space. (3) Calcium ions, diffusing about in the cytoplasmic space, act to block pores in the surface membrane which permit the passage of Na^+ between the extracellular and cytoplasmic spaces.

I suggest that a major reason why the hypothesis has now fallen out of favour is the excessive enthusiasm with which it was previously advanced. In its heyday it was offered as the complete explanation of visual transduction. This ignored complications at both (2) and (3) of the hypothetical mechanisms. Measurement of the electrical properties of rods (Falk & Fatt *J. Physiol. Lond.* **299**, 195; 1973) gave evidence that pore formation in the disk membrane did not follow directly the bleaching of rhodopsin; the number of rhodopsin molecules that could be engaged in the process over a period of 10 min was limited to 0.05–0.1 of the number initially available for bleaching. Also, the ability to create pores was lost when the surface membrane was damaged, implying that some diffusible material was required. The attribution of a direct role to Ca^{2+} in blocking the Na pores of the surface membrane also had its problems. Ca ion might act either by entering the pore and so clogging it or by combining with a nearby membrane site so as to alter the electric field at the cytoplasmic end of the pore. However, by neither of these means would the Ca^{2+} released from the disks be likely to be as efficacious as quantitative application of the theory required. An indirect mode of action would be less demanding.

In a scheme that has recently come to the fore and is investigated by Miller and Nicol (*Nature op. cit.*) the role of Ca^{2+} is envisaged to end in the activation of the enzyme phosphodiesterase (PDE) which catalyses the destruction of cyclic guanosine monophosphate (cyclic GMP), which is in turn required for activation of another enzyme that leads eventually to opening of Na pores in the surface membrane. Miller and Nicol examine the control of the Na permeability of the surface membrane by experimentally introduced cyclic GMP and observe the latter's destruction by light-induced activation of PDE. The authors make a passing reference to Ca^{2+} as among the possible activators of PDE.

Robinson and Hagins also give the Ca hypothesis a sideways acknowledgement. This time Ca^{2+} is mentioned as a possible candidate for the transmitter substance, which when pumped back into the disks after its release is considered to account for the observed hydrolysis of GTP to GDP with a time constant of decay of about 10 s following flash illumination. There is also a smaller component of GTP loss attributed to an accelerated turnover of cyclic GMP. As the authors point out, their most unexpected finding is a change in the state of binding of GDP occurring within 4 s of the flash (and possibly within a much shorter period). One may presume this to be related to events taking place in the disks. On the Ca hypothesis the binding of GDP would be involved in the creation of pores in the disk membrane. What is surprising is that the magnitude of the effect is such that some 50 GDP molecules are taken up for each rhodopsin molecule bleached. The question then arises whether all 50 molecules are required to create a single pore or whether the bleaching of a single rhodopsin molecule results in 50 pores, providing chemical amplification.

The latter question leads to the immediate cause of the decline in popularity of the Ca hypothesis. During the past year Liebman and Pugh (*Vision Res.* **19**, 375; 1979) have proposed a model of visual transduction in which events between bleaching of rhodopsin and activation of PDE are considered to take place through the diffusion and chemical interaction of these and associated molecules in the disk membrane. An initial stage of chemical amplification is proposed, its gain being given by the ratio of PDE molecules activated to rhodopsin molecules bleached, a number which may well be the same as the factor of 50 found for GDP uptake. In this model there is no role for the pumping of Ca^{2+} or any other transmitter substance across the disk membrane, nor for the creation of pores in the membrane consequent on the bleaching

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of rhodopsin. Liebman and Pugh attempted to test the Ca hypothesis and claim to have falsified it by measuring light-induced hydrolysis of cyclic GMP in the absence of free Ca^{2+} and showing in a quantitative argument that the observed rate of hydrolysis is of the correct magnitude to account for visual transduction. On the strict application of the scientific method, this should signal the abandonment of the Ca hypothesis. The numerous experimental findings on the involvement of Ca^{2+} in the response of rods to light, as well as the existence of pumping activity and a light-induced permeability increase of the disk membrane, would have to be relegated to the status of inconsequential side-effects. But science, especially biology, does not always work that way. The reason it does not in the present case is that the system under investigation is unlikely to be as simple as the model of Liebman and Pugh attempts now to represent it, or as the Ca hypothesis did earlier. There is in addition the disturbing factor that the biological material on which the different studies have been made is not the same. Liebman and Pugh used finely fragmented rods to examine the behaviour of separated disks, whereas studies in support of the Ca hypothesis have often been constrained to use undamaged rods in order to yield results consistent with the hypothesis. Under these circumstances the hypothesis has not been quite abandoned, as the letters of both Miller and Nicol, and Robinson and Hagins show. One may expect that it will continue to fare thus until further investigation either causes it to drop from sight completely or permits its reassertion as its rival encounters difficulties. By then, perhaps, its limitations will be better appreciated. □

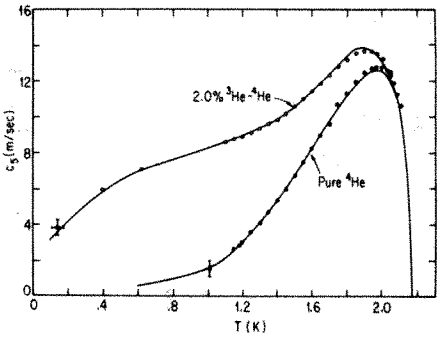


Fig. 1. Experimental values of the fifth sound velocity C_5 (points) compared with the theoretical predictions (full curves) for pure ^{4}He and a dilute ^{3}He - ^{4}He solution, plotted as a function of temperature (T). (From Williams, Rosenbaum & Rudnick *Phys. Rev. Lett.* **42**, 1283; 1979).

New wave of superfluid ^{4}He

from P. V.E. McClintock

A NEW type of propagating wave mode has been found in thin films of superfluid helium-4. Christened 'fifth sound', its discovery is reported simultaneously by two separate research groups, writing in the same issue of *Physical Review Letters*: by Gary A. Williams, Ralph Rosenbaum and Isadore Rudnick of the University of California at Los Angeles (**42**, 1282; 1979); and by G.J. Jelatis, J.A. Roth and J.D. Maynard working at Pennsylvania State University (*op. cit.*, 1285).

At least four different types of wave motion were previously known to occur in liquid helium-4 below its superfluid transition temperature at 2.2 K. The reason for this extraordinary richness of the liquid's vibrational characteristics lies, of course, in the fact that it behaves as though composed of two quite separate but freely interpenetrating components, the normal fluid components and the superfluid component. The former has properties much like those of any other liquid whereas the latter, having zero viscosity and carrying no heat, is largely responsible for the well known exotic properties of liquid helium. Depending on the applied experimental conditions, vibrations can travel through the liquid in a number of different ways, each with its own name and characteristic (but temperature dependent) propagation velocity. The two components can move together (so-called first sound) or in antiphase (second sound) or the superfluid can be made to vibrate by itself while the normal fluid remains clamped in position, by placing the liquid helium in a porous medium (fourth sound). The newly discovered wave mode is a fairly close relation of third sound, which is a wave on the surface of a thin helium film.

A helium film quickly forms on any surface whose lower part dips into the liquid. It is typically up to 200 Å in thickness, depending on the height above the free surface in the helium bath or on the ambient pressure in the case of unsaturated films, and it can support long wavelength modes rather similar to gravity waves on the surface of a classical fluid, but with the van der Waals attraction to the substrate filling the role normally played by gravity. Because viscous forces clamp the normal fluid component and prevent it from moving parallel to the solid substrate, there will be a tendency for the wave to be accompanied by associated temperature changes: at peaks, where the film is thick, there will be extra superfluid and a lower density of heat-carrying normal fluid, corresponding to a lower temperature; at troughs, where the density of superfluid is depleted, the film will tend to be hotter. Normally, however, an additional mechanism comes into play and effectively irons out all such incipient temperature differences. The film is in thermal equilibrium with the surrounding helium vapour, and so a hot spot on the film results in increased evaporation leading to local cooling, whereas the vapour tends to condense preferentially on to any cold spots, thereby warming them. Thus a wave on the surface of the film is usually a pure thickness mode, not accompanied by any changes in temperature: it is known as third sound.

If, however, the evaporation/condensation phenomenon could somehow be inhibited, the wave would then be different in nature and would propagate at a quite different characteristic velocity, because of the additional restoring force arising from the temperature differential between the troughs and peaks. To this end, the Los Angeles and Pennsylvania groups have both resorted to experimental geometries in which the space available for the vapour is carefully restricted. The basic principle is that the separation between the surface of the helium film and the nearest other surface should be made smaller than the mean free path of helium atoms in the vapour. Under such conditions, the vapour is no longer able to transport heat in the direction parallel to the film. Thus the surface wave, instead of being isothermal as in third sound, becomes adiabatic, with temperature minima at the peaks and maxima at the troughs. For relatively thick helium films, where the surface is only weakly influenced by the van der Waals attraction of the substrate, the temperature differential provides the dominant force driving the wave and determining its propagation velocity. It is this thermally driven surface wave which has now been observed for the first time, and which is being called fifth sound.

The two groups used quite different techniques, however, for restricting the vapour. The Los Angeles group worked with standing wave resonances on helium adsorbed on fine alumina powder, compressed so that the average diameter of the pores between the particles was comparable with the mean free path of helium atoms in the vapour. The Pennsylvania group, on the other hand, have succeeded in setting up a more difficult, though conceptually more straightforward, experiment using travelling waves in which a flat plate is placed very close and accurately parallel to the substrate carrying the helium film. They used optically flat quartz plates, and achieved an average spacing of only 5 μm by sandwiching a few grains of powder between them to stop them touching.

In both cases the experimenters found a wave which had a propagation velocity

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much faster than that of third sound and, after making due allowance for certain complicating factors, they could deduce the velocity of fifth sound from the measurements. Results from the Los Angeles experiment are shown in Fig. 1. Agreement with the theoretical prediction (lower full curve) is quite remarkably good, and this clearly remains the case even when a little helium-3 is added to change the relative proportions of the normal fluid and superfluid components (the helium-3 contributing only to the former).

Fifth sound constitutes an interesting addition to the rich variety of diverse phenomena already known to occur in liquid helium-4. No doubt efforts will soon be made to observe an equivalent mode in the case of superfluid helium-3: for temperatures around 2 mK the mean free path of vapour atoms is effectively infinite and, to this extent (if not in any other), the experimental design will be simpler. □

Measuring weak absorption spectra

from T.F. Hunter

THE past decade or so has seen a major advance in our ability to measure very weak absorption spectra. Such measurements are needed for analysis of trace amounts of materials or for studying intrinsically weak transitions. In the latter category come vibrational transitions to high energy overtone and combination bands, say in the visible region of the spectrum, and of considerable importance to an understanding of chemical kinetics; spin-forbidden electronic transitions; and transitions due to non-linear coupling of the radiation field with a molecular system, for example, two-photon absorption.

Normal absorption spectroscopy, with its measurement of incident and transmitted intensities, can be of value in this area if sufficiently long pathlengths are used; turntable laser sources, allowing multiple-passage of the radiation beam to be set up more readily, have helped. However, very long optical pathlengths are awkward experimentally, and, in measuring the difference between the incident and transmitted beams, no distinction is made between absorption and scattering, a distinction which may be of consequence for very weakly absorbing samples.

Modern photo-electric detection systems measure the intensity of emitted photons with high sensitivity and, for some time, weak absorptions have been followed by so-called excitation spectroscopy where the intensity of emission is plotted as a function of excitation wavelength. For example, two-photon absorption studies in vapours (Hochstrasse *et al. J. Chem. Phys.* **60**, 317; 1974; Schlag *et al. J. Chem. Phys.* **66**, 386; 1977) have been studied with high resolution and sensitivity using fluorescence monitoring of this kind. However, many classes of compound show effectively no fluorescence because the dominant deactivation process from the excited state is a non-radiative coupling to other states and other forms of energy.

In any system where the quantum yield of emitted photons is less than unity (and this means essentially all systems), the non-radiative coupling leads to excess heat being produced following the absorption process. An increase in temperature or pressure in the system results. Two techniques, using this flow of heat energy, have been developed.

Thermal lensing spectroscopy (Gordon *et al. J. appl. Phys.* **36**, 3; 1965; Albrecht *et al. J. Chem. Phys.* **65**, 179; 1976) uses a powerful beam of radiation from a tunable dye laser for example. Following the absorption of some radiation by the weak transition under study, the temperature of the sample fluid rises; since the cross-sectional profile of the intensity of the laser beam is Gaussian in shape so is the temperature distribution. Such temperature variation leads to a concomitant variation in the refractive index, a phenomenon which constitutes a thermal lens. The laser beam diverges slightly due to the flow of heat energy. By intensity-modulating the incident beam and thus enabling lock-in devices to minimise interference from noise (thermal, optical, electrical), and by monitoring a part of the transmitted beam through a pin-

T.F. Hunter is in the School of Chemical Sciences, University of East Anglia, Norwich.

Eukaryotic transcription *in vitro*

by Eleanor Lawrence

IN an unscheduled talk at the recent ICRF Tumour Virus meeting* R. Roeder announced to an appreciative audience the development of an *in vitro* transcription system in which initiation of the adenovirus major late gene transcription unit at the 'correct' site has been unequivocally demonstrated.

He and his colleagues P.A.Weil, D.Luse and Jacqueline Segall at the Washington University School of Medicine, St Louis, have obtained specific initiation and transcription of part of the adenovirus late genes, using either full-length purified adenovirus DNA or restriction fragments containing the start of the transcription unit, in a system consisting of a crude extract (the soluble fraction, S100) of uninfected human KB cells to which purified human RNA polymerase II has been added. Purified polymerases II from other vertebrate cells are also effective but that from wheat germ is not.

Although still in a primitive state this *in vitro* transcription system promises to provide the means by which the basic requirements for the transcription of eukaryotic 'class II' genes can be determined. Class II genes are those transcribed by RNA polymerase II and include many of the single copy sequences in eukaryotic cells, as opposed to the ribosomal RNA genes for example, which are transcribed by RNA polymerase III.

Roeder has yet to test his system on other eukaryotic genes, but the close resemblance of the putative 'promoter' sequences for the adenovirus late genes to those of beta-globin and ovalbumin amongst others, suggests that the system may work equally well for them. Successful transcription of the adenovirus late genes in the absence of any components from infected cells is of particular interest as the late genes are not transcribed *in vivo* until replication of the adenovirus DNA has begun. This suggests either that these genes are being transcribed at a low level even early in infection, or, more attractively, that control over their expression is exerted at a higher level, that of chromatin organisation perhaps, and that these controls are bypassed when purified DNA is transcribed.

The site at which transcription starts *in vivo* has been mapped precisely (see Ziff & Evans *Cell* **15**, 1463; 1978) and so Roeder was able to determine that the 5′ end and oligonucleotide fingerprints of the RNA produced in his system matched those of transcripts *in vivo*. Although initiation is specific, transcription does not continue very far into the gene *in vitro* and is also slower than *in vivo*. So one task is to determine whether this premature *in vitro* termination can be overcome, by adding extracts from adenovirus-infected cells for example. The long term aim is gradually to refine the system until it is fully characterised (as with the *in vitro* assays for DNA replication developed for the small bacteriophages). Determination of which regulatory DNA sequences preceding and within eukaryotic genes are essential for faithful transcription is already well underway in the *Xenopus* oocyte transcription/translation system and more recently through the insertion of defined fragments of eukaryotic genes into mammalian cells using SV40 vectors. A fully characterised *in vitro* assay however, could also identify the array of cell proteins that might be expected to be needed.

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hole onto a photo-cell, the resultant signal as a function of the radiation wavelength gives good spectral information on weak transitions. Absorptivities of around 10^{-4} cm^{-1} are readily determined, most measurements having been done on liquids.

The other technique, using the flow of heat energy, is that due to the optoacoustic effect. This effect is of long standing (Bell *Amer. J. Sci.* **20**, 305; 1880) and has been much used, especially in gas-phase studies, for examination of molecular relaxation processes, for example, vibrational–translational energy transfer, de-activation of triplet states and photofragmentation processes (Read *Adv. Mol. Relaxation Processes* **2**, 257; 1967; Hunter *et al. J. Chem. Soc.* (Faraday II), **70**, 1010; 1974; Hunter *et. al. Chem. Phys. Lett.* **58**, 291; 1978). the optoacoustic (OA) effect consists of detecting the oscillatory pressure changes in a system following absorption of intensity-modulated radiation. This is most commonly done in the gas-phase using condenser or electret microphones, and the combination of this technique with laser excitation has enabled the detection of trace gases with high sensitivity, of the order of 1 p.p.b. in many cases (Kreuzer *et al. Science* **173**, 45; 1971; Patel *et al. Appl. Phys. Lett.* **30**, 578; 1977). Absorptivities as low as 10^{-10} cm^{-1} have been measured. Such trace gas detection has normally been done at one, carefully selected, laser wavelength, but obviously, with tunable lasers, weak absorption spectra can be studied. The most sensitive arrangement is to have the OA cell within a dye laser cavity, where a high level of circulating radiation power is present, and high resolution spectra of methane and ammonia in the visible have been so obtained (Stella *et al. Chem. Phys. Lett.* **39**, 146; 1976).

Two recent papers in *Nature* by C.K.N. Patel and A.C. Tam (**280**, 302, 304; 1979) describe important extensions of the OA technique to the measurement of very weak absorptions in liquids. These authors have used as their periodic excitation source a pulsed tunable dye laser, with pulse width of 1 μs and pulse repetition rate at 10 pulses per second, and have developed a very useful pressure transducer based on a lead zirconate titanate cylinder which is submerged in the liquid. Accurate absorption data are given for pure water over the range 400 to 700 nm, and, perhaps more important, details of two-photon absorption in liquid benzene are presented both for linearly and circularly-polarised excitation. The high photon density, both in space and time, required for two-photon studies is achieved by using pulses and by careful focusing of the laser beam; the sample cell is external to the laser cavity.

OA measurements on liquids have been carried out before but in general have used the same technique as that used in the study of solid samples (Rosencwaig *et al. J. appl. Phys.* **47**, 64; 1976; McDonald *et al. J. appl. Phys.* **49**, 2313; 1978). The surface of the solid, or liquid, is irradiated and the periodic flow of heat is transmitted to a gaseous column containing the microphone. Although widely used in examining solids (several commercial instruments are available) where scattering is a major difficulty with other techniques, the OA method, with a gas as intermediary, has limitations in sensitivity and overall resolution. This is particularly true for solids of high thermal conductivity. The use by Patel and Tam of pressure transducers actually in the liquid, or, for solids, bonded to the surface and thus measuring the sound waves in the solid directly (Farrow *et al. Appl. Opt.* **17**, 1093; 1978), promises to be much more sensitive than the normal approach.

I have concentrated here on the use of the OA technique to measure weak absorptions; its use in the more specialised areas of energy transfer such as photofragmentation shows equal promise. As a detection system it covers all excitation wavelengths, the only requirement being that the excited states produced relax, in whole or in part, to heat energy; thus advances in laser technology (the production of fully tunable infrared lasers) and further improvements in the electronic ability to recover signals from noise will ensure progress in the range and sensitivity available. □

Implications of immunological heterogeneity of tumours

from Robert S. Kerbel

THE concept of 'tumour progression', coined some 25 years ago by Leslie Foulds, has stood the test of time. Its importance cannot be overestimated: to understand tumour progression is to understand why cancer is such a frustratingly difficult disease to treat. Simply stated, tumour progression is the process of independent, stepwise and permanent changes in the many different properties of a malignant tumour. Immunogenic specificity, karyotypic diversity, drug-resistance, hormone-dependence, metastatic potential, degree of cellular differentiation and conversion to ascitic growth are some of the properties which can change as the tumour develops. The process of progression is thought to have its basis in the continuous emergence of successive clones of tumour cell variants, one gradually replacing another, through the intervention of natural or artificial selection pressures.

Progression implies that a tumour cannot be simply a homogeneous population of malignant cells. The undoubted monoclonal origin of most tumours in turn implies an inherent instability (probably genetic, as argued persuasively by Nowell (*Science* **194**, 23; 1976)) which results in tumours continually throwing out mutant cells, a few of which have a selective advantage and become the progenitors of new clones which eventually predominate. The result is the slow but inexorable evolution of an increasingly malignant and autonomous tumour, one not subject to most of the normal constraints imposed by the host.

Immunogenic heterogeneity within a tumour obviously has profound implications not only for scientists studying the immunological relations between host and tumour but also for the practical application of specific immunotherapy, especially in relation to tumour recurrence and metastasis.

The first indications of immunogenic heterogeneity were provided by Prehn in 1970 (*J. Natn. Cancer Inst.* **45**, 1039; 1970) who established two sublines of cells with different antigenic specificities each derived from different fragments of tissue taken from opposite sides of a primary methylcholanthrene-induced mouse sarcoma. A pair of cell lines established in a similar manner from later serial passages of the same tumours did not differ, however. Prehn concluded that primary or early-passage tumours possess marked, random antigenic heterogeneity which can be lost during subsequent passage as the result of the immune selection exerted against the more immunogenic clone(s).

The importance of such results to the problems of tumour recurrence and metastasis were nicely illustrated by subsequent work performed in many laboratories. It is well known that if cells from established transplantable tumours are inoculated subcutaneously into mice and the resultant tumour removed early enough, the tumour does not regrow at the primary site. The animals also become highly resistant to a later injection of cells from the same tumour line. However, if primary methylcholanthrene rat sarcomas were surgically resected, secondary regrowths almost invariably developed at the resection site (Pimm & Baldwin *Int. J. Cancer* **20**, 37; 1977). Furthermore, cell lines established from such recurrent tumours were found to be immunologically distinct, as assessed by immunisation–protection experiments, from lines established from their original primary

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sarcomas. Similarly, metastases, that is, tumour recurrences found at a site distant from the original tumour, may contain antigens different from those of the primary tumour, or may have lost some antigens completely — the so-called deletion mutants, for example (Sugarbaker & Cohen *Surgery* **72**, 155; 1972).

The significance of such immunological heterogeneity should not be lost on those who use established transplantable tumours to study the immunology of the tumour–host relationship. What it means in relation to the specific immunotherapy of experimental tumours is made abundantly clear by some recent experiments reported by Olsson and Ebbesen (*J. natn. Cancer Inst.* **62**, 623; 1979). They attempted to prevent the growth of AKR mouse thymic lymphomas (of recent origin), by repeatedly injecting young mice with irradiated AKR thymoma cells, after the recipients had been injected with untreated cells of a primary AKR thymoma. Not surprisingly, the procedure was generally unsuccessful; about 70% of the treated mice developed thymomas. What was surprising was that the thymomas which arose in these mice were immunologically distinct from those in untreated control recipients, as assessed by antibody and cytotoxic T cell assays. After analysing many tumours, the authors concluded that virtually all AKR thymomas are immunologically polyclonal, consisting of a dominant clone, and three distinct minor subclones — the latter group comprising less than 3% of the original tumour cell population. By treating animals with unfractionated thymoma cells, effective immunity is induced only against the dominant clone since the cells of any minor clone are present in too small a number to stimulate the immune system. This merely allows the cells of a minor or 'silent' clone to flourish, so that it eventually becomes dominant. When Olsson and Ebbesen treated their animals with a mixture of equal and large numbers of cells from each clone, so that effective immunity would be produced against all the clones, they produced a cure rate of greater than 90%.

These results also suggest that tumour progression may be more complex than previously thought. It may be that newly emerging clones do not always have to replace one another successively or die off. Rather, many may lead a chronic but cryptic existence, content to live in the shadow of the dominant clone. Only with a favourable change in the immunological *status quo* do they get a chance to flex their muscles.

It would be tempting to blame the failures of specific tumour immunotherapy on immunological heterogeneity and the existence of 'silent' subclones. There are, however, many who would say this is nonsense since most naturally arising tumours are not demonstrably immunogenic (Hewitt *et al. Br. J. Cancer* **33**, 241; 1976). Indeed, the Kleins have stressed the importance of the unrelenting process of tumour progression in the evolution of non-immunogenic spontaneous tumours, whereby successive immunogenic clonal variants replace each other as a result of selection, until a variant emerges which, lacking tumour antigens, becomes independent of the usual restrictions imposed by the immune system (*Proc. natn. Acad. Sci. U.S.A.* **74**, 2121; 1977). This remains a sensitive issue, and the antigenic properties of human tumours are still far from clear. Perhaps the extensive use of hybridomas to produce monoclonal antibodies against putative human tumour-specific antigens will help eventually to resolve this controversy (Herlyn *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 1438; 1979).

The non-immunogenicity of many primary spontaneous tumours and the polyclonal nature of those that are immunogenic, do not auger particularly

Is NGF an enzyme?

NERVE growth factor (NGF) is a protein with a key role in the growth and differentiation of immature sympathetic nerve cells and in the maintenance of fully differentiated sympathetic neurones.[1] Since its discovery in several organs, cell lines and body fluids, biochemists have faced the apparently simple but actually very difficult task of isolating and identifying the polypeptide responsible for such impressive biological action. Attempts to purify this 'molecule' from cell-free extracts have been hampered by the tendency of NGF to associate with other components of the homogenates. Thus the apparent size and sedimentation constant of the NGF 'molecule' have varied according to isolation procedures used. The molecular identity of NGF has changed from a protein of molecular weight 44,000 (ref. 2) to a component of molecular weight 40,000 which is strongly associated with other components of the extract so that together they elute as a protein larger than 100,000 (ref. 3). A form of NGF defined as 7S on the basis of its sedimentation behaviour has also been isolated and found to be[4] a mixture or complex of three proteins defined as α, β, γ. While γ is an enzyme with arginine esteropeptidase activity and α has no detectable biological or biochemical action, β is the only component of the complex endowed with nerve growth promoting acitvity. Subsequent studies suggested that the function of the γ component in an intact cell is to cleave a pro-NGF chain of larger size, as demonstrated for hormones such as insulin[5].

Finally, a 30,000 molecular weight NGF consisting of two identical polypeptide chains held together by non-covalent bonds was isolated in pure form and defined as 2.5S NGF[6]. The β and 2.5S NGF are almost identical proteins differing only in an N-terminal octapeptide with no detectable relevance to nerve growth promoting activity. This latter preparation has been sequenced and studied extensively[7]. All attempts to detect enzymatic activity in 2.5S NGF have given negative results.

In a recent article NGF has been reported to be a specific protease although the experiments supporting this conclusion leave room for doubt[8]. The authors isolated from the same source as that of the 2.5S or β NGF, the mouse salivary gland, a protein(s) with molecular weight of 116,000. In acrylamide gel electrophoresis in the absence of detergents or other denaturing substances, this protein(s) runs as a single band which exhibits a protease activity with an almost evanescent degree of specificity for plasminogen (880 ng of this preparation have an activity comparable with 0.083 ng of urokinase). The same band cross-reacts with antibodies directed against 2.5S NGF, a finding which is taken as evidence that NGF is a protease. The authors have overlooked the possibility that the apparently homogeneous 116,000 molecular weight component with protease activity, may be a complex of 2.5S or β NGF plus some other protein(s) easily identifiable with the many proteases present in the salivary glands. Their conclusion requires support from evidence obtained in carefully controlled dissociating conditions that the 116,000 molecular weight complex is indeed composed of a single polypeptide chain and is not a mixture of at least two different entities: one with properties of the 2.5S or β NGF but devoid of enzymatic activity and the other(s) with protease activity. The same authors while reporting this enzymatic property in the 116,000 molecular weight protein acknowledge that the 2.5S NGF alone does not possess such activity. Although all previous work indicates that NGF is associated in mouse salivary glands with proteases, this association is not evidence that NGF itself is an enzyme.

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well for the future of specific immunotherapy. One possible route to overcoming this problem, as the Kleins have pointed out, is tumour cell modification effected by various artificial means. Another is to concentrate more effort on non-specific immunotherapy. The rationale is fairly simple: a few types of lymphoreticular cell, namely activated macrophages and natural killer (NK) cells, seem capable of spontaneously killing various kinds of tumour cell populations. Because classical antigens are not involved in the recognition process — at least as far as activated macrophages are concerned — variants resistant to this form of effector cell killing may arise far less frequently, if at all. Fidler, for example, has shown that it is possible to derive tumour variant sublines resistant to conventional lymphocyte-mediated killing by progressive selection *in vitro* (Fidler *et al. Cancer Res.* **36**, 3160; 1976); the variants appeared to have lost the tumour antigens (Fidler & Bucana *Cancer Res.* **34**, 3945; 1977). There are no reports (yet) of the derivation of similar macrophage-resistant (Mac^R) variants. Whether this is from lack of trying is not clear, but if Mac^R tumour variants prove to be rare, it would provide a ray of hope in the fight against the forces of tumour progression. □

Stellar rotation in Erice

from Evry Schatzman

EVEN stars of the most classical types, if they are rotating, are extremely complicated. The convection zones of any star are difficult enough to understand; nevertheless, the problems facing astrophysicists begin to multiply when the stars are rotating. The generation of a magnetic field, for example, is certainly related to rotation — but how?

The workshop* was restricted to the problems of the instabilities associated with rotation and to the effect of rotation on turbulence. The problem of magnetic fields, which is itself formidable, was purposely limited to a few basic questions concerning its relation to turbulence and rotation. On the other hand, getting hydrodynamicists and astrophysicists together was a good way of finding out which kinds of astrophysical problems can be approached by laboratory experiments, and of determining how far we can go in applying present hydrodynamical knowledge to astrophysical problems.

It is naturally the duty of the astrophysicists to list the problems which seem to depend on processes associated with rotation. In other words, what kinds of observations inform us directly about the physics of the interiors of stars? These observations can be roughly classified according to the depth at which the processes take place, as follows:

(1) magnetic fields in the filaments at the surface of the Sun (perhaps a few scale heights);

(2) supergranulation (the depth of the convective zone);

(3) symmetry properties between the North and South hemispheres of the Sun (flow throughout the whole convective zone);

(4) lithium depletion by transport from the bottom of the convective zone to the lithium burning level (about one scale height below the convective zone);

(5) $^{12}C/^{13}C$ anomalies in giant stars on the first ascending branch of the Hertzsprung-Russell diagram (region in which ^{13}C nuclear processing takes place in main sequence stars, that is, about 1/3 of the stellar radius in from the surface.

In addition, the problem of the origin of white dwarfs' magnetic fields involves the dynamo process in the convective core before large mass loss and white dwarf formation, and consideration of the properties of angular momentum of stars addresses the problem of transfer of angular momentum in the whole star, during formation and on the main sequence.

Only partial answers can be given to these questions. N. Weiss (University of Cambridge) and Galloway (Boulder, Colorado) presented several aspects of the dynamo mechanism, and it is worth stressing that, after years of optimism, the existence of the turbulent dynamo does not seem to be so firmly established. Galloway described the first results of a very large numerical experiment of Gilman (Boulder, Colorado) to simulate the building of the solar-cycle, the significance of which turns out to be very difficult to determine. F. Busse (University of California, Los Angeles) described the role of boundaries on the production of rolls in a rotating thermally unstable region and suggested very strongly that symmetry properties in the solar hemisphere and in the hemispheres of Jupiter can be due to the presence of these rolls.

All the problems of mass transport can be interpreted in terms of meridional circulation or of mixing associated with various instabilities (Rayleigh, shear flow, thermal enhanced). Busse revived the controversy (between Randers, Temesvary, Roxburgh and Baker-Kippenhahn) about the existence of Eddington-Sweet currents by showing that the only stationary solution for a rotating star is a distribution of angular velocities which balances exactly the force induced by stellar oblateness, and cancels the Coriolis force by the suppression of the meridional velocity. S. Temesvary (Tubingen) showed, after Busse, that all non-stationary stream flows decay with the time scale of viscous dissipation, so that the Sun can have any stream flow.

Similarly, for heavier stars (radiative viscosity, electron scattering), the ratio of the viscous dissipation time to the stellar life time grows like M^{-1}! It seems likely that no star lives long enough on the main sequence to reach the magic stationary situation. Therefore, meridional circulation seems to be there. However E. Schatzman (Observatoire de Nice) showed, using Michaud *et al*'s arguments that, if the meridional circulation is responsible for the lithium depletion in the Sun, this contradicts the fact that there is almost no beryllium depletion. J. P. Zahn (Observatoire de Nice) described the conditions for various shear instabilities and Schatzman showed that a consistent picture can be given, with the same turbulent process, probably related to shear flow instability, which explains a variety of abundance peculiarities for elements nuclearly processed deep inside the stars.

The general properties of stellar rotation on the main sequence are now understood, but the detailed mechanisms remain to be established. Then one would not have to rely merely on phenomenology, as one does in discussions of magnetic field generation.

A discussion on the condition in which angular momentum transport takes place in a turbulent flow showed clearly that this problem is still far from being solved. □

Evry Schatzman is at the Observatoire de Nice

*A workshop on rotating stars was held on 2-10 June in Erice.



A hundred years ago

The Americans have stolen a march upon us in sanitary matters, as they have done in so many other things. In March of this year an Act passed the US Congress organising a Board of Health to look after all matters relating to the public health, and to advise Congress what steps should be taken for the promotion of so all-important a matter. We have just received the first number of the weekly *National Board of health Bulletin*, published by this body, who have 50,000 dollars appropriated to them for salaries and expenses. Besides mortality statistics the *Bulletin* contains a number of rules and regulations with respect to the sanitary condition of ships.

Under the date of April 30, a correspondent of the *North China Herald* at Wladivostock, in Russian Manchuria, writes that, although the mountain tops were still covered with snow, the winter had at length come to an end, the ice in the harbour having on that day broken up and floated out to sea. The winter began in October and has been the severest known for many years. The bay was entirely covered with ice from two to three feet thick. Wladivostock is said to be improving, though slowly, and there are signs of an attempt at road-making.

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review article

Population biology of infectious diseases: Part I

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If the host population is taken to be a dynamic variable (rather than constant, as conventionally assumed), a wider understanding of the population biology of infectious diseases emerges. In this first part of a two-part article, mathematical models are developed, shown to fit data from laboratory experiments, and used to explore the evolutionary relations among transmission parameters. In the second part of the article, to be published in next week's issue, the models are extended to include indirectly transmitted infections, and the general implications for infectious diseases are considered.

ANY contemporary ecology text contains at least one chapter devoted to predator–prey interactions. The discussion typically embraces field and laboratory observations along with simple mathematical models, and emphasizes how the densities of both prey and predator populations may be regulated by their interaction.

In natural communities, however, an accumulating body of evidence suggests that parasites (broadly defined to include viruses, bacteria, protozoans, helminths and arthropods) are likely to play a part analogous, or at least complementary, to that of predators or resource limitation in constraining the growth of plant and animal populations. Examples from the laboratory are Park's[1] experiments in which the sporozoan parasite *Adelina* drastically reduced the population density of the flour beetle *Tribolium casteneum*, and in certain circumstances reversed the outcome of its competition with *T. confusum*, and Lancinani's[2] studies of the way the ectoparasitic water mite *Hydryphantes tenuabilis* influences the population dynamics of the aquatic insect *Hydrometra myrae*. Various studies have indicated the importance of infectious disease as a mortality factor in populations of wild mammals[3,4], and as possibly the predominant such factor in bird populations[5–7]. For example, among bighorn sheep in North America the main cause of death probably is infection by the lungworms *Protostrongylus stilesi* and *P. rushi*, which then predispose the hosts to pathogens causing pneumonia[8,9]. On a grand scale, Pearsall[10] and others suggest that the geographical distribution of most artiodactyl species in Africa today is largely set by a pandemic of rinderpest that occurred towards the end of the nineteenth century; the numerical simulations of Hilborn and Sinclair[11] confirm that rinderpest can have a big influence on wildebeest population levels. Several authors[3,5,12–20] have argued the general case for infectious diseases as regulators of their host populations.

More broadly, it is likely that interplay between the pathogenicity of viral, bacterial, protozoan or helminth infections and the nutritional state of the host contributes importantly to the density-dependent regulation of natural populations[13], with the parasites greatly amplifying the effects of low levels of nutrition. Such phenomena are largely responsible for the dramatic differences between age-specific survival probabilities for people in developed and underdeveloped countries[21,22]. Indeed, McNeill[23] and others[24,25] have speculated that many of the broader patterns of human history are to be interpreted in terms of the evolving relationships between man and his diseases.

Although there does exist a large and mathematically sophisticated literature dealing with the transmission dynamics of parasitic infections of many kinds, this literature[26–34] almost invariably assumes the host population to have some constant value, and then seeks to answer such questions as: Can the infection be stably maintained in the population? Is it endemic or epidemic? What is the time course (in terms of susceptibles, infectives and recovered individuals) of the infection when introduced into a virgin population? This assumption that the total host population is effectively constant derives from a history of medical interest in human diseases (predominantly in developed countries), where population densities do usually remain roughly constant on the time scale appropriate to the pathology of most diseases. On the other hand, in the ecological and parasitological literature attention has recently been given to the population dynamics of host–parasite associations, with particular emphasis on the way protozoan, helminth and arthropod parasites can depress the natural growth rate of their host populations[13–17,35]. Our review aims to weave together these medical and ecological strands, concentrating on the way parasitic infections can influence the growth rate of their host populations.

The article is being published in two parts. This first part begins with a survey of the diverse array of infectious organisms and of their associated life cycles. We then show how a very simple dynamic model can provide a remarkably detailed explanation of a classic series of experiments on infections in laboratory populations of mice. This success gives the confidence to enable us to proceed into areas less well supported by good data, and we next discuss microparasitic infections with direct life cycles in natural populations; particular attention is given to the evolutionary relations among transmission parameters, the factors which determine the pattern of disease behaviour within populations of hosts and the population consequences of acquired immunity.

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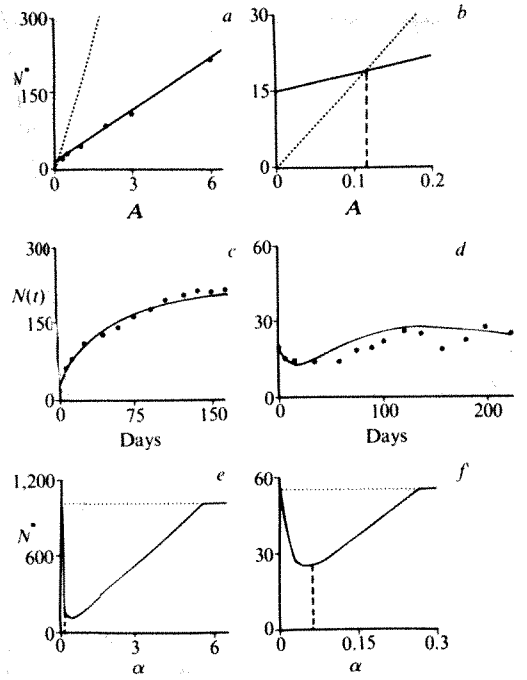


Fig. 1 Population dynamics of *Pasteurella muris* in colonies of laboratory mice. *a*, Relationship between the equilibrium population of mice N^* and the daily rate of input of susceptible mice A (solid dots are observed levels[46,47]; the solid line is the best linear fit, equation (6)). The dashed line shows the estimated relationship between N^* and A in the absence of the disease (the slope is $1/b$, where $b = 0.006$). *b*, An enlargement of that portion of (*a*) where solid anddashed lines intersect, determining the threshold level of immigration A_T, equation (5), below which the disease will not persist. *c*, *d*, Growth of mouse colonies harbouring the disease, from an initial population of 20 mice, for $A = 6.0$ and 0.33, respectively (again, solid dots are the experimental data, and the solid lines are the theoretical predictions described in the text). *e*, *f*, Relationship between the equilibrium population of mice N^* and the disease-induced mortality rate α, as predicted by equations (1)–(3), for $A = 6.0$ and 0.33, respectively. The dotted vertical line shows the actual value of α for *P. muris*.

In the second part of the article[36], we begin with a discussion of macroparasitic infections with direct life cycles in natural populations. Extensions to parasites with indirect life cycles are then briefly indicated, with emphasis on the way the ecology of the general evolutionary trends. Finally, we survey the main mechanisms that can produce cyclic patterns, or multiple stable states, in the levels of infection in the host population.

Diversity of agents causing disease

By using the term 'parasitic infection' to include all organisms—viruses, bacteria, protozoans, helminths and arthropods—on the US Centre of Disease Control's list, we are encompassing a great diversity of life forms and of associated population parameters. Broadly, however, two classes may be distinguished:

Microparasites (viruses, bacteria, protozoans) are characterised by small size, short generation times, extremely high rates of direct reproduction within the host, and a tendency to induce immunity to reinfection in those hosts that survive the initial onslaught[37]. The duration of infection is typically short in relation to the expected lifespan of the host, and therefore is of a transient nature (there are, of course, many exceptions, of which the slow viruses[38] are particularly remarkable).

Macroparasites (parasitic helminths and arthropods) tend to have much longer generation times than microparasites, and direct multiplication within the host is either absent or occurs at a low rate. The immune responses elicited by these metazoans generally depend on the number of parasites present in a given host, and tend to be of relatively short duration[39,40]. Macroparasitic infections therefore tend to be of a persistent nature[13], with hosts being continually reinfected.

Both microparasites and macroparasites may complete their life cycles by passing from one host to the next either directly or indirectly via one or more intermediate host species. Direct transmission may be by contact between hosts (for example, venereal diseases) or by specialised or unspecialised transmission stages of the parasite that are picked up by inhalation (such as common colds), ingestion (such as pinworm) or penetration of the skin (such as hookworm). Indirect transmission can involve biting by vectors (flies, mosquitos, ticks, and others) that serve as intermediate hosts, or penetration by free-living transmission stages that are produced by molluscan or other intermediate hosts. In other cases, the parasite is ingested when an infected intermediate host is eaten by the predatory or scavenging primary host. A special case of direct transmission arises when the infection is conveyed by a parent to its unborn offspring[41] (egg or embryo), as can occur in syphilis and rubella and for many viral infections of arthropods; this process has been termed 'vertical transmission', in contrast to the variety of 'horizontal transmission' processes discussed above.

The natural historian's main concern is often the recondite biological details that make each parasitic infection unique. In contrast, our aim is to understand the basic similarities and differences in terms of: the number of population variables (and consequent equations) needed for a sensible characterisation of the system; the typical relations among the various rate parameters (such as birth, death and recovery rates, transmission coefficients); and the form of the expressions describing the transmission processes. In the absence of such a unified framework, each disease tends to develop its own arcane literature.

Experimental epidemiology: infectious diseases as regulators of laboratory populations of mice

Although there are relatively few studies of the influence of disease upon the dynamics of laboratory populations[1,2,42–45], there is a remarkably detailed body of work of Greenwood *et al.*[46,47], subsequently extended by Fenner[48,49]. These experiments, on laboratory populations of mice infected with various viral and bacterial diseases, have some simplifying features which make them particularly amenable to theoretical analysis. Specifically, the space available to the mice was adjusted to keep the population density constant as absolute levels changed; in addition adult mice were introduced at specified rates, so that the basic process was an immigration–death one (removing the time lags and other complications attendant upon recruiting to the population by natural birth processes). In short, many density-dependent complications are avoided by the design of the experiments.

We now outline a simple model that captures the essentials of these experiments, and discuss its fit to the data for two microparasites: one a bacterium (*Pasteurella muris*); the other a virus (ectromelia, a poxvirus). Both parasites multiply directly within the host and induce a long-lasting immunity to reinfection (mice show some loss of immunity to reinfection by *Pasteurella*, but the immunity to ectromelia seems to be lifelong).

Using notation that will be standard throughout this review, we define the absolute number of susceptible (uninfected), infected and immune mice to be X, Y and Z, respectively. The total number of mice, $N = X + Y + Z$, is not assumed to be some independently-set constant, but is set by the dynamics of the infection. A is defined as the rate at which mice are introduced ($A = 2$ means 2 mice introduced per day), and b the natural mortality rate; in the absence of the disease, the mouse population will equilibrate at around $N^* = A/b$. The infection is a direct one, for which the conventional assumption is that the rate at which mice acquire the infection is proportional to the number of encounters between susceptible and infected mice, being βXY where β is some 'transmission coefficient'. The mortality rate for infected mice is taken to be $b + \alpha$, with α

Table 1 The influence of various types of directly transmitted microparasites on host population growth

Type of disease	Growth characteristic (disease regulates host population if expression is negative)	Threshold host population, for successful introduction of the disease
Horizontal transmission		
No immunity ($\gamma \to \infty$)	$r-\alpha$	$(\alpha+b+v)/\beta$
Life-long immunity ($\gamma = 0$)	$r[1+(v/b)]-\alpha$	$(\alpha+b+v)/\beta$
Transient immunity (duration $1/\gamma$)	$r[1+v/(b+\gamma)]-\alpha$	$(\alpha+b+v)/\beta$
Transient immunity and an incubation (latent) period of duration $1/\sigma$	$r\left[1+\frac{v}{(b+\gamma)}+\frac{(\alpha+b+v)}{\sigma}\right]-\alpha$	$\frac{(\alpha+b+v)(b+\sigma)}{\beta\sigma}$
Transient immunity and disease eliminates reproduction of infected class	$rv/(b+\gamma)-(b+\alpha)$	$(\alpha+b+v)/\beta$
Transient immunity and disease reduces birth rate of infected class to fa	$r\left[\frac{fa-b}{r}+\frac{v}{(b+\gamma)}\right]-\alpha$	$(\alpha+b+v)/\beta$
Vertical (and horizontal) transmission		
Transient immunity and all births from infected class are also infected	$r[1+v/(b+\gamma)]-\alpha$	$(\alpha+b+v-a)/\beta$; threshold is zero if $a>\alpha+b+v$
Transient immunity and a fraction f of births from infected class are also infected	$r[1+v/(b+\gamma)]-\alpha$	$(\alpha+b+v-fa)/\beta$; threshold is zero if $fa>\alpha+b+v$

representing the mortality caused by the disease; there is also a recovery rate v. Recovered mice are initially immune, but this immunity can be lost at a rate γ (for permanent immunity, as for ectromelia, $\gamma = 0$). These assumptions lead to the following equations for the dynamics of the infection:

$$dX/dt = A - bX - \beta XY + \gamma Z \quad (1)$$

$$dY/dt = \beta XY - (b+\alpha+v)Y \quad (2)$$

$$dZ/dt = vY - (\gamma+b)Z \quad (3)$$

Adding all three, the equation for the total population of mice is

$$dN/dt = A - bN - \alpha Y \quad (4)$$

This system of equations (which is similar to that illustrated schematically by Fig. 3) differs from usual epidemiological models in that N is a dynamical variable, rather than some specified constant.

The equations have a stable equilibrium solution with the disease maintained in the population if, and only if,

$$A/b > (\alpha+b+v)/\beta \quad (5)$$

Failing this, the disease dies out, and the population settles to its immigration–death equilibrium value at $N^* = A/b$. If equation (5) is satisfied, the disease persists, and the total population is depressed below this infection-free level to the lower value

$$N^* = \frac{A + D(\alpha+b+v)/\beta}{b+D} \quad (6)$$

Here D is defined for notational convenience as

$$D = \alpha/[1+v/(b+\gamma)] \quad (7)$$

Note that the important threshold phenomena, which enter directly when N is a specified constant[26,31–34,50], appear in a more subtle form when N is itself determined by the dynamics of the disease.

In their experiments on the maintenance of pasteurellosis, *P. muris*, in mouse populations Greenwood *et al.*[45,46] introduced new mice at rates ranging from $A = 0.33$ to $A = 6$ mice per day. The quantities b, α and v can be crudely estimated from life tables for uninfected populations, and from case mortality and recovery rates (we get $b \simeq 0.006$, $\alpha \simeq 0.06$, $v \simeq 0.04$ days^{-1}). Direct estimate of the parameters β and γ is more difficult.

Using data from Greenwood *et al.*[46,47] (and reanalysing to discard the transient initial population values en route to the steady state), we obtain the experimental results shown in Fig. 1*a* for the equilibrium mouse population N^* as a function of A. These data accord well with the linear relation between N^* and A predicted by equation (6). Furthermore, the parameters β and γ may now be roughly estimated from the fit between the theoretical straight line, equation (6), and the data for N^* versus A (we estimate $\beta \simeq 0.0056$, $\gamma \simeq 0.021$ days^{-1}).

In Fig. 1*a*, the dashed line depicts the equilibrium mouse population in the absence of the disease, $N^* = A/b$. The intercept of this line with the linear fit to the data for N^* in the presence of the disease yields the threshold immigration rate, A_T, below which equation (5) is violated and the disease cannot persist; Fig. 1*b* magnifies this aspect of Fig. 1*a*. We estimate $A_T \simeq 0.11$ mice per day (corresponding to an equilibrium population of about 19 mice). Greenwood *et al.* suggested *P. muris* was always maintained in mice populations, but their lowest introduction rate was $A = 0.33$.

With β and γ determined from Fig. 1*a*, we now have a parameter-free prediction of the temporal development of the infection for any initial number of mice $N(0)$ and introduction rate A. Two such fits between theory and data are shown in Figs. 1*c* and 1*d*, for $A = 6$ and $A = 0.33$, respectively. Note the propensity to damped oscillations at relatively small A values. Bearing in mind the complete absence of adjustable parameters, both the fits are extremely encouraging, and strongly suggest that simple deterministic models can be useful even when the host population is small.

How much does the disease depress the mouse population below the level that would pertain in its absence? This general question is answered in Fig. 1*e* and *f*, which shows N^* as a function of disease pathogenicity α, for $A = 6.0$ and $A = 0.33$ respectively. Two significant points emerge.

First, the maximum depression of the host population is achieved by a disease of intermediate pathogenicity[51]. Too small an α has little effect on N^*, while too large an α violates equation (5) and makes it impossible for the disease to persist. The dashed vertical lines in Fig. 1*e* and *f* show the actual value for α for *P. muris*.

Second, note that the higher the immigration rate A, the greater the degree of depression of the host population (relative

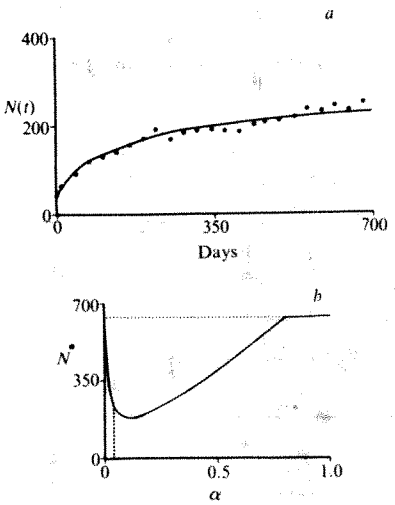


Fig. 2 Population dynamics of ectromelia in colonies of laboratory mice. The data[46,48,49] indicate $b = 0.005$, $\alpha = 0.042$, $\beta = 0.0013$, $v = 0.014$, $\gamma = 0$; the rate of introduction of susceptible mice was always $A = 3$ (all quantities in units of day^{-1}). *a*, Growth of a mouse colony harbouring the disease, from an initial population of 45 mice (dots and solid curve as in Fig. 1*c*, *d*). *b*, Depression of the equilibrium population of mice N^* as a function of pathogenicity α for ectromelia, analogous to Fig. 1*e*, *f*.

to the disease-free equilibrium value). This suggests that diseases caused by microparasites are more likely to persist within, and cause severe reduction of, host populations with high birth (or immigration) rates; this phenomenon derives essentially from the high inflow of susceptibles.

Greenwood *et al.*[46], and later Fenner[48,49], also studied the effects of the mouse pox virus, ectromelia. An analysis, akin to that just outlined, leads to similarly encouraging agreement between our simple theory and the experimental data for ectromelia in laboratory populations of mice. Some of these results are summarised in Fig. 2.

For both *P. muris* and ectromelia, the actual value of the pathogenicity parameter α (indicated by the dashed vertical lines in Figs 1*e*, *f* and 2*b*) lies around the value that induces maximum depression of the host population. Is this coincidence, or does it reflect evolutionary pressures? The question is intriguing, but difficult to pursue in the absence of a larger body of information about a wider range of diseases.

In brief, the theory and the facts of these experiments are in accord in showing how infectious diseases can stably regulate their host populations below disease-free levels. They also show the existence of a critical host density (directly tied to the rate at which new susceptibles are introduced, either artificially in the laboratory, or by births in the natural world), below which the infection cannot be maintained. In this sense, equation (5) replaces the threshold condition of conventional epidemiological models in which the host population is an independently determined constant.

Microparasitic infections as regulators of natural populations

The models discussed above are only half-way to a fully dynamic description of host–parasite interactions. Although the death rates are set by natural processes, and are influenced by the parasites, the 'birth' processes are determined artificially by the rate of introduction of new mice. We now consider what happens when the birth rates are also set by natural processes intrinsic to the host population.

To begin with, we focus on diseases caused by microparasites that are transmitted directly, and ask three main questions: what biological characteristics of an infection determine its impact on host population growth; what are the population consequences of immunological responses; and what conditions lead to endemic or to epidemic infections?

Consider the simple situation of an infection whose transmission processes are as described by equations (1)–(3), except that now the new individuals arise by natural births. This situation is illustrated schematically in Fig. 3. If the *per capita* birth rate is a, independent of whether the individual is susceptible, infected or immune, then the net birth rate term is $a(X+Y+Z)$, and the dynamical system of equations (1)–(3) is replaced by

$$dX/dt = a(X+Y+Z) - bX - \beta XY + \gamma Z \quad (8)$$

$$dY/dt = \beta XY - (\alpha + b + v)Y \quad (9)$$

$$dZ/dt = vY - (b+\gamma)Z \quad (10)$$

The total population of hosts, $N = X+Y+Z$, obeys

$$dN/dt = (a-b)N - \alpha Y \quad (11)$$

Equivalently it is useful to define the intrinsic growth rate $r = a-b$ of the disease-free population and to write $y = Y/N$ as the 'prevalence', or fraction of the host population that are infected. This gives

$$dN/dt = (r - \alpha y)N \quad (12)$$

One of two circumstances now arises. If

$$\alpha > r\left[1 + \frac{v}{b+\gamma}\right] \quad (13)$$

the disease regulates the host population to a stable value N^*. This disease-determined population level is

$$N^* = \frac{\alpha(\alpha+b+v)}{\beta[\alpha - r(1+v/\{b+\gamma\})]} \quad (14)$$

Of this steady population, the fraction infected is given trivially from equation (12) as

$$y^* = r/\alpha \quad (15)$$

Conversely, if equation (13) is not satisfied, the system of equations (8)–(10) eventually settles to a state in which the total population grows exponentially at a rate ρ given by

$$\rho = [B^2 - (b+\gamma)(\alpha - r) + rv]^{1/2} - B \quad (16)$$

with $B = \frac{1}{2}(\alpha + b + v + \gamma - r)$. This population growth rate is necessarily less than the disease-free one, $\rho < r$. Asymptotically, the exponentially growing total population contains a constant number of susceptibles X, with essentially all individuals being infected or immune. The asymptotic prevalence of infection is

$$y \equiv Y/N \to (r-\rho)/\alpha \quad (17)$$

Note the similarities and differences between these conclusions and those for conventional models[26,31–50] in which the total population is set at some constant value. In this crude model there are no density-dependent regulatory effects other than the

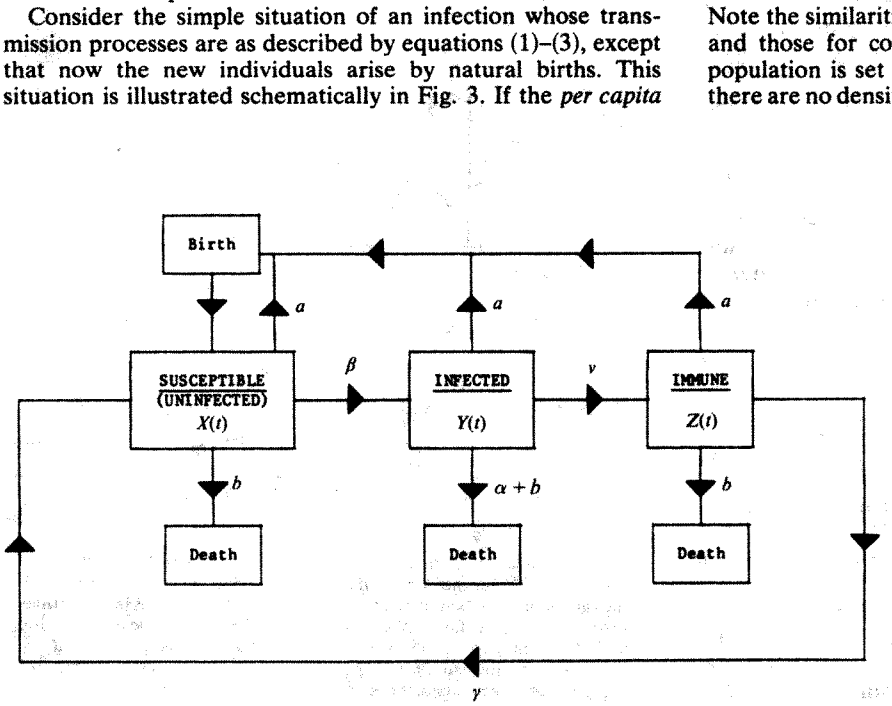


Fig. 3 Diagrammatic flow chart for a directly transmitted infection, described by a compartment model with susceptible (X), infected (Y), and immune (Z) hosts. The flow of individual hosts between compartments is controlled by a set of rate parameters: *per capita* birth rate, a; natural death rate of hosts, b; disease-induced mortality, α, acting on infected hosts; recovery rate, v; transmission rate per encounter between susceptible and infected hosts, β; rate of loss of immunity, γ.

Table 2 Population characteristics of some common directly transmitted microparasites of man (data from refs 66–69)

Parasite	Incubation period (days)	Duration of infectiousness (communicability) (days)	Infectiousness	Duration of immunity	Lifespan of infective stage	Case mortality rate (pathogenicity)	Transmission (H = horizontal V = vertical)
Measles virus	9–12	5–7	High	Lifelong	Very short	Low–high	H
Smallpox virus	12–14	10	Medium	Lifelong	Long	High	H
Rubella virus	17–20	14	Medium	Lifelong	Very short	Low	H, V
Mumps virus	10–20	7	Medium	Lifelong	Short	Low	H
Bordetella pertussis (whooping cough)	7–10	14+	High	Lifelong	Very short	Medium	H
Polio virus	5–20	Long	High	Lifelong	Medium	Medium	H
Varicella zoster virus (chicken pox and shingles)	13–17	20–30	High	Lifelong	Very short	Low	H (V)
Herpes simplex virus	5–8	Long	Medium	Lifelong	Very short	Very low	H, V
Cytomegalovirus	Long?	Long	Medium	Lifelong	Very short	Very low?	H, V
Epstein–Barr virus	10?	Long?	Medium	Lifelong	Very short	Very low?	H
Clostridium tetani (tetanus)	7+	21–30	Low	Lifelong	Long	High	H
Salmonella typhi (typhoid)	10–14	30+	Low	Short	Medium	High	H
Bacillus anthracus (anthrax)	3–7?	?	Low	Long	Very long	Very high	H
Corynebacterium diphtheriae (diptheria)	2–6	20	Medium	Long	Medium	High	H

disease itself and the population 'runs away' (maintaining the disease within it) at the diminished rate ρ if α is too small to satisfy equation (13). Conversely, if equation (13) is fulfilled, the population settles to the value N given by equation (14). In either case, if N is initially less than a threshold value,

$$N_T = (\alpha + b + v)/\beta \qquad (18)$$

then initially Y will decrease and X will increase exponentially at the rate r. However, once X exceeds N_T on this trajectory of exponential increase, then Y will increase, and the system either will converge (steadily or with damped oscillations) on the N^* of equation (14), or will grow at the slower rate ρ of equation (16). Thus the familiar threshold phenomena are found within the more dynamic system of equations (8)–(10).

Equation (13) is clearly a key one. It can be modified to take into account the known biology of a wide range of directly transmitted microparasitic infections. Without discussing the derivations, Table 1 lists the criterion for ability to regulate the host population (generalising equation (13)), and the threshold expression (generalising equation (18)), for a variety of such refinements, including *inter alia* the effects of incubation periods, vertical transmission, and infections that reduce host reproduction.

Several general points emerge from Table 1. (1) For a disease to regulate the host population, the case mortality rate α must be high relative to the intrinsic growth rate r of the disease-free host population. Ability to achieve this degree of regulation is decreased by lasting immunity (γ small) and high rates of recovery from infection (v large, corresponding to infections of short duration). (2) Diseases with long incubation periods, where hosts are infected but not infectious, have less impact on population growth. (3) Diseases which affect the reproductive capacities of infected hosts are more liable to suppress population growth. (4) Vertical transmission lowers the magnitude of the threshold population, N_T needed for successful introduction of the disease; vertical transmission also lowers the equilibrium population of the host in those cases where it is regulated by the disease. (5) The threshold density below which the disease cannot persist within the host population is set by the rate of loss of hosts from the infected class divided by the rate of transmission; high threshold densities are therefore required for the maintenance of diseases with short durations of infection, long incubation periods and high case mortality rates.

Population consequences of immune responses

Although the nature of immunological responses by individual hosts to specific pathogens has received much attention in recent years[37,40,52], relatively little thought has been given to the population consequences of acquired immunity[26,32,53] (sometimes called 'herd immunity' effects). The general insights just culled from equations (13)–(18) can be usefully illuminated by a numerical example. Figure 4*a* shows the growth of a fictitious human population (from an initial size of 50,000) subject to a virus disease and under various assumptions about the duration of immunity. The vital rates and transmission parameter values are as detailed in the figure caption. In more homely terms, they represent a growth rate of the disease-free population of around 3% per annum, a case mortality of about 30% (similar to measles in malnourished human populations with no previous exposure[54]), duration of the infection around 4 weeks, and a transmission coefficient β that implies a threshold population density of $N_T \simeq 380{,}000$ people.

In all cases in Fig. 4*a*, the disease is not maintained and the population grows at its intrinsic 3% rate if it is below the threshold value N_T. Above this point, the population's fate depends on the nature of the immune response. If the duration of the immunity to reinfection ($1/\gamma$) is of short to medium length (less than about 20 yr), the disease is able to regulate the host population at the stable level N^* of equation (14). If the disease induces hardly any immunity (γ large), this equilibrium level N^* will be close to the threshold N_T for maintenance of the disease. Conversely, if the duration of immunity is above 20 yr, the population continues to grow exponentially at some rate lower than 3%; life-long immunity ($\gamma = 0$, as for measles) results asymptotically in 1.6% per annum growth. This example makes plain the important part immunity plays in determining the population consequences of a disease.

The qualitative patterns revealed in Fig. 4*a* are reminiscent of those shown by human population growth[55,56] between the beginning of the Agricultural Revolution (some 10,000 years ago) and the onset of the Industrial-Scientific Revolution (around 300 years ago). In the first 5,000 yr, the global population increased about 20-fold, from around 5 million to around 100 million. The next 5,000 yr saw only a roughly 5-fold increase to around 500 million in the sixteenth century. It may not be unduly fanciful to speculate that the rise of human conglomerations to levels capable of maintaining directly transmitted microparasitic diseases, and the accompanying depression of population growth rates, is at least partly responsible for the observed patterns.

Epidemic and endemic patterns of diseases

Epidemic diseases are characterised by rapid changes in the prevalence of infection. Often such infections disappear from a particular host population for short or long periods. Conversely, endemic infections persist for long times, showing relatively little fluctuation in prevalence. Note that in our dynamic models, equations (8)–(10), the disease always becomes endemic, in the sense that the host population grows to the level $N > N_T$, whereupon the disease is maintained. The prevalence settles to the steady value $y^* = r/\alpha$ if the disease controls the population, and to $y^* \to (r - \rho)/\alpha$ if the population still grows.

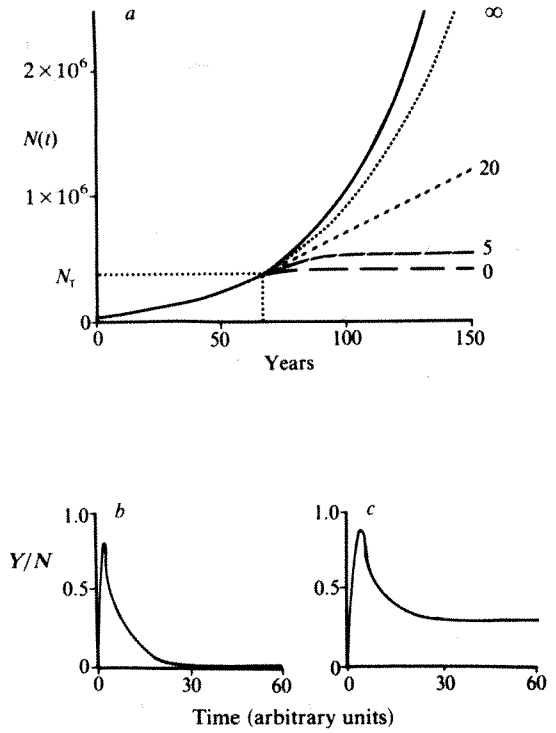


Fig. 4 *a* An illustration of the way the duration of immunity influences the dynamics of host population growth, $N(t)$, for a hypothetical disease; the various population parameters are assumed to be $r=0.03$, $b=0.015$, $v=13.0$, $\alpha=6.0$, $\beta=5\times10^{-5}$ (all per year), and $N(0)=50{,}000$. The solid line depicts the population growth in the absence of the infection. The four broken lines depict the effects of immunity of varying duration, namely (as labelled): $1/\gamma=\infty$ (lifelong); $1/\gamma=20$ yr; $1/\gamma=5$ yr; and $1/\gamma=0$ (no immunity). *b*, Temporal changes in the prevalence of infection, Y/N, following the introduction of the above disease into a virgin population of hosts where the equilibrium prevalence level is low. *c* As for (b), except now the equilibrium prevalence is relatively high.

It is well known, however, that diseases which induce long-lasting immunity often exhibit periodic or episodic 'face out', even within relatively large host populations[26,30,57–61]. In particular, the classic work of Bartlett[62,63] on measles epidemics has suggested the importance of stochastic effects in determining whether a disease will persist endemically or as recurrent epidemics.

Without entering into the detailed complications of a stochastic formulation, we can use the above model to get some qualitative insights about these patterns. Of particular importance is the rate at which new susceptibles appear; hence the general correlation between endemicity and host population size[64], and the observation that the host birth rate is central. Specifically, consider the case where the hosts' intrinsic growth rate is much smaller than the case mortality rate, $r \ll \alpha$. Then, if $N > N_T$, introduction of the infection results in a classical epidemic (see Fig. 4*b*): the prevalence first rises, attains a peak, and then falls to the value given by equation (15) or equation (17), which in either case is very small. That is, if $r \ll \alpha$, it is likely that prevalence settles to be so small as to give a high probability for stochastic 'fade out' and epidemicity. This can be true even for diseases potentially capable of regulating the host population, if r/α and N^* are both sufficiently small. In addition, epidemics can occur even when $r > \alpha$ if the disease does not regulate the host population but merely slows its growth rate slightly, so that $r-\rho \ll \alpha$ (the details of the interplay among parameters that leads to $\alpha+\rho \gg r > \alpha$ are complicated, and can be deduced from equation (16) for ρ). On the other hand, if neither r nor $r-\rho$ is a lot smaller than α, the disease is likely to be endemic, with relatively high values of y^* making stochastic extinction of the disease improbable[62,63,65,66]. This circumstance is depicted in Fig. 4*c*.

Thus infections of short duration which induce lasting immunity will tend to exhibit epidemic patterns. The classic 'epidemic' disease such as measles, rubella and pertussis are of this character[67–70]. As also stressed by Yorke *et al*[32], a broader examination of viral and bacterial infections of man clearly supports this point (see Table 2). Many authors[57,58,71] have observed that such infections are probably diseases of modern societies; in primitive societies the net inflow of susceptibles into small communities was probably too low to maintain the diseases.

Other infectious agents (for example, herpes simplex virus, cytomegalovirus, Epstein–Barr virus) persist in the host for long periods and are of low pathogenicity. Such diseases are usually endemic in character[57] (see Table 2). Moving beyond human populations, it is important to remember that hosts with high rates of reproduction, such as arthropods, may be able to support endemic disease even if host density is low[72]. Furthermore, infectious organisms that induce life-long immunity, or are of high pathogenicity, can be endemic if they produce free-living infective stages which can survive for a long time in the external environment (anthrax bacillus is an example).

There is no doubt that microparasitic infections can slow population growth[73–75]. Whether a given disease will regulate the host population or merely slow its growth, and whether the infection will be endemic or epidemic, depends on the interplay of many biological parameters[32,70]. Unfortunately, our quantitative knowledge of these parameters is limited, even for viral and bacterial diseases of man.

Conclusion

The effects of microparasitic infections on the dynamics of animal populations depend on the ecology of the interactions between host and parasite. These patterns of disease behaviour involve four principal factors, namely: the host providing a habitat for the parasite; the degree to which the parasite induces host mortality (or diminishes the reproductive capability of the host); the extent to which the host acquires immunity; and the necessity of transmission from one host to the next. Overlaid on these factors are many biological complications, specific to individual host–parasite associations, whose sequential action is determined by life cycle structure.

In the second part of this article, we show how a common set of factors are involved in the dynamics of all infectious diseases, whether they are caused by viral or helminth agents, and whether they are transmitted directly or indirectly between hosts.

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articles

Possible optical observation of the companion star to the binary pulsar PSR1913+16

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Deep R band CCD photographs of the region surrounding the binary pulsar PSR1913+16 have been obtained with the 4-m telescope of the Kitt Peak National Observatory. Using an accurate astrometric solution, we find a star with $M_R = 20.9$ *at the precise coordinates of the pulsar. Possible interpretations of this result, including the unlikely* (<3%) *possibility that it is an accidental superposition of a field star, are discussed. If this object is the physical companion to the pulsar, it is probably a helium star.*

THE discovery of the binary pulsar PSR1913+16[1] has created substantial interest as a system in which various general relativistic effects[2–4] may be measured. The ability to perform these experiments relies critically on two factors: (1) that the pulsar clock should behave in a regular way and (2) that the companion star must be treatable as a point mass. The former point seems to be satisfied[5] so the major remaining issue for tests of relativity to proceed is how to determine the nature of the companion star. Since the optical extinction is probably modest ($A_V = 3.3$; ref. 6) we expect that a non-compact companion should be observable with a sensitive detector on a large telescope. Thus, sensitive observations could establish valuable constraints on the size of the companion star. The small observed apsidal motion in the system already limits the companion star to being either a helium star or a compact object[3].

The observations

We have used the JPL CCD array (400×400 pixels; 0.45″ per pixel) at the prime focus of the 4-m Mayall telescope to observe the region of the binary pulsar PSR1913+16. Our

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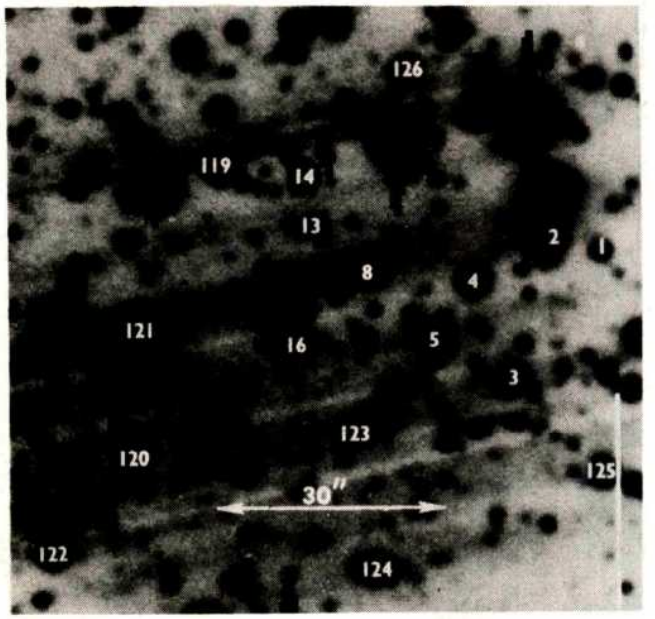


Fig. 1 Finding chart for all the transfer stars on the CCD field (raw data frame). The positions of each star are listed in Table 1. North is up, and east is left. The faint stars 7 and 9 in Fig. 2 are just south of star 8 in this figure.

observational sensitivity would allow us to detect an object with $M_R \leqslant 23.7$. Within our measurement error of 0.32″ we find an object with $M_R = 20.9$ at the precise pulsar radio coordinates given by Taylor *et al.*[7]. This is the same object identified by Kristian and Westphal[8] (also J. Kristian, personal communication) with their positional accuracy of ≃1″.

The region of the pulsar was observed on the nights of 8 and 9 May 1978 and was made with an *R* filter of the system described by Bessel[9]. This passband is a compromise between going as red as possible to reduce the galactic absorption, while avoiding bright variable red OH features in the sky which cause problems with reduction as well as degrade the intrinsic signal-to-noise ratio.

Figure 1 shows a reproduction of a portion of a CCD frame of the area of PSR1913+16. This is a raw data frame and has not been flattened to remove the variations in sensitivity. Also shown are the 17 transfer astrometric stars which were used to determine the position of the very faint objects in the CCD frame. This is one of a set of three 16-min exposures of this field. Figure 2 shows a more detailed reproduction of the CCD frame which has been processed to remove background variations. The faintest objects visible (such as object 22) have $M_R = 23.8$, or are about 3 mag fainter than the limit of Shao and Liller[10].

The astrometric solution has been obtained using the Palomar Sky Survey plates and the SAO star catalogue. This was done on the IPPS and Grant engine at KPNO, and independently at ESO. Our solution differs significantly from that of Shao and Liller[10]. On Kristian's (personal communication) suggestion we performed independent astrometry on copies of the Palomar Sky Survey plates. We put objects 1.6 arc s further east than Shao and Liller[10] and with this solution, we found a star (object 21 in Fig. 2) located within 0.05″ of the pulsar radio coordinates. The radio coordinate uncertainties given by Taylor *et al.*[7] are 0.04″. The positions of 25 objects in the CCD field are listed in Table 1 where the objects numbered <20 correspond to those of Shao and Liller, and the letters correspond to the objects of Kristian *et al.*[11].

We now briefly consider the uncertainties in our stellar position. The Palomar Sky Survey plate was measured and fit with a 6 parameter linear function using 29 SAO stars. The scatter in the fit showed an r.m.s. uncertainty of 0.54″ in each star's position, resulting in an expected r.m.s. uncertainty of 0.11″ for a stellar position determined by this solution. This may be due to SAO star coordinate errors or PSS plate distortion or measurement error. Since the CCD picture contains no SAO stars, secondary astrometric standards are needed to establish the CCD picture coordinates. The SAO coordinate grid was transferred to the CCD picture using 17 stars common to both pictures. These transfer stars are numbered in Fig. 1. This transfer process introduced an estimated error ≤0.1″. The overall coordinate grid error in the CCD picture then becomes 0.15″ r.m.s. The measurement uncertainty of star 21 found experimentally by measuring two CCD frames is ≤0:2″. To check for systematic errors we constructed a coordinate grid using the AGK3 stars and found the errors between the SAO and AGK3 solutions to be 0.2″. Thus we expect the accumulated error (systematic and statistical) of our coordinate solution for star 21 to be ≤0.32″ r.m.s.

Using a standard star (Kopf 27) observed in the *R* band with the CCD detector, we have reduced our measurements to spectrophotometric *R* magnitudes for several stars near the pulsar position. These are also given in Table 1. Our *R* magnitudes are close (±0.2 mag) to the *I* magnitudes given by Shao and Liller[10]. For the candidate star we find $M_R = 20.9$.

Interpretation

Within the tight limits set by the accuracy of the pulsar coordinates and our astrometry, several interpretations of our results are possible: (1) the light seen comes from the pulsar itself; (2) a third star in the system produces the light; (3) a field star accidentally lies along our line of sight; (4) the companion star is the light source. We will now consider these possibilities.

(1) If the light comes from the pulsar, one might expect it to be strongly modulated at the pulsar frequency of 16.9 Hz. However, the observations of Nather *et al.*[12] show the upper limit on optical pulsations is $M_R \simeq 26.2$. This would imply the pulsed fraction of PSR1913+16 must be ≤1%. As the two

Table 1 Positions and magnitudes of objects in the vicinity of PSR1913+16

Star	RA (1950.0)	Dec (1950.0)	Magnitude (*R*)
1	19 h 13 min 10.32 s	+16° 01′ 17.6″	
2	10.80	19.1	
3	11.10	01.3	
4	11.44	13.6	
5	11.79	06.4	
8 (H)	12.36	14.6	
13	12.86	20.6	
14 (J)	12.92	26.9	
16	12.96	05.6	
21	12.47	01 08.0	20.9
22	13.03	00 59.1	23.8
23	12.62	01 16.6	20.5
24	13.04	15.7	20.2
25	13.27	15.5	19.6
26	12.42	03.9	21.5
27	12.61	02.4	21.6
28	12.80	00 59.5	22.8
119	13.69	01 28.2	
120	14.39	00 50.9	
121	14.33	01 06.8	
122	15.05	00 38.6	
123	12.43	54.1	
124 (F)	12.28	36.6	
125	10.28	00 49.2	
126	12.04	01 40.4	
Pulsar radio position*	12.474	01 08.02	

Note that stars numbered less than 20 correspond to those of Shao and Liller[10] and letters correspond to Kristian *et al.*[11]. Star 21 is the candidate originally suggested by Kristian and Westphal[8].

* From ref. 7.

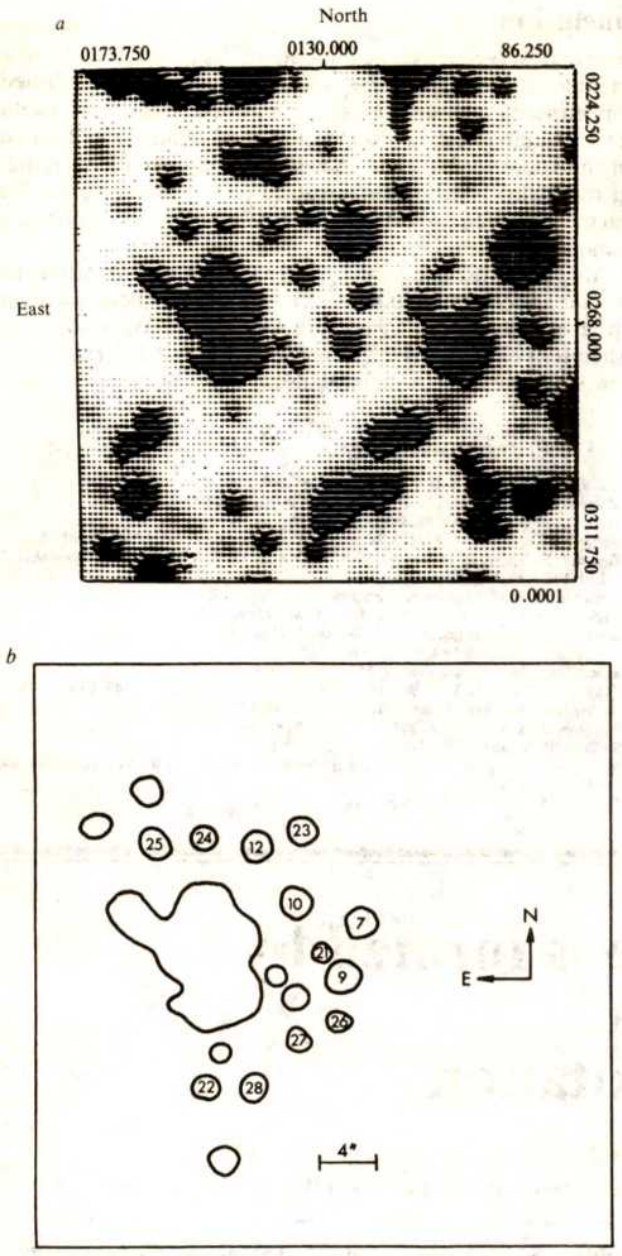


Fig. 2 *a*, Reproduction of the CCD data processed for removal of fixed pattern noise, in the region of the binary pulsar PSR1913+16. Star 21 is the object within 0.05 arc s of the pulsar position. We find a 97% probability that this star is a real association, based on star counts in the field and our measurement uncertainties. Star 22 is the object used as a reference for the limiting magnitude. Other stars correspond to those of Shao and Liller[10]. The positions and magnitudes are listed in Table 1. *b*, Identification chart for faint stars of Table 1 and *a*.

known optical pulsars, the Crab and Vela pulsars[13], show pulsed fractions of ~100% and 50% respectively, this upper limit makes this interpretation unlikely.

(2) If a third star is in the system, it must have survived at least one supernova in the system without being ejected, and must also have produced sufficiently subtle effects on the orbit of the pulsar as to remain undetected until now. This possibility seems extremely unlikely.

(3) To evaluate the possibility that we are seeing a field star, we must consider our measurement uncertainties and the density of field stars. A star would be a valid candidate if that object lay within our error circle 90% of the time. Assuming gaussian errors, this sets the error circle radius as 1.72 $\theta_{\text{r.m.s.}}$,

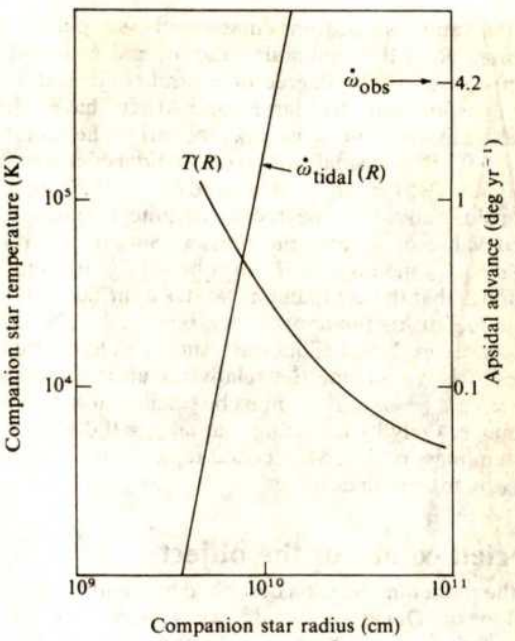


Fig. 3 The relationship between the companion star's radius (bottom scale) and its temperature (left vertical scale) is shown. It is based on our observed flux from the candidate star, an assumed distance and extinction (see text) and the assumption that it radiates as a black body. The apsidal advance (right vertical scale) due to tidal effects is also shown as a function of the companion star radius. The structure of a helium star was assumed (see text). The total observed apsidal advance is also indicated. Simply requiring that the tidal effect be $\leqslant\dot{\omega}_{\text{obs}}$ implies $T_c \geqslant 23{,}000$ K.

where $\theta_{\text{r.m.s.}}$ is the r.m.s. radial uncertainty due to all statistical and systematic effects. In this case, $\theta_{\text{r.m.s.}} \leqslant 0.32''$ so the 90% error circle radius is 0.55". Since our observed coordinate difference is $\leqslant 0.05''$, we (formally) accept the candidate star. Using the CCD picture, we have established the density of stars with $M_R \leqslant 20.9$ at 0.037 stars per arc s^2, so there is a 3% chance that the star is an accidental association and a 97% chance that it is a real association.

(4) The most likely result is that we have detected the companion star of PSR1913+16. The small observed apsidal motion in the system of $\dot{\omega} = 4.24°\ \text{yr}^{-1}$ prohibits hydrogen main sequence stars, since their tidal effects would cause $\dot{\omega} > \dot{\omega}_{\text{obs}}$. Only helium stars and degenerate objects are small enough to be allowed (see ref. 4 and refs therein). We will now estimate some properties of this companion star.

If we assume a distance[1] of 5 kpc and an extinction[6] of $A_V = 3.3$ and further assume the object radiates as a black body, we can establish a relationship between the radius of the object and its temperature using our observed R flux at 0.68 μm of $f_\nu = 1.58 \times 10^{-28}$ erg cm^{-2} s^{-1} Hz^{-1} as shown in Fig. 3. This relation implies unrealistically high temperatures for a compact object. If $r = 10^9$ cm (a typical white dwarf) we find a temperature of 2×10^6 K. If the object is a neutron star ($r = 10^6$ cm) we find $T = 2 \times 10^{12}$ K. Thus, if the object is a thermal radiator, it cannot be degenerate and is expected to be a helium star.

A non-compact companion star will, in general, give a tidal contribution to the apsidal motion of the orbit given by (ref. 3, equation (3.8))

$$\dot{\omega}_{\text{tidal}} = 11.2\left(\frac{k_2}{0.01}\right)\left(\frac{M_p}{M_c}\right)\left(\frac{M_p + M_c}{M_\odot}\right)^{-5/3}\left(\frac{R_c}{0.2R_\odot}\right)^5 \quad \text{deg yr}^{-1}$$

in addition to the general relativistic contribution (ref. 3, equation (3.5))

$$\dot{\omega}_{GR} = 2.11\left(\frac{M_p + M_c}{M_\odot}\right)^{2/3} \quad \text{deg yr}^{-1}$$

where M_p and M_c are the masses of the pulsar and its companion, R_c is the companion radius, and k_2 is a structure function measuring the degree of central condensation of the companion star. Roberts, Masters and Arnett[4] have calculated, using stellar models, the value of k_2 for various helium stars and find $k_2 \simeq 0.05$. The apsidal motion due to tidal effects is shown in Fig. 3, assuming $M_p = M_c = 1.4 M_\odot$ and $k_2 = 0.05$. Note that for a given apsidal advance, the radius is quite insensitive to the assumed value of k_2 and the masses. Simply requiring that $\dot{\omega}_{tidal} \leq \dot{\omega}_{observed}$ means that R_c must be $\leq 1.2 \times 10^{10}$ cm. This in turn implies that the companion star has a surface temperature $T \geq 23{,}000$ K using the above black-body result. Note that, at least for more massive helium stars, such high temperatures are expected[14]. If we include the relativistic apsidal motion then, $\dot{\omega}_{tidal} = \dot{\omega}_{observed} - \dot{\omega}_{GR}$, so R_c must be smaller, and T larger than given above. Only by assuming that $\dot{\omega}_{tidal} = 0$ does one obtain the system mass of $2.85 M_\odot$ needed to account entirely for the $\dot{\omega}_{observed}$ by the relatistic effect.

Expected colour of the object

Using the reddening relation described by Nandy *et al.*[15] and the extinction of Davidsen *et al.*[6] we expect $E(V-R) \simeq 1.0$. Johnson[16] gives $V-R$ for stars in the above temperature region of $\simeq -0.1$. To convert our spectrophotometric magnitude to a Johnson R magnitude we must add -0.2. We thus roughly expect $V \simeq 21.6$. Kristian and Westphal[8] reported $V = 22.5$. If the given V and R are correct and the object is the companion star, this result suggests that the extinction is greater than that assumed and the temperature of the companion star is hotter.

Conclusion

We have observed a likely candidate (97% probability) for the companion star of PSR1913+16. This result could be confirmed by spectroscopic observations. If confirmed, this system might not be a suitable testing ground of general relativity and would bring into question the claimed evidence for gravitational radiation by Taylor *et al.*[7]. The existence of a helium star for the binary companion would also be interesting on evolutionary grounds.

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A κ-immunoglobulin gene is formed by site-specific recombination without further somatic mutation

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The active gene for a κ light chain is formed by a somatic recombination event that joins one of several hundred variable region genes to one of a series of recombination sites (J-segments) encoded close to the κ constant region gene. The nucleotide sequences of cloned germ line and somatically recombined genes define the precise organisation of these genetic segments and the site and nature of the recombination event that joined them. Apart from somatic recombination, no further alteration of the germ line sequence has occurred. The J-segment is of special interest as it encodes signals for both DNA and RNA splicing and provides a means of generating further immunoglobulin gene diversity.

A COMPLETE explanation of antibody gene expression must take into account at least four criticaLobservations—the striking variable/constant structure of antibody molecules, the diversity of their sequences, the fact that only one member of an allelic pair of antibody genes is expressed in a given lymphocyte, and the curious process by which a given lymphocyte seems to be able to sequentially associate a single variable region with two or more different classes of heavy chain constant regions (see complete bibliography in ref. 1). The argument advanced by Dreyer and Bennett[2], namely, that variable and constant regions are separately encoded in the germ line and are probably drawn together by somatic recombination, is likely to be relevant to each of these features of antibody gene expression.

Most recently, a series of experiments involving the analysis of DNA fragments encoding variable and constant region immunoglobulin genes has convincingly borne out the predictions of the Dreyer–Bennett hypothesis[3–5]. In particular, an examination of cloned DNA fragments has added considerable and unexpected detail to our understanding of the arrangement of genes corresponding to immunoglobulin λ and κ light chains[1,6–9]. Our own experiments have concerned the κ light chain genes of the mouse[1,4,8]. (Antibody diversity in the mouse depends almost entirely on κ, as opposed to λ, light chains[10].) By cloning individual genes and using *in situ* hybridisation of DNA restriction fragments, we have shown that a large repertoire of κ variable region genes is encoded in the germ line genome. Saturation hybridisation experiments by Valbuena *et al.*[11] support this view. In addition, those genes that we have sequenced seem to be foreshortened, that is, they fall 13 amino acid codons short of the conventionally defined variable region sequence[4]. In the case of the MOPC-41 κ chain, we were able to show that formation of the active gene involved a recombination event that joined such a foreshortened variable region gene to a segment of DNA (called the J or joining segment) encoding

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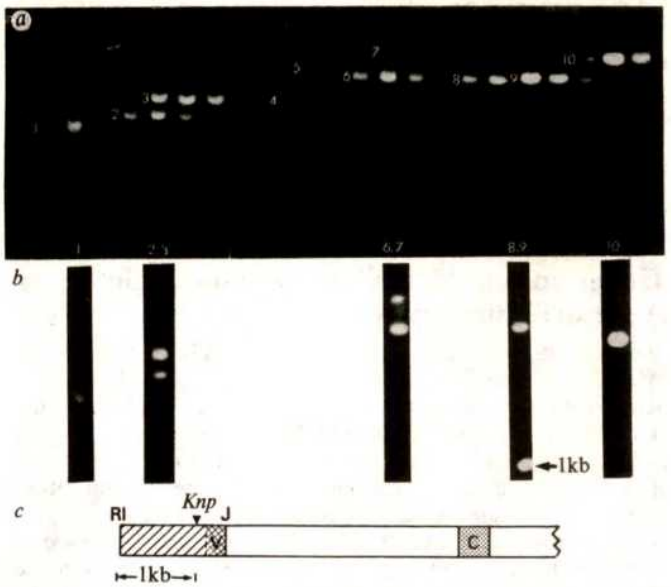


Fig. 1 Identification of the *Eco*RI fragment of embryonic DNA that contains the germ line MOPC-41 variable region gene. *a*, *Eco*RI-digested embryonic DNA was fractionated first by RPC-5 column chromatography and then by agarose gel electrophoresis, transferred to a nitrocellulose filter and hybridised to the ^{32}P-labelled 1 kilobase *Eco*RI-*Kpn*I (see map, bottom panel) fragment from the recombined gene as described previously[1,8]. *b*, Separate fractions from the RPC-5 column containing MOPC-41-like variable-region sequences were pooled as indicated, cleaved with *Kpn*I, fractionated on agarose gels, similarly transferred to nitrocellulose filters and hybridised to the ^{32}P-labelled *Eco*RI-*Kpn*I fragment. *c*, Location of the sequence within the MOPC-41 recombined gene (the *Eco*RI-*Kpn*I fragment) that was used as probe to detect the germ line gene.

these last variable region sequences[8]. Even in the active form, however, the fused V/J segment was separated from the constant region gene by a 3.7 kilobase intervening sequence. Bernard *et al.*[6] have shown that analogous mechanisms are involved in rearrangement of λ light chain genes, although there may be only one variable region gene for each of the two λ classes.

But many important questions remain. Does the germ line gene undergo substantial somatic mutation before expression? Can recombination between variable and J-segment serve to generate additional diversity? What is the structure of an active, as opposed to an inactive or germ line, immunoglobulin gene? Which sequences define the boundaries of the coding and other functional domains of the gene? And finally, what can these observations tell us about allelic exclusion and/or heavy chain switching?

We have attempted to answer some of these questions by cloning and determining the sequence of major portions of an active κ gene and its germ line precursors. In its germ line form, this sequence is encoded in at least four discrete segments of DNA. A somatic recombination event joins two of these segments, creating the expressed gene which then consists of three discontinuous coding segments. This recombination occurs between one of many germ line variable region genes and one of five short joining regions that we find close to the κ constant region gene[18]. The adjoining or J-region is especially interesting in that it must encode two specialised recombination sites, one for DNA joining and another for RNA splicing. Apart from this recombination event, the sequence of the expressed variable region gene is identical to the germ line sequence, that is, no somatic mutation has occurred. A comparison of these sequences with other κ and λ genes suggests that this general structure and mechanism have been preserved in evolution. Further, it suggests that antibody diversity arises principally from three sources: a large and diverse germ line repertoire of

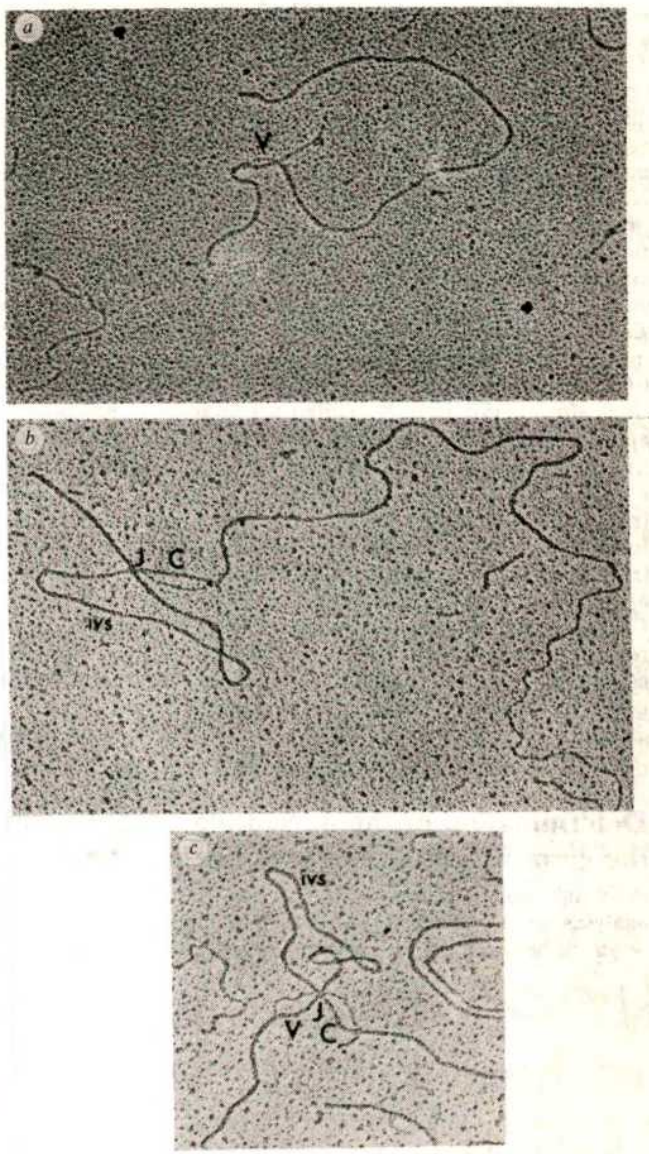


Fig. 2 R-loops formed between MOPC-41 light chain mRNA and the cloned germ line and recombined MOPC-41 light chain genes. Panels represent R-loops formed between MOPC-41 mRNA and the following: *a*, MOPC-41 variable region fragment derived from embryonic DNA (6.8 kilobases); *b*, constant and J region fragment derived from embryonic DNA (16 kilobases); and *c*, fragment derived from MOPC-41 DNA (16.5 kilobases) that contains recombined variable and constant region genes[8]. The R-loops corresponding to the variable region (V), J region (J) and constant region (C) are indicated. IVS identifies the 3.7 kilobase intervening sequence between J region and C region. At least 20 molecules of each type were measured to determine the distances of the genes from one another, and from the ends of the fragments. The R-loops shown in panels *b* and *c* have been published previously[8] and are shown for comparison. The methods of R-loop formation and characterisation have been described previously[4]. The structures were confirmed by direct sequence determination (see Fig. 4).

variable region genes, a smaller set of J-regions, and a recombination mechanism that joins these sequences in different combinations in the somatic cell.

Cloning the germ line precursor of the active MOPC-41 κ chain gene

The complete MOPC-41 κ chain gene is formed by a recombination event that joins the germ line variable region sequence to a J-region segment located about 3.7 kilobases from the κ constant region gene[8]. We have previously cloned the germ line J and constant region genes on a single segment of embryonic

DNA. We have also cloned the recombined MOPC-41 gene on a segment of DNA derived from a MOPC-41 plasmacytoma[8]. These clones constitute the product, but only one of the two precursors involved in the formation of the light chain gene. A difficulty arose in cloning the remaining germ line precursor, the variable region gene. It—like other κ variable region genes—is a member of a small set of approximately 10 closely related genes, all of which cross-hybridise to a MOPC-41 variable region probe[1]. Fortunately, the parental fragment could be distinguished from the other related sequences by restriction endonuclease digestion (Fig. 1). The recombinant clone had a characteristic *Kpn*I site which occurred 1 kilobase from the *Eco*RI site at the 5′ end of the variable region sequence. Only one of the ten hybridising *Eco*RI fragments of embryonic DNA (that were separated by two-dimensional analysis[1] and detected by *in situ* hybridisation[12]) yielded a 1 kilobase fragment after appropriate digestion. The fragment (no. 8 in Fig. 1) was tentatively identified as the parental variable region gene.

The partially purified fragment was cloned in λgtWES·λB (ref. 13) and analysed by visualisation of R-loop structures formed by annealing MOPC-41 κ chain mRNA to the cloned fragment (Fig. 2). A single variable region R-loop was visualised approximately 1 kilobase from the 5′ end of the fragment. R-loop structures formed between the germ line J/constant region fragment and the active, recombined gene are shown for comparison (Fig. 2). The separate variable and constant region genes, on separate *Eco*RI DNA fragments in the germ line (Fig. 2*a* and *b*) are recombined so as to reside on the same DNA fragment in the antibody producing cell (Fig. 2*c*).

Determination of the nucleotide sequences of the germ line and rearranged genes

Although our electronmicroscopic and restriction enzyme analyses were consistent with a recombination event having occurred between the two parental genes, the fine structure of the precursor genes and their recombination product as well as the nature of somatic changes, if any, that accompanied the recombination event could only be shown by direct sequence determination. The procedure used in determining the sequence was the partial chemical degradation method of Maxam and Gilbert[14]. The restriction enzyme strategy, the overlapping sequences determined and the extent of bi-directional sequencing are shown in Fig. 3. The sequences determined are shown in Fig. 4.

The germ line MOPC-41 variable region gene: evolutionary conservation

Inasmuch as the amino acid sequence of both the mature MOPC-41 protein[15] and its hydrophobic leader[16] have been determined, the coding sequence is easily identified within the cloned fragment. The germ line MOPC-41 variable region gene (VK41) is arranged in a fashion analogous to that of the mouse germ line λI and λII variable region genes[6,9] and other sequenced germ line κ variable region genes (VK2 and VK3)[4]. An 18 amino acid hydrophobic leader coding sequence is separated by a 129 base-pair intervening sequence from the main variable region coding block. [We do not believe that the in-phase initiation codon present close to the analogue of codon −4 in K2 and K3 is the true initiation codon[4]. Rather, further sequencing has revealed that the VK2 gene also has a separately encoded hydrophobic leader sequence (Y. Nishioka, unpublished).] The coding sequence resumes at coding position −4 of the leader sequence and continues to position 95 in the variable region. As we have shown for other germ line κ variable region genes[4], the last 13 amino acid codons commonly associated with the variable region are missing. Thus, the general structure of these genes seems to have been strongly conserved in the hundred or so million years that κ and λ sequences have undergone separate evolutionary development. It is also worth noting that the sequence homologies, both between other λ and

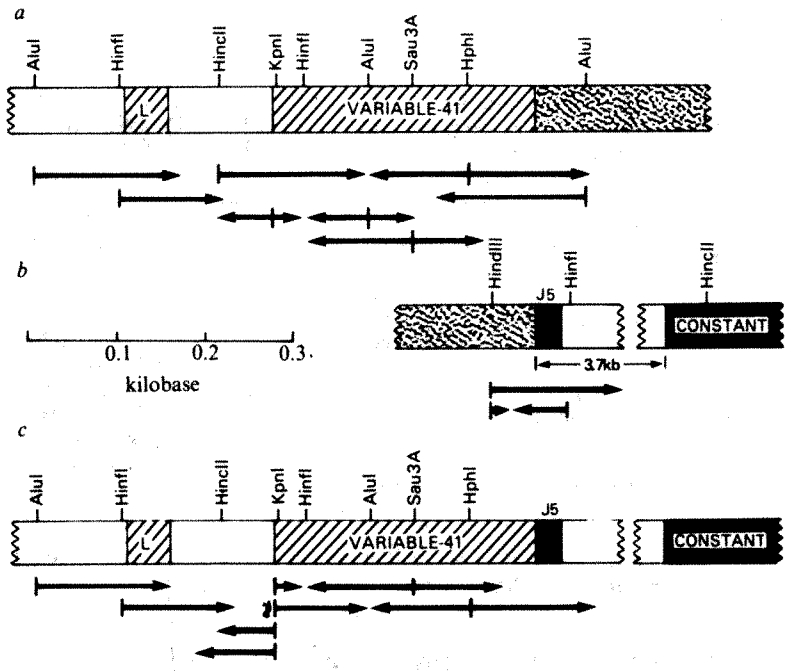


Fig. 3 The strategy used to determine the nucleotide sequences of cloned immunoglobulin genes. The basic approach and methodology have been described by Maxam and Gilbert[14]. Our application of their technique has also been described previously[8]. The 6.8 kilobase fragment containing the germ line MOPC-41 variable region gene[6,8], the 6.5 kilobase *Bam*HI-*Eco*RI fragment of the 16 kilobase *Eco*RI fragment carrying the germ line J and constant region genes[8], and the 7 kilobase *Bam*HI-*Eco*RI fragment of the 16.5 kilobase fragment carrying the recombined gene[8] were re-cloned in the bacterial plasmid pBR322. Restriction fragments between 500 and 2,400 base pairs were excised from these plasmids by cutting with the enzymes *Eco*RI, *Kpn*I, *Xba*I, *Hin*dIII and *Bam*HI. (The *Eco*RI site is 1.2 kilobases to the 5′ side of the variable region and recombined genes.) *Xba*I cleaved the embryonic derived fragment 300 base pairs to the 3′ side of the variable region gene and 1.2 kilobases to the 3′ side of the recombined gene. *Hin*dIII cleaved the constant region fragment gene containing fragments between J1 and the constant region gene. *Bam*HI cleaved 1 kilobase to the 3′ side of the constant region gene (ref. 8 and our unpublished results). These fragments were cleaved with the indicated enzymes and end-labelled with ^{32}P by the action of T4 polynucleotide kinase; finally a smaller fragment labelled at only one end was obtained by re-cleaving the labelled fragments with a third restriction enzyme. The labelled fragments were isolated from polyacrylamide gels and subjected to base-specific chemical degradation and subsequent fractionation on polyacrylamide–urea gels. The derived sequences are indicated in Fig. 4. The accuracy of any sequence is probably greater than 95%; sequences which have been determined in both directions are more accurate than sequences which have been determined in only one direction. The structural gene sequences have been indicated by diagonal lines (leader and variable region) and solid lines (J and constant region genes). The portions of the fragments that are removed by the recombination event are indicated by stippled regions. The clear portion of the fragments are sequences which become the 5′ flanking and intervening sequences of the recombined gene. The scale shows the distance represented by 0.5 kilobase on the cloned fragment.

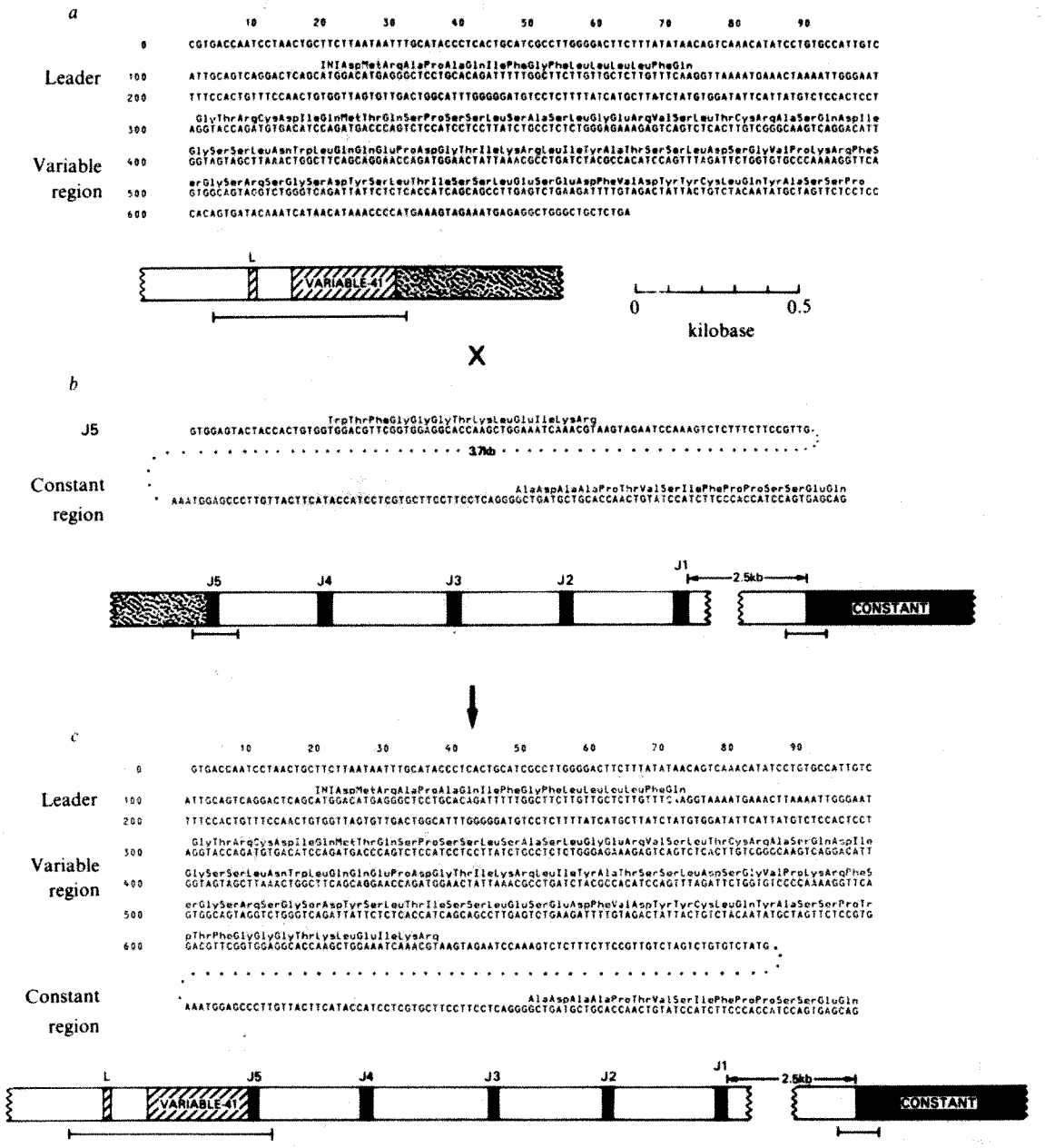


Fig. 4 Nucleotide sequences of the germ line and expressed genes encoding the MOPC-41 light chain. *a*, Germ line leader and variable region gene; *b*, germ line J region and constant region genes (the leader/variable gene and the J/constant gene are encoded on separate *Eco*RI fragments cloned from embryonic DNA); *c*, recombined gene: the light chain gene expressed in the MOPC-41 cell has been formed by fusing the germ line variable and J region genes. Gene segments are diagrammatically coded as described in Fig. 3. The location of each sequence with respect to the structural gene sequence is indicated on the figure below each sequence. The locations of four additional J-region genes, J4, J3, J2 and J1 in the intervening sequence were determined by Max *et al.*[18] using nucleotide sequence analysis.

other κ genes, are most strongly preserved in the coding sequences (85%, VK41/VK2) and least well in the intervening sequences (25%, VK41/VK2), a pattern which was noted originally between closely related β globin genes[12].

The recombination event and the J region: homologies and inverted repeats

As regards the premature termination of the germ line MOPC-41 variable region gene at codon 95, our electronmicroscopic R-loop analysis (Fig. 2) suggests that the remaining codons occurred at a region 3.7 kilobases from the germ line constant region gene. In fact, direct sequence analysis confirmed that the J coding sequence occurs in this region, extending from codon 96 (Trp-TGG) to 108 (Arg-CGT). The precise point at which the recombination event occurred can also be specified by comparing the sequences of the germ line genes to that of the recombined gene (Fig. 5). The codon in position 95 (Pro-CCT) of the germ line variable region gene has been converted to a different proline codon (CCG) by the recombination event. The G in the third position is therefore derived from the J-region fragment. Obviously a shift of the recombination site would create a different codon and this could be a source of additional immunoglobulin diversity (see below and ref. 18).

The nature of all recombination events between V and J regions cannot be confidently inferred from the single recombination event analysed here. However, our further sequence studies located at least four additional and different potential J sequences between the germ line MOPC-41 J and the constant region gene[18]. These sequences occur at regular intervals about 320–360 nucleotides apart and are indicated in the map shown in Fig. 4. Clearly, if all combinations are permitted, considerable diversity can be generated by simply combining the multiple variable region germ line genes with these J segments. This

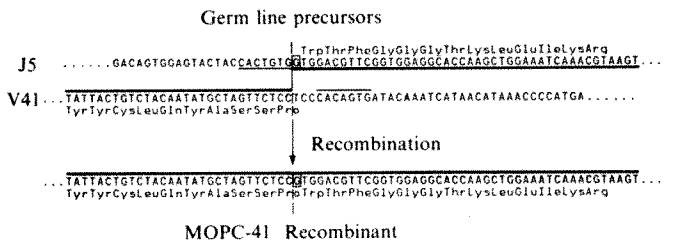


Fig. 5 Nucleotide sequences involved in the recombination of germline precursors of the active MOPC-41 gene. The precise location of the recombination event is indicated by the cross-over line. The amino acid sequences encoded by each segment are indicated. The locations of a 7 base pair sequence to the 5′ side of the J region and a homologous 7 base pair sequence to the 3′ side of the variable region are underlined. The sequence is also an inverted repeat; that is, the V region segment is complementary to the J-region segment. Notice that the recombination event occurs in the middle of a proline codon, generating a new proline codon.

possibility has been suggested by a comparison of amino acid sequences of κ light chains in two inbred strains of mice[19,20]. Diversity would be even more greatly amplified if the recombination event could use different splicing frames at each V/J junction[18].

A comparison of the MOPC-41 variable and J sequences indicates that the recombination event does not depend on simple generalised recombination such as that which requires extensive, in-phase sequence homology. There are several short regions of homology in the variable and J-region, but none align the two sequences in phase. Perhaps one of the most attractive of these homologies is a seven base sequence CAC($\frac{T}{A}$)GTG which occurs immediately adjacent to the splice site in both variable and J regions (see underlined sequences, Fig. 5). The sequences are short palindromes or inverted repeat sequences and interestingly appear to border all variable and J regions thus far sequenced[18]. Because these are inverted repeats, they are mutually complementary and can be drawn as a short stem structure between V and J genes. We have considered the implication of this possibility and specific molecular mechanisms for recombination in detail elsewhere[18].

It is also likely to be significant that the J-region structure has been closely preserved between κ and λ genes. We have compared our κ J-region sequence to that determined for the λ (type I) J by Bernard *et al.*[6] (Fig. 6) and find that not only are they identical in length but they are also closely homologous in sequence. In fact, the homologous inverted repeat sequence referred to above is largely preserved adjacent to the recombination site in both the λ J and variable region genes (Fig. 6). It is simplest—perhaps too simple—to think of such sites as recognition core sequences for specialised, site-specific recombination. Nevertheless, these small inverted repeats are formally analogous to the inverted repeats seen at the ends of bacterial insertion sequences[21,22].

Except for recombination, the germ line variable region sequence is unaltered in the active gene

Whereas one of a large number of germ line κ variable region genes recombining with one of several J-segments might suffice to generate the complete κ chain repertoire, studies of λ light chains strongly suggest that point mutations arise somatically, producing additional diversity[23]. Sequence determination of λ light chain genes[6] supports the notion that simple somatic base alterations are responsible for the amino acid differences noted among λ chains. Still, even in the λ chain system, a predominant sequence ascribed to the unaltered germ line gene was seen in about two-thirds of the chains examined[23]. Although the single position changes noted in myeloma proteins would increase the light chain repertoire and might be relevant to the immune response (they might also be irrelevant, the result of a transcriptionally active gene being repeatedly replicated during prolonged passage in a tumour), the germ line sequence remains the major λ sequence expressed.

Such protein sequence comparisons have been much more difficult in the mouse κ system, for—in contrast to the λ light chains—mouse κ light chains are extremely diverse in sequence, a fact that is consistent with their role as the major (>95%) light chain in all mouse antibodies. This diversity is further consistent with a large number of germ line variable region genes[1,6,11]. It was therefore of particular interest to determine whether additional somatic mutations had occurred in the germ line VK41 gene. A comparison of the germ line and expressed sequences (Fig. 4) indicates that—with the exception of the V/J recombination event—they are identical in every position. The expressed gene has, therefore, undergone no further somatic alteration.

The recombined MOPC-41 gene remains an interrupted gene: intervening sequences

The large intervening sequence (IVS-2) that separates the recombined variable-J sequence from the constant region gene in MOPC-41 DNA was first identified by visualising R-loop structures formed between the gene and its mRNA (ref. 8). Both this sequence and the smaller intervening sequence (IVS-1) that separates the major portion of the leader from the variable region and are strictly analogous to the corresponding structures of the λ light chain gene[6,7]. Their borders are established by our nucleotide sequence analysis and these seem to occur within codon −4 and within codon 108 for the small and large intervening sequences, respectively (Fig. 4). Evidence from the mouse globin system[25–27], the ovalbumin system[28,29] as well as the immunoglobulin system[30–32], indicates that these intervening sequences are transcribed and spliced out during maturation of a large mRNA precursor. The nature of the RNA splice signal is not yet known, as there is limited conservation of sequence between these sites among different genes. However, as a general rule, the data are consistent with the elimination of

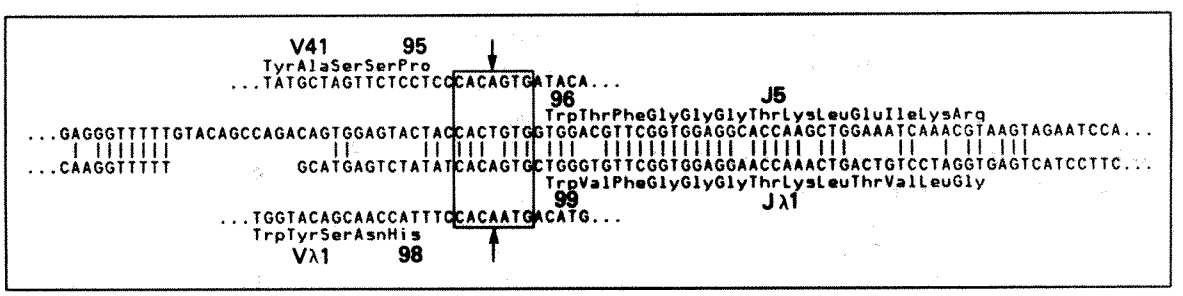


Fig. 6 Nucleotide sequence homologies between κ and λ variable and J-region genes surrounding the DNA recombination site. J1 and Jλ1 have been aligned to indicate the close relationship between these two DNA sequences (homologies are indicated by vertical lines). The 3′ ends of the κ (MOPC-41) and λ I variable region genes are indicated so that the location of the common sequence, CAC($\frac{A}{T}$)GTG, is aligned (boxed sequence). The arrows indicate the centre of this small palindrome that constitutes an inverted repeat noted in Fig. 5 and present in both κ and λ variable and J-region genes. The λ gene sequences are from Bernard *et al.*[6].

sequence beginning with [GT] and ending with [AG] (ref. 28). The sequence of the κ light chain gene is consistent with this rule as follows:

IVS-1, CAA G(GT . . . AG)GT ACC,

IVS-2, AAA C(GT . . . AG)GG GCT

where the underlined sequences represent codons and the sequences within parentheses, excised segments.

Antibody diversity, allelic exclusion and the heavy chain switch

We have discussed the role that recombination might play in creating antibody diversity—joining one of hundreds of variable regions to one of several J-segments, each of which is linked, in turn, to the single constant region gene[18]. It is clear that this recombination event is critically linked to antibody gene expression. If recombination occurs (or occurs properly) on only one of a pair of alleles, presumably only the recombined gene will be expressed. Allelic exclusion may therefore be a consequence of a single authentic recombination event affecting only one member of a chromosome pair[8]. Preliminary evidence suggests that germ line genes and/or aberrant recombinants occur together with active genes in myeloma tumours[8,33].

The problem of moving a single heavy chain variable region from one heavy chain constant region to another—the so-called heavy chain switch—may also involve the use of a J-like segment and a site-specific recombination event. However, the need to distinguish a stable recombinant (as in a light chain gene) from an unstable one (as in a μ chain gene) suggests that two independent rearrangements are involved in switching from a μ to a γ or an α chain. The first is probably analogous to what we see in the light chain gene, but the second might be quite different, involving an alternative mechanism of DNA rearrangement.

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letters

Persistent emission from X-ray burst sources and the nature of galactic bulge X-ray sources

TWO principal types of theories have been proposed to explain X-ray bursts: instabilities in the accretion flow of matter onto a collapsed object[1–9], and thermonuclear flashes in the freshly accreted material on the surface of a neutron star[10–16]. Both mechanisms probably occur in nature but account for different observed phenomena[17]. The available evidence [17–19] leaves little doubt that the Type II bursts[17], which are emitted by MXB1730−335 (the Rapid Burster), are due to an accretion instability. The properties of Type I bursts[17], which are emitted by all burst sources (including the Rapid Burster), are naturally explained by the thermonuclear flash model[15]. In the light of this model, we discuss here the intrinsic persistent X-ray luminosity and the ratio of the average persistent X-ray luminosity to the time-averaged burst luminosity associated with Type I burst sources[17,20,21].

Following van Paradijs[22], we assume that the peak burst luminosity is a standard candle. The intrinsic persistent X-ray luminosity L_x in units of this standard candle is thus given by the ratio γ of the persistent X-ray flux to the average peak burst flux. On the basis of the results given below we argue that the bright non-bursting galactic bulge X-ray sources[23,24] are accreting neutron stars, which differ from X-ray burst sources in that L_x is above a certain critical value.

An earlier compilation of values of the ratio, α, of the average persistent X-ray luminosity to the time-averaged burst luminosity has been given by Lewin[20]; the α-values presented in Table 1 include more X-ray burst sources and are based on a larger collection of SAS 3 data on all well-studied burst sources. We confirm the earlier conclusion[15,21] that in general the observed values of α are consistent which the thermonuclear flash model.

Detailed calculations of thermonuclear processes in the surface layers of an accreting neutron star were recently reported by Joss[15] (see also refs 14 and 16). Joss's results indicate that such objects undergo helium-burning flashes, which are visible to an external observer as bursts of X rays. This model can account for most of the basic features of Type I X-ray bursts, and leads to the following predictions concerning the persistent X-ray luminosity of the Type I burst sources: (1) every burst source is also a source of persistent (accretion-driven) X-ray emission; hence, apart from efficiency factors[12], α is just the ratio of the gravitational potential energy liberated by accretion to the nuclear energy released in flashes and should have a value of ~100 (refs 12, 13, 15); and (2) bursts occur only when the accretion rate is below a certain critical value and the corresponding persistent luminosity is below a critical value L_c. This effect can be qualitatively understood as resulting from an increase in the pre-flash temperature and a decrease in the level

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of degeneracy of the helium-burning shell, and from a decrease of the temperature gradient above the helium-burning shell, with increasing mass accretion rate[25]. The precise value of L_c is not yet well determined and may depend on other parameters (such as the strength of the neutron-star magnetic field). However, the available calculations[12,15] suggest that $L_c \approx 0.1\ L_{ed}$, where

$$L_{ed} = \frac{4\pi cGM}{\kappa} \simeq 1.25 \times 10^{38} \left(\frac{M}{M_\odot}\right)\left(\frac{\kappa}{0.4\text{cm}^2\,\text{g}^{-1}}\right)^{-1} \quad \text{erg s}^{-1} \quad (1)$$

is the Eddington luminosity for an object of mass M and an opacity coefficient of κ (normalised to Thomson scattering opacity in pure hydrogen). Bursts also should not occur when the accretion rate is below another critical value (at least two orders of magnitude smaller than L_c)[13], because the helium-burning will then be driven by pycnonuclear reactions[13].

In Table 1 we have collected the values of the observed persistent X-ray flux F_{per} and the ratios α and γ for 10 burst sources. Most of these results are based on data obtained from pointed SAS 3 Y-axis[26,27] observations; previous data sources are also listed in Table 1.

The data on MXB1820−30 have been taken from the SAS 3 rotation modulation collimator observations by Clark *et al.*[28]. The upper limit to the persistent X-ray flux from MXB1659−29 has been obtained from changes in the observed X-ray count rate when the source enters and leaves Earth occultation[18,21]. In the case of the Rapid Burster, we have used the time-averaged Type II burst flux for the persistent flux, as the Type II bursts presumably[17] replace the persistent flux. In April 1977, the observed upper limit to a truly persistent X-ray flux from this source was 20% of the average Type II burst flux[29]. For the faint X-ray source MXB1906+00, a determination of the persistent X-ray flux from the SAS 3 observations is difficult (due to contamination by Aql X-1 in the field of view[26]); we therefore used the average persistent X-ray flux of Seward *et al.*[30]. Because of source confusion problems and the transient character of the possible persistent X-ray counterparts, we have not included in Table 1 the three galactic centre burst sources[31–34]. Two sources (MXB1659−29 and MXB1820−30) became inactive when F_{per} rose above a certain value[28,35]; the corresponding values of γ are also given in Table 1. When different values of α were measured during observations of a given source, the range of observed values is presented.

According to calculations by Joss[12,15], the maximum luminosity in an X-ray burst is comparable to the Eddington limit. This result is supported by the empirical results obtained by van Paradijs[22], which strongly suggest that the average maximum luminosity L_0 of X-ray bursts is indeed about the same for all burst sources and is of the same order as the Eddington limit. Scaling their distances such that the burst sources are distributed symmetrically about the galactic centre yields $L_0 \sim 2 \times 10^{38}$ erg cm^{-2} s^{-1}. The intrinsic persistent X-ray luminosity L_x of a given burst source, in units of the luminosity standard L_0, is given by γ (see Table 1).

The following trends are visible in the distribution of the values of γ. First, the sources all have $\gamma < 0.3$ when active. This is consistent with the values for which the calculations by Joss[15] and Joss and Li[16] predict bursting behaviour. Second, the two sources which show the most irregular burst behaviour (MXB1735−44 and MXB1837+05[18,36–38]) have the largest values of γ of all sources in Table 1. The irregular burst behaviour of MXB1735−44 and MXB1837+05 may be related to the rather large persistent luminosity of these sources, which may lie near the critical value above which X-ray bursts are expected to be absent. (Note, however, that the behaviour of X-ray burst sources must depend on additional complications beyond these general considerations; in particular, there are few correlations between the level of burst activity and the instantaneous persistent X-ray luminosity in burst sources[37–40].

The values of α given in Table 1 are averages over time intervals of typically a few days. For most of the sources α is a relatively well defined quantity; the average total energy per burst from a given source, the time intervals between them and the value of the persistent flux do not vary greatly. We estimate that the values of α typically have a possible uncertainty of $\lesssim 50\%$. In some cases, however, the burst behaviour is quite irregular. An example is the source MXB1735−44, where the burst intervals vary from ~1–2 h to more than 50 h[18,21,36,37]. In those cases where appreciable irregularity of the burst activity is apparent, we have given separately in Table 1 the values of α during times of exceptional burst activity.

Table 1 X-ray burst source parameters

Source	F_x^* (erg cm^{-2} s^{-1})	α	γ	Refs
MXB 0512−40	8.0 (−10)	75	0.05	F. Li (personal communication)
MXB 1636−53	6.3 (−9)	300; 50‡	0.09	22, 40
MXB 1659−29	<3.9 (−10); 1.7 (−9)†	<25	<0.04; 0.15†	17, 21, 22
MXB 1728−34	3.9 (−9)	120	0.05	20, 22, 39
MXB 1730−335	1.2 (−9)§	130	0.06	18, 19, 21
MXB 1735−44	5.1 (−9)	380–1580; 85–200‡	0.18	22, 36, 37
MXB 1820−30	1.5–4.0 (−9); 6.0 (−9)†	40–100	0.01–0.15; 0.06†	20, 28
MXB 1837+05	9.1 (−9)	780–1100; 190‡	0.30	20, 22, 38
MXB 1906+00	2.1 (−10)	70	0.08	20, 22
MXB 1916−05	2.8 (−9)	270	0.13	22, 49

* Numbers in parentheses indicate powers of 10.
† No bursts observed above this value of the persistent X-ray flux.
‡ When two values are shown, the latter corresponds to intervals of high burst activity.
§ Time-averaged Type II burst flux.

Very high values of α are obtained for the irregular sources MXB1735−44 and MXB1837+05, when averaged over the entire period of observation; values between 100 and 200 are observed, however, during the periods of high burst activity. In the context of the thermonuclear flash model, these values for α suggest that during periods of burst inactivity the material in the freshly accreted layers on the neutron star undergoes relatively steady nuclear burning. This result is strengthened by the observation that the energy of the first burst after a long period of burst inactivity is about the same as for the bursts that occur throughout intervals of high burst activity. It may not, therefore, be necessary to invoke the "storage model," introduced by Woosley and Taam[10], in which nuclear fuel is accumulated during burst-inactive periods and is consumed only during periods of burst activity.

With one exception, the values of α in Table 1 fall in the range of ~50–~200 (during periods of high burst activity) which agrees well with the values predicted by the thermonuclear flash model. The low upper limit of 25 in the case of MXB1659−29 may perhaps result from bolometric corrections and anisotropic emission of the persistent flux[12,41,42], or from the mixing of hydrogen into the helium-burning shell[14].

Lamb and Lamb[13] suggested that the observed values of α were too small to be consistent with any thermonuclear flash mechanism, unless one invokes the "storage model" described above. Our conclusions differ from those of Lamb and Lamb[13] principally for the following reasons: (1) More detailed computations[15] indicate that the nuclear processing in a helium-burning flash proceeds all the way to iron-peak nuclei, thereby releasing ~2.7 times more energy per unit mass than in the simple fusion of helium into carbon. (2) We exclude the Type II bursts from the Rapid Burster, as these bursts are produced by a different physical mechanism than that responsible for Type I bursts[17]. (3) Preliminary estimates (see Lamb and Lamb[13]) of the

values of α from some sources were lower than the more accurate values given in Table 1.

The burst sources resemble the bright galactic bulge X-ray sources[23,24] in their galactic distribution[43] and in the spectra of their persistent X-ray fluxes[44,45]. In fact, some of the steady counterparts of the burst sources (such as MXB1735−44) can be categorised as bulge sources (see refs 24, 46).

Lewin *et al.*[43] suggested that the galactic distribution of X-ray burst sources was consistent with their being a sub-class of already known low galactic latitude X-ray sources, while Ostriker[44] and Markert *et al.*[45] have proposed that the burst sources are a subset of the galactic bulge and globular cluster X-ray sources on the basis of their spectral properties. However, the brightest of the galactic bulge sources, with luminosities of $\sim 10^{38}$ erg s^{-1}, have never been found to burst.

The classification of the galactic bulge sources into two groups by Parsignault and Grindlay[47], which is based on their X-ray spectra (Fe line emission) and the character of their brightness variations, largely coincides with a grouping of these sources according to absolute X-ray luminosity. This is shown by histograms of the apparent brightness[48] of the sources in these two classes, given that the galactic longitude distribution of the sources ($|l^{II}| \leq 20°$) indicates a small range of distances. Apparently the mass accretion rate is one of the most important parameters that determine the variability (including the presence of Type I X-ray bursts) and spectral properties of bulge sources.

In view of the results presented here we suggest more specifically (compare refs 43–45) that the majority of the galactic bulge X-ray sources are neutron stars that are undergoing accretion, and that the burst sources are a subset of the bulge sources whose accretion rate is below a critical value (and, perhaps, with other parameter values that permit bursting behaviour, such as weak magnetic fields[16]). For accretion rates above this value, the structure of the freshly accreted envelope on the neutron star is changed such that nuclear burning proceeds relatively steadily. Below this value, thermonuclear flashes occur which are observed as X-ray bursts.

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The ring systems of Jupiter, Saturn and Uranus

THE recent direct observation by the Voyager spacecraft of a ring around Jupiter[1] makes a comparison between the jovian ring and the rings of Saturn and Uranus possible, and leads to certain conclusions about their origins which are reported here.

Studies made by Vsekhsviatsky[2] and described by Struve[3] of the position of an irregular dark line on Jupiter with respect to the subsolar point suggested the presence of a ring less than two jovian radii above the surface. This radius falls, however, beyond the Roche limit and beyond the satellite Amalthea. On the other hand Acuña and Ness[4] proposed that certain minima in the charged particle fluxes observed by Pioneer 11 are caused by a ring at 1.83 times the jovian radius[5], a value which agrees well with the Voyager results. Comparing the size of the minima caused by the ring with those caused by the nearby Amalthea one can conclude that the solid surface area of the ring should be about 20,000 km^2 assuming that only the geometrical cross-section plays a part.

It is interesting to compare the location and width of the rings of Jupiter, Saturn and Uranus with the various theoretical limits. Besides the classical Roche[6] limit a_R, which is defined as the smallest distance at which a liquid self-gravitating body remains spheroidal and does not breakup, there are the limits a_i and a_0 indicating the distances at which an incident or orbiting, spherical, homogeneous and defectless solid body will break up in a most fracture-resistant mode[7]; non-spherical bodies and those containing unavoidable defects will breakup, depending on their mode of fracture, at much larger distances. There are also two accretion limits[8], the lower one, a_l, denoting the distance below which only molecular accretion onto an orbiting particle is possible and the upper one, a_u, which is the smallest distance at which two particles of the same radii can accrete. For rotating particles, a between a_l and a_u, there is an upper limit of the radius of the larger particle onto which a smaller one can accrete. Beyond a_u all modes of accretion are possible and thus larger bodies can form. The various limits can be expressed as $a = C(M/\pi\rho)^{1/3}$ with $C_R = 2.21$, $C_0 = 1.25$, $C_i = 1.08$, $C_l = 1.31$ and $C_u = 2.62$; here M is the mass of the planet and ρ is the density of the ring particles. Figure 1 shows the location of the rings and the various limits for the three planets are indicated assuming that the rings are made either of ice ($\rho = 1$ g cm^{-3}) or of carbonaceous chondrites ($\rho = 3$ g cm^{-3}); the location of the nearest satellites is also shown. All the rings lie outside the a_i, a_0, a_l, limits some of which are even smaller than the indicated planetary radii. The jovian ring is below the Roche limit for both ice and chondrite, Saturn would agree with this limit if the density of ice and chondrite were 0.77 g cm^{-3} while Uranus would agree if the density were less than 2.2 g cm^{-3}. The upper accretion limit a_u is satisfied for both ice and chondrite for Jupiter and Uranus but only for ice for Saturn.

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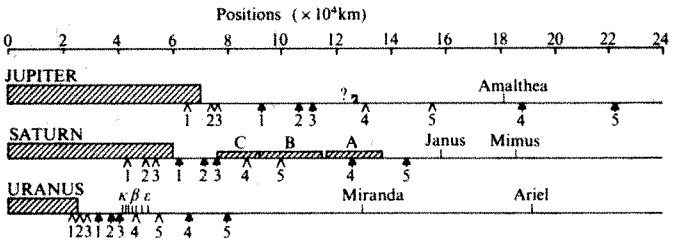


Fig. 1 The positions of the rings of Jupiter, Saturn and Uranus as well as those of their nearest satellites (above the lines) and of the various theoretical limits for densities 1 (▲) and 3 (△) g cm^{-3} (below the lines). The inner boundary of the jovian ring is uncertain. 1, Breakup of incident body a_i; 2, breakup of orbiting body a_0; 3, lower accretion limit a_l; 4, Roche limit a_R; 5, upper accretion limit a_u.

A ring which was formed by accretion of particles formed in the protoplanetary nebula would be expected to be located between a_l and a_u to make the process possible and to ensure that continuous collisional comminution does not lead to its disappearance through the action of radiation pressure, the Poynting–Robertson effect, and so on[9]. One would not expect the ring to be outside a_u because in that position accretion should be rapid and lead to the breakup of the ring into larger clumps and bodies. On the other hand a ring which was formed by the breakup of a body under the action of tidal forces should lie near a_i and a_0 only if the body was spherical and broke in a particular fracture-resisting manner[7]. Unless the body were very large, of the order of a few hundred km, one would not expect it to be spherical and it could thus breakup at any distance larger than a_i or a_0. The resulting ring would be rather narrow, it would tend to be lumpy, uneven in thickness, and if the initial fracture had produced a few larger pieces they may have formed distinct separate rings.

The above considerations agree with the conclusion that the saturnian rings, which fit so well between a_l and a_u, are made primarily of ice and that they are the product of condensation from the protoplanetary nebula and subsequent collisional spread. The 10 or so rings of Uranus are probably the result of a breakup of one or more bodies which were non-spherical and mechanically weak and probably of carbonaceous composition[9]. The formation and maintenance of distinct, separated rings was probably the result of resonances with various satellites[10]. The breakup of the body occurred probably not less than 10^7 or 10^8 yr ago because the rings seem to be continuous. The jovian ring is probably an example of a much more recently fragmented body which was non-spherical, mechanically weak and probably also of the carbonaceous kind. As pointed out by Pollack[11] the thermal history of Jupiter is unfavourable for the ring to form by condensation. If the width of the ring were 20–200 km, combining it with the total solid surface of the ring of 20,000 km^2 leads to an opacity one or two orders of magnitude lower than that of the weakest rings of Saturn which would explain the observational difficulties.

The tenuous nature of the ring may be due to continuous bombardment and erosion by the intense flux of protons in the jovian Van Allen belt. An estimate of this erosion can be made using a flux $\Phi = 2\times10^6$ cm^{-2} s^{-1} at 1–2 MeV and assuming $d\Phi/dE \sim E^{-1.5}$ dependence (ref. 12 and J. A. van Allen, personal communication), using Draine and Salpeter's[13] sputtering yields for $MgSiO_3$ and graphite and the sputtering yields for ice of Brown *et al.*[14]. One obtains an average erosion of 50–100 Å for ice, 2–3 for silicate and 0.2–0.5 Å yr^{-1} for graphite. Many small grains would thus be eliminated from the ring on the time scale of 10^6 yr once their size became small enough to be affected by radiation pressure, the Poynting–Robertson effect, and so on. In these circumstances the ring may show more rapid evolutionary changes than those expected to occur on the saturnian ring A[15] and may have a much shorter life than the rings of the two other planets.

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Cyclic reaction mechanism in the electrodeposition of aluminium

DENDRITIC growth is one of the most serious problems encountered in the galvanic deposition of metals. For many applications a high deposition rate is required but the rate cannot exceed the transport rate of metal ions to the electrode. A method of solving the transport problem by a cyclic mechanism is presented here, which combines the electrode process with a subsequent chemical reaction.

Metallic layers deposited in diffusion-controlled conditions are incoherent and powdery and are therefore not suitable for normal applications. But even at lower deposition rates the formation of the metallic layer will be influenced by diffusion. Because of the concentration gradient due to the electrode reaction, metallic nuclei that protrude in the depleting solution will grow faster than the others. The roughness of the electrode surface will be magnified and after a certain time dendritic growth will be observed. Levelling agents are added to industrial galvanic solutions to avoid dendrites. In most cases the levelling effect of these agents is due to their adsorption on a growing nucleus and the resulting deactivation of the nucleus. New nuclei have to be formed continuously and the result is a smoothly growing surface.

Instead of poisoning a growing nucleus we propose to solve the transport problem itself by combining the electrode process

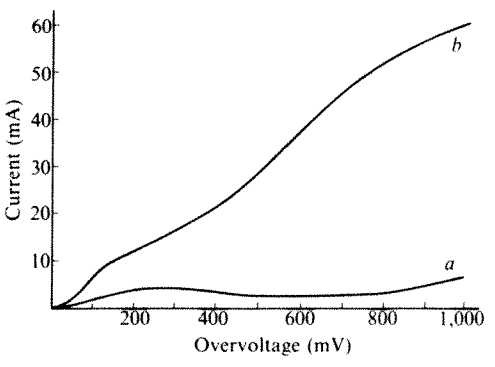


Fig. 1 Current–overvoltages curves for the deposition of aluminium at 25 °C from: *a*, 0.17 M $AlH_3 \cdot 2Et_3N$ in $LiAlH_4$–THF; *b*, 0.17 M $AlH_3 \cdot 2Et_3N$, 0.44 M $AlCl_3 \cdot 2THF$ in $LiAlH_4$–THF.

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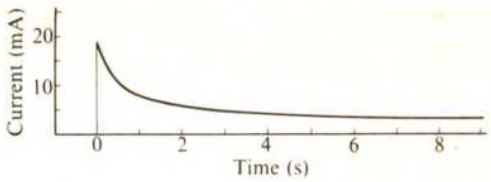


Fig. 2 Chronoamperogram for the deposition of aluminium in a 0.17 M $AlH_3 \cdot 2Et_3N$–$LiAlH_4$–THF solution at −403 mV and 25 °C.

with a subsequent chemical reaction so that the concentration of the active compound remains constant at the electrode surface:

$$A + ne^- \rightarrow M + B \quad (1)$$

$$Z + B \rightarrow A + D \quad (2)$$

where A represents the active compound, M the deposited metal and Z an electrochemically inactive compound present in the solution at high concentration and is capable of producing A. In a cyclic process the compound A is reduced at the electrode (1) but regenerated in a fast chemical reaction (2).

Reaction mechanisms of this type are well known in catalysis where a low activation energy is required for the reaction (1)

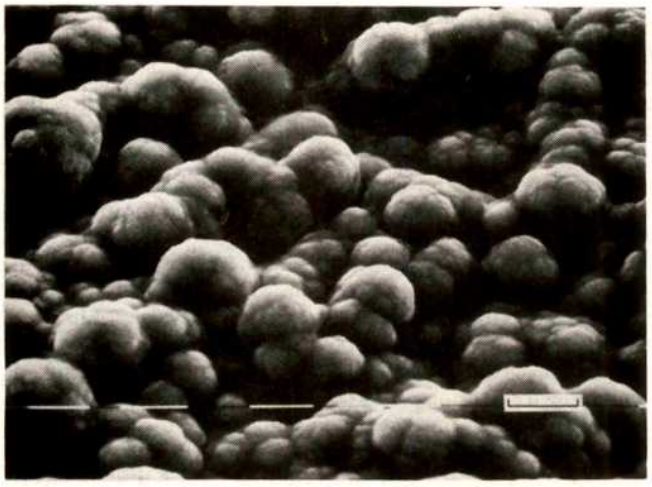


Fig. 3 Scanning electron micrograph of aluminium deposited at 3.5 mA cm^{-2} and 25 °C for 90 min from a solution of 0.17 M $AlH_3 \cdot 2Et_3N$ in $LiAlH_4$–THF. Scale bar, 10 μm.

promoting the conversion of Z into M. To obtain a cyclic electrodeposition process it is essential that the overpotential for the deposition of M from A (1) is smaller than that for the reduction of Z into M.

For the electrodeposition of aluminium Brenner[1] introduced a solution of $LiAlH_4$ and $AlCl_3$ in diethylether (Et_2O). Very pure aluminium with a high cathodic efficiency can be obtained with this solution. Levinskas[2] proposed the following reaction for the electrode process:

$$AlCl_2(Et_2O)_2^+ + 3e^- \rightarrow Al + 2Cl^- + 2Et_2O$$

We think, however, that the electrode process occurs by means of a cyclic mechanism as given in reactions (1 + 2) where AlH_3 is the active compound 'A' in the electrodeposition. The compound is regenerated in the reaction of $AlCl_2 \cdot (Et_2O)_2^+(Z)$ with hydride ions (B). The first evidence is the observation that no aluminium metal can be deposited from a solution of $AlCl_3$ in diethylether without decomposing the solvent. This rules out the scheme of Levinskas. In contrast very pure aluminium is deposited from a solution of $AlH_3 \cdot 2Et_3N$, $LiAlH_4$ in diethylether[3]. This shows that the overpotential for the aluminium deposition is larger for $AlCl_3$ than for AlH_3. The deposition rate is limited by mass transport as shown from the diffusion plateau of the current–overvoltage curve (Fig. 1*a*). The chronoamperogram (Fig. 2) measured at −403 mV also shows this diffusion dependence.

The variation of the current as a function of time is in good agreement with the Cottrell equation:

$$i = \frac{nFSD_A^{1/2}C_A^0}{(\pi t)^{1/2}}$$

Electrodeposition at a current density of 3.5 mA cm^{-2} results in a rough and spongy surface as shown in Fig. 3. This structure is a precursor of dendritic growth.

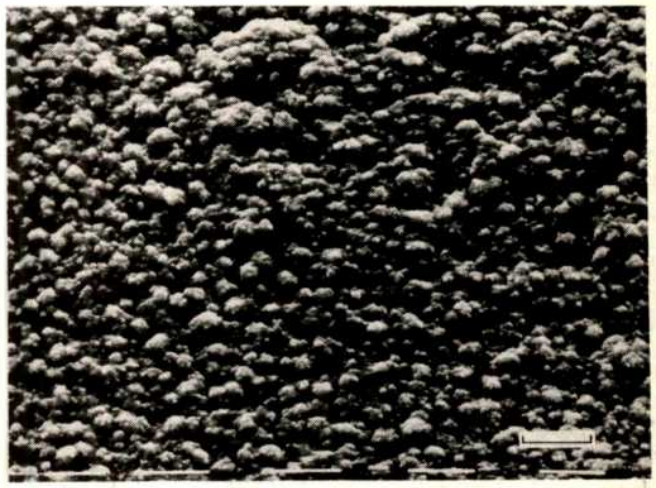


Fig. 4 Scanning electron micrograph of aluminium deposited at 21 mA cm^{-2} and 25 °C for 15 min from a solution of 0.17 M $AlH_3 \cdot 2Et_3N$, 0.44 M $AlCl_3 \cdot 2THF$ in $LiAlH_4$–THF. Scale bar, 10 μm.

The compound AlH_3 was obtained in a pure and crystalline form as an adduct with triethylamine: $AlH_3 \cdot 2Et_3N$. For the electrochemical measurements the less volatile tetrahydrofuran (THF) was used instead of diethylether as the solvent. The measurements were carried out with a special cell design in which the aluminium reference electrode was located in a cavity behind the working electrode ensuring a very small ohmic drop between working and reference electrodes.

When $AlCl_3 \cdot 2THF$ is added to the previous solution of $AlH_3 \cdot 2Et_3N$, $LiAlH_4$ in THF the situation is completely changed. There is no diffusion plateau in the voltammogram

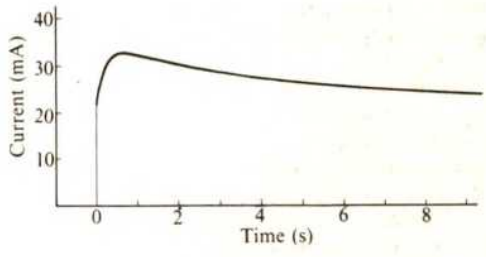


Fig. 5 Chronoamperogram for the deposition of aluminium in a 0.17 M $AlH_3 \cdot 2Et_3N$, 0.44 M $AlCl_3 \cdot 2THF$ solution at −403 mV and 25 °C.

(Fig. 1*b*) and compact aluminium layers are obtained even at higher current densities (Fig. 4).

The chronoamperogram (Fig. 5), also measured at −403 mV, indicates that in this case the current is no longer diffusion controlled. We explain this by the following cyclic process:

$$AlH_3 + 3e^- \rightarrow Al + 3H^-$$

$$AlCl_2(THF)_4^+ + 3H^- \xrightarrow{k_2} AlH_3 + 2Cl^- + 4THF$$

The chemical reaction maintains a constant concentration of the active compound at the electrode and the result of the process is the deposition of a compact aluminium layer. The measured values of the current as a function of time are in good agreement with the equations derived by Delahay[4].

$$i = nFSD_A^{1/2}C_A^0(k_2C_Z^0)^{1/2}\,erf\,(k_2C_Z^0t)^{1/2} + \frac{nFSD_A^{1/2}C_A^0}{(\pi t)^{1/2}}\exp(-k_2C_Z^0t)$$

The rate constant $k_2 = 11.7\ s^{-1}\ mol^{-1}\ l$ (in THF at 25 °C) of the chemical process can be calculated from the chronoamperometric measurements. The value of the rate constant k_2, together with the concentration of $AlCl_3$ (C_Z^0), defines the limits between which the cyclic process can function.

We believe that a cyclic mechanism can also explain the effect of adding cyanide compounds to an aqueous solution used for the electro-deposition of zinc. It is found[5] that $Zn(OH)_2$ is the active compound in the electrode reaction but it is also known that the presence of cyanide ions gives smoother deposits than those obtained from zincate alone[6].

In a cyclic process the diffusion limitation of $Zn(OH)_2$, which is only present at a low concentration level, is removed by the chemical regeneration of $Zn(OH)_2$ in the reaction of $Zn(CN)_4^{2-}$ with hydroxide ions.

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Effects of acetylene and soil water content on emission of nitrous oxide from soils

THERE are fears that increased nitrogen fertilisation of soils may increase release of N_2O from soils through denitrification of nitrate and thereby pose a threat to the stratospheric ozone layer protecting the Earth from biologically harmful UV radiation from the Sun[1–6].

Most of the fertiliser nitrogen added to soils is in the form of ammonium or of ammonium-producing compounds such as urea. Before this nitrogen can be converted to N_2O by denitrifying microorganisms, it must be oxidised to nitrite or nitrate by the nitrifying microorganisms in soils. Studies with pure cultures[7] have shown that at least one of these nitrifying microorganisms (*Nitrosomonas europaea*) can produce N_2O during oxidation of ammonium to nitrite, so the possibility that nitrifying as well as denitrifying microorganisms contribute to N_2O emissions from soils must be considered. Recent work[8,9] has shown that N_2O emissions from well aerated soils treated with ammonium sulphate greatly exceeded N_2O emissions from the same soils treated with an equivalent amount of nitrogen as potassium nitrate and were greatly reduced by addition of nitrapyrin [2-chloro-6-(trichloromethyl)-pyridine], which inhibits nitrification of ammonium in soils[10,11] but has little or no effect on denitrification of nitrate by soil microorganisms[12]. These observations indicated that most of the N_2O evolved from well aerated soils treated with ammonium or ammonium-producing fertilisers is formed during oxidation of ammonium to nitrite by nitrifying soil microorganisms and that these microorganisms contribute significantly to N_2O emissions from soils. We report here further evidence for these conclusions. This evidence was obtained by studying the effects of acetylene (C_2H_2) on N_2O emissions from soils incubated at different water tensions after treatment with ammonium sulphate or potassium nitrate. The effects of C_2H_2 were studied because recent work has shown that this gas inhibits reduction of N_2O to N_2 by denitrifying microorganisms in soils or pure cultures without affecting reduction of nitrate to N_2O by these microorganisms[13–17] and also inhibits oxidation of ammonium by *Nitrosomonas europaea*[18] and the nitrifying microorganisms in soils[19].

The soils used (Table 1) were screened (<2 mm) samples of field-moist surface soils selected to obtain a range in properties. Subsamples containing 30 g of soil were incubated at 30 °C after treatment with ammonium sulphate or potassium nitrate (100 μg N per g) and adjustment of their water contents to the equivalent of 0, 10 or 100 cm of water tension. Incubations were performed under air or air containing 0.1% (v/v) C_2H_2 in 1.2-l glass bottles fitted with ground glass joints and glass stopcocks, and the atmospheres in the bottles were sampled for N_2O analysis and renewed after various times. Nitrous oxide was determined by a gas chromatographic technique that utilises the xenon in air as an internal standard[20]. Soil water tension was determined by the tensiometer described by Rose[21], and nitrogen as (nitrite + nitrate) present in soils before and after incubation was determined as described by Bremner and Keeney[22].

Table 1 shows the effects of C_2H_2 on the amounts of nitrogen as N_2O and as (nitrite + nitrate) produced on incubation of well aerated samples of ammonium-treated soils. Besides confirming that C_2H_2 is a potent inhibitor of nitrification in soils, the data reported show that this gas inhibited production of N_2O in ammonium-treated soils. This is evidence that most, if not all, of the N_2O evolved from these soils in the absence of C_2H_2 was produced by nitrifying microorganisms.

Further evidence for this conclusion is reported in Table 2, which shows the effects of C_2H_2 and soil water content on the amounts of N_2O evolved from soils treated with ammonium sulphate or potassium nitrate. The data reported show that the amounts of N_2O evolved from ammonium-treated soils at water tensions ranging from 0 to 100 cm increased with increase in soil water content and greatly exceeded the amounts evolved from the corresponding nitrate-treated soils. Table 2 also shows that

Table 1 Effects of acetylene on amounts of nitrogen as N_2O and as (nitrite + nitrate) produced on incubation of ammonium-treated soils under aerobic conditions

Soil	*p*H	Organic carbon (%)	Clay (%)	Sand (%)	N_2O nitrogen evolved in 12 days (ng per g soil)		(Nitrite + nitrate) nitrogen produced in 12 days (μg per g soil)	
					$-C_2H_2$	$+C_2H_2$	$-C_2H_2$*	$+C_2H_2$*
Storden	8.2	0.4	12	53	170	<1	83 (4)	<1 (<1)
Harps	8.1	4.2	37	18	208	<1	105 (11)	<1 (<1)
Webster	6.8	3.8	25	28	30	<1	82 (9)	<1 (<1)
Nicollet	6.2	2.6	27	37	24	<1	67 (6)	<1 (<1)

Soils treated with ammonium sulphate (100 μg N per g soil) were incubated at 100 cm water tension under air or air containing 0.1% (v/v) C_2H_2. Atmospheres in incubation flasks were renewed at 3-day intervals.

* Figures in parentheses indicate the amount of nitrogen as (nitrite+nitrate) produced in control (no ammonium added) (μg N per g soil).

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Table 2 Effects of soil water tension and acetylene of N_2O emissions from soils treated with ammonium sulphate or potassium nitrate

Soil	Form of N added	Soil water tension (cm water)	N_2O nitrogen evolved (ng per g soil) 2 days $-C_2H_2$	2 days $+C_2H_2$	7 days $-C_2H_2$	7 days $+C_2H_2$	14 days $-C_2H_2$	14 days $+C_2H_2$
Harps	Ammonium	0	625	82	1,170	96	1,175	103
		10	270	2	399	8	401	12
		100	130	<1	202	<1	210	<1
	Nitrate	0	1	71	6	73	7	74
		10	1	1	1	4	2	5
		100	<1	<1	<1	<1	1	<1
Webster	Ammonium	0	34	1	42	2	52	4
		10	29	1	35	1	44	2
		100	18	<1	23	<1	31	<1
	Nitrate	0	2	<1	6	2	6	3
		10	1	<1	3	1	4	2
		100	1	<1	2	<1	3	<1

Soils supplemented with N (100 μg per g soil) as ammonium sulphate or potassium nitrate were incubated at various water tensions under air or air containing 0.1% (v/v) C_2H_2. Atmospheres in incubation flasks were renewed after 2, 4, 7, 9 and 11 days.

C_2H_2 greatly reduced evolution of N_2O from ammonium-treated soils and that the amounts of N_2O evolved from these soils in the presence of C_2H_2 were similar to, or greater than, the amounts evolved from the corresponding nitrate-treated soils. Since C_2H_2 inhibits production of N_2O via oxidation of ammonium by nitrifying microorganisms but promotes release of N_2O during reduction of nitrate by denitrifying microorganisms, these observations seem to leave no doubt that most of the N_2O evolved from the ammonium-treated soils in the work reported was produced during oxidation of ammonium by nitrifying soil microorganisms and that very little of this gas was produced through reduction of nitrate by denitrifying microorganisms.

Our finding that N_2O emissions from ammonium-treated soils increased markedly as the soil water tension decreased (that is, as the soil water content increased and the availability of oxygen to soil microorganisms decreased) is readily explained if nitrifying microorganisms are responsible for these emissions. Ritchie and Nicholas[23] have shown that *Nitrosomonas europaea* produces an enzyme that allows it to reduce nitrite to N_2O during oxidation of ammonium and thereby utilise nitrite as a terminal electron acceptor instead of oxygen when the supply of oxygen is limited.

Our conclusion that nitrifying microorganisms contribute substantially to N_2O emissions from soils is supported by field studies showing that N_2O emissions from plots fertilised with nitrogen as ammonium sulphate exceeded those from corresponding plots treated with an equivalent amount of nitrogen as calcium nitrate (A. D. Matthias, A.M.B. and J.M.B., unpublished).

Other evidence that nitrification deserves much greater attention in research on sources of atmospheric N_2O has been provided by recent work[24,25] indicating that nitrification may be the major source of N_2O in the oceans.

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Non-uniform vertical distribution of fine sediment in the Amazon River

IN studies of sediment transport in rivers, it is frequently assumed that suspended particles finer than a certain size (usually of the order of 60 μm) are uniformly concentrated from river bed to water surface[1,2]. The assumption seems to be mostly a matter of convenience; it is well established that the vertical concentration gradient for sediment particles suspended in rivers is directly proportional to fall velocity of the particles and inversely proportional to shear velocity of the flow[3]. For very fine particles suspended in highly turbulent flows, concentration gradient may be small; the assumption has been used, therefore, to justify collection of near-surface samples in streams where velocities are high and turbulence is intense[2]. Our recent studies of sediment in suspension in the Amazon River show that this assumption is not valid for deep rivers and that appreciable errors result from using concentrations of surface samples to represent concentration of suspended sediment and its associated adsorbed constituents through the entire stream depth.

During high-water seasons of 1976 and 1977, we collected samples of water and sediment in the Amazon River between the mouth and Iquitos, about 3,700 km upriver from the mouth. We used a variety of sampling equipment aboard RV Alpha Helix: Niskin bottles, a point sampler, a depth-integrating

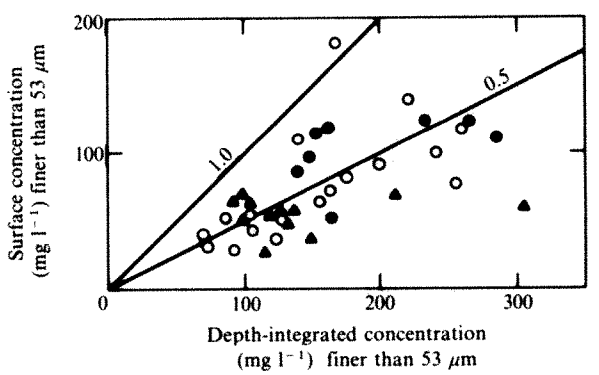


Fig. 1 Relations between concentrations of fine-grained suspended sediment at the river surface and in depth-integrated samples in the Amazon River mainstem near Iquitos, at São Paulo de Olivença, at Santo Antônio do Içá, at Itapeúa (near Coari), near Manacapuru, and at Óbidos. Samples collected between 20 May and 2 June 1977. Points are coded by river depth: ●, depths ≤24 m; ○, 24–49 m; ▲, ≥50 m. Lines through the graph are not regression lines, but are reference lines to show ratios of surface to depth-integrated concentrations.

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sampler, and a bucket. The Niskin bottle, which has become standard sampling equipment in most US-based research on the chemistry of the oceans, is lowered open to the desired depth and then closed by a messenger sent down the wire. The point sampler we used is a modified version of the US P-63 sampler that is used in deep rivers of North America[2]: it has a nozzle that faces directly into the flow and that is designed to admit water at its ambient velocity in the river. The nozzle has a valve that can be opened and closed by remote control after the sampler has been lowered to the desired depth. As the sampler is lowered into the flow, air is compressed in the sample container so the pressure inside the container and in the flow are equal. This is to avoid an initial surge into the sampler when the valve is suddenly opened, which will bias the sample[2]. Our sampler had an auxiliary air chamber to allow pressure equalisation to depths of 100 m. The depth-integrating sampler we used is an enlarged modification of the type of sampler with which most river-sediment samples are collected in the US[2]. It has the same type of nozzle as the point sampler (but without a valve that closes it), and it is lowered to the river bed and back to the surface at a uniform vertical rate. The resulting depth-integrated sample is thereby weighted for differences in velocity in the vertical, and the concentration of sediment in the sample is a velocity-weighted average concentration. Our simplest piece of equipment was a bucket with which we dipped some of the samples from the river surface. Details of our sampling and analytical procedure including a list of our data are given elsewhere[4,5].

The particle-size analyses represented in Figs 1 and 2 were carried out by several procedures. Samples collected in 1976 were separated entirely aboard ship by sieve (sizes >63 μm) and pipette (sizes <63 μm). The sieved and pipetted fractions were preserved (latter on preweighed Nucleopore filters having a pore diameter of 0.4 μm) for later weighing in the laboratory in Edinburgh. Samples collected in 1977 were all passed through a 53-μm sieve aboard ship, and material caught on the sieve was preserved for more detailed sieve analysis in the laboratory in Denver. Suspensions of fine sediment that passed the 53-μm sieve were mostly filtered aboard ship onto preweighed Millipore filters (nominal pore diameter 0.45 μm) for later weighing in Denver. However, some suspensions of particles finer than 53 μm were bottled and returned to the US for detailed pipette analysis of the silt and clay sizes. Shipboard pipette analyses made in 1976 used river water without added dispersants. Pipette analyses made in the US in 1977 used distilled water and dispersing agents[6].

Figure 1 compares concentrations of fine-grained (less than 53 μm) suspended sediment collected within 1 m of the water surface with the concentrations collected at the same sites from the full depth of water by the depth-integrating sampler. Samples represented in Fig. 1 were collected at six cross-sections spaced along 3,000 km of the Amazon mainstem between Iquitos, Peru, and Óbidos, Brazil. In general, concentration of fine material in suspension at water surface is about half the average fine-material concentration in the full depth of the river. This does not support Gibbs' report[7] that samples collected at 0.9 total depth (0.1 of the distance from river bed to water surface) in the Amazon had only a 20% greater concentration of suspended sediment than samples collected from river surface at the same sites. Samples taken at 0.9 total depth should have even greater concentration than depth-integrated average concentrations shown in Fig. 1. Furthermore, when total suspended concentration is considered (that is, when material >53 μm is included), the difference between near-surface and near-bottom concentrations is even greater[4].

Is there a minimum particle size below which concentration in suspension from bed to river surface is uniform? Figure 2 shows the results of size analyses of samples taken at three different localities with three different types of equipment. The analyses show that particles even as small as 10 μm are not uniformly distributed. In two of the three sets of analyses shown in Fig. 2, concentrations of particles as small as 1 or 2 μm show slight but discernible differences between bottom and surface.

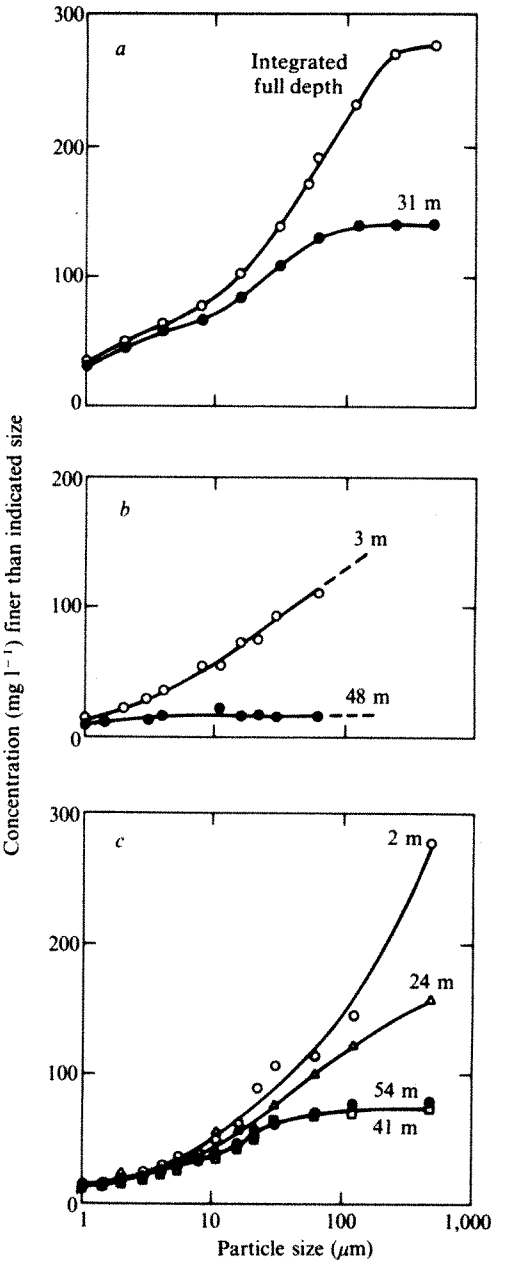


Fig. 2 Particle-size distributions of suspended sediment collected by different sampling methods in the Amazon River. Numbers by each curve indicate distances of samples above the river bed. *a*, Samples collected by depth-integrating sampler through the full depth of the river (from surface to bed and back to surface) and by the integrating sampler at a single depth of 1 m below surface (31 m off river bed). Each curve represents a composite of two samples taken in left centre part of the channel at São Paulo de Olivença, 22 May 1977. *b*, Samples collected by 30-l Niskin bottles 3 m above river bed and at river surface (48 m off river bed) in centre of channel near Manacapuru, 22 June 1976. *c*, Samples collected by point sampler at 2 m, 24 m, 41 m, and 54 m above river bed in left centre part of channel at Óbidos, 14 June 1976. Particle-size distributions at 41 m and 54 m were not sufficiently different to warrant separate lines through their points. Uppermost sample (54 m above the bed) was taken at a depth of 9 m below water surface.

We conclude that, in a river as deep as the Amazon, one cannot assume that suspended particles coarser than 1 μm are uniformly distributed in suspension. In the Amazon, as well as in other large rivers of the world, estimates of sediment yield and flux of suspended chemical constituents that are based only on surface samples are likely to contain significantly large errors.

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Palaeomagnetism of stalagmite deposits

VARIOUS sedimentary deposits have recently been studied with the aim of producing geomagnetic field curves representing the Brunhes epoch. Unfortunately the magnetic recording process tends to be subject to several depositional and post-depositional errors and in the light of these Watkins[1], Verosub[2] and Verosub and Banerjee[3] have reviewed many of the claims that have been made for secular variation and anomalous field behaviour. The corresponding age data, provided by ^{14}C, uranium series disequilibrium and other methods may also suffer from such errors as bioturbation and migration of radionuclides. It has been shown that most speleothems (here stalagmites and flowstones) may be dated by uranium series methods to an age limit of 350,000 yr BP[4]. With the aim of finding an alternative continuous magnetic record we have studied the natural remanent magnetism (NRM) and ages of speleothems from the Canadian Rockies, the UK and elsewhere. We report here briefly on two of them and in greater detail on a third.

Each speleothem was oriented in the cave using mounts carrying a compass and a clinometer. The sample was cast in plaster of Paris in the laboratory where it was then sawn parallel and perpendicular to growth horizons, usually into 2-cm cubes. The procedure consisted of measuring the NRMs, carrying out a pilot alternating field demagnetisation and then remeasuring the specimens, alternating field cleaned to an optimum level. Fisher statistics were used to find a mean direction for all specimens from the same growth horizon and this direction was then reoriented from its laboratory to its site value.

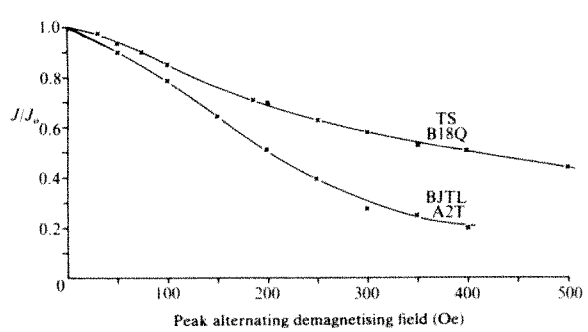


Fig. 1 Specimen demagnetisation curves, where J/J_0 is the ratio of the cleaned magnetisation to the NRM.

A flowstone BJTL, 7 cm thick, was collected from Bear Jaw cave near Crow's Nest Pass, Alberta, Canada (Table 1). $^{230}Th/^{234}U$ ratios obtained for the upper growth horizons showed that it was older than 350 Kyr BP (Table 2). Nevertheless, the magnetic results were encouraging in that (1) the major part of the NRM appeared to be stable, the median demagnetising fields of four test specimens all being greater than 180 Oe (Fig. 1 specimen BJTL A2T), with little shift of the concurrent vector direction; (2) the declination (D) and inclination (I) values were apparently not influenced by surface effects (the flowstone growth horizons dipped at about 28.5° in a direction of 298°). The magnetic intensity ranged from 3×10^{-9} to 2×10^{-8} emu g^{-1} (the Toronto ScT magnetometer used here has a quoted sensitivity of 2×10^{-7} emu).

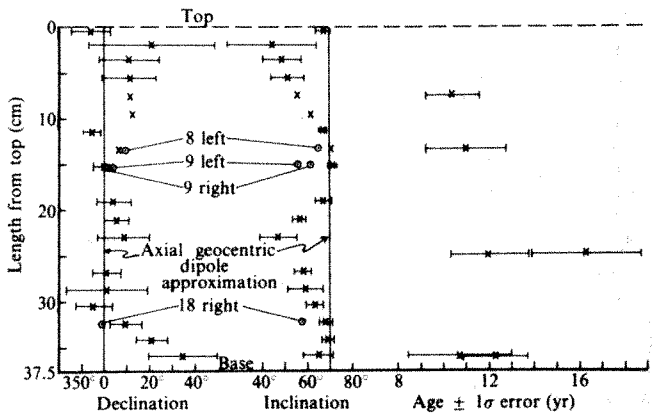


Fig. 2 Declination, inclination and dates of the Kingsdale stalagmite TS. (Direction errors of the lateral specimens have been omitted for clarity.)

A 13-cm thick flowstone from Eagle Cave, Crow's Nest Pass, was reversely magnetised throughout and together with its radioisotope ratios, this suggests that it grew near the end of the Matuyama epoch.

Several speleothems collected subsequently showed very low magnetic intensities. Where the signal was low but the growth layers well defined, greater signal strength was obtained by stacking adjacent specimens of the same growth horizon (suggested by M. L. Glasser). Such was the case of sample TS, a 37-cm high stalagmite from Kingsdale, Yorkshire, UK. Three vertical slices were obtained from the centre of the stalagmite and specimens from the middle cuts were stacked in two's and three's for NRM measurement (Table 1 and Fig. 2). Visual examination of the sample indicated continuous growth from the base, possible flood events having occurred at the levels of cuts 18 and 19, where there is dark opaque detrital material containing quartz grains. Because of their higher intensity, specimens from these lower cuts did not need to be stacked for measurement. Between 1 and 2 cm from the top, at cut 2, there is a concave upwards layer and irregular lateral crystal growth which probably indicates dissolution and hence a growth hiatus. At the time of collection the upper surface was wet, indicating that the top centimetre was probably of recent origin.

Figure 1 shows the alternating field demagnetisation of specimen TS B18Q; the median demagnetisation field was about 400 Oe and the direction moved only slightly from D = 6.4°, I = 60.0°, to settle around D = 10° and I = 63°. In view of

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Table 1 NRM of samples and analyses of directions

Sample	Cut no.	Distance from top (cm)	NRM N	NRM D	NRM I	NRM α_{95}	NRM K	Alternating field demagnetised H (Oe)	Alternating field demagnetised D	Alternating field demagnetised I	Alternating field demagnetised α_{95}	Alternating field demagnetised K	Alternating field demagnetised δD^*	Alternating field demagnetised δI^*
BJTL	1	1.0	9	38.0	77.2	10.6	13	50	42.0	80.5	2.3	410	14	2.3
Bear Jaw	2	2.9	10	355.7	75.2	12.4	11	100	10.9	78.1	3.1	200	15	3.1
Cave	3	4.8	9	357.7	69.7	2.5	347	100	355.8	72.0	1.2	1570	3.8	1.2
	4	6.8	8	5.1	65.3	1.5	1072	100	7.3	67.1	1.1	2011	2.8	1.0
TS	1	0.5	3	3.5	64.0	7.3	121	100	354.0	67.2	3.5	529	9.0	3.5
Kingsdale	2	1.9	3	11.3	58.0	32	6.5	100	21.0	44.3	20.2	16	28.2	20.2
	3	3.7	6	355.2	49.7	120	120	50	10.8	49.0	8.3	47	12.6	8.3
	4‡	5.65	4	12.3	44.1	11	37	50	11.1	51.5	7.4	90	11.9	7.4
	5†	7.55	1	14.8	47.5	—	—	50	11.2	55.5	—	—	—	—
	6†	9.55	1	14.8	53.4	—	—	50	12.1	61.8	—	—	—	—
	7‡	11.4	4	355.6	63.0	1.6	2010	50	354.5	66.9	1.7	1670	4.3	1.7
	8†	13.45	1	11.5	63.5	—	—	50	6.4	70.6	—	—	—	—
	9‡	15.2	4	357.7	70.5	1.5	2156	50	359.7	71.2	1.7	1737	5.3	1.7
	10‖	17.2		Missing										
	11§	19.15	4	(2) 356.5	40.6	16.6	18	50	3.7	66.9	3.4	850	8.7	3.4
	12‡	21.15	4	3.1	56.2	2.5	779	50	5.1	56.5	2.9	583	5.3	2.9
	13‡	23.0	4	10.1	46.0	8.9	62	50	8.8	47.0	8.1	76	11.9	8.1
	14‖	24.85		Missing										
	15	26.85	3	6.0	55.7	4.9	271	100	0.7	58.3	3.2	626	6.1	3.2
	16	28.7	3	5.2	55.3	9.0	80	100	1.1	59.0	7.9	104	15.3	7.9
	17	30.55	3	3.3	61.5	6.4	158	100	355.4	63.3	3.6	496	8.0	3.6
	18¶	32.4	4	10.0	65.7	3.6	380	100	9.3	67.7	2.7	661	7.1	2.7
	19	34.2	3	20.8	67.2	2.4	1090	100	21.0	69.4	2.7	925	7.7	2.7
	20	35.85	3	35.2	64.3	5.0	263	100	34.7	65.0	6.7	148	15.9	6.7
TS lateral specimens	8‡ Left face	13.45	4	0.0	62.9	2.7	678	50	9.7	63.7	1.5	2227	3.4	1.5
	9‡ Left face	15.2	4	335.7	39.4	22.5	9.7	50	3.5	55.6	5.4	168	9.6	5.4
	9‡ Right face	15.2	4	12.0	59.6	1.5	2053	50	1.3	61.8	2.6	709	5.5	2.6
	18 Right face	32.4	3	0.9	58.3	3.3	618	80	358.6	57.4	2.2	1317	4.1	2.2

* Errors from $\alpha_{95} = \cos I.\delta D = \delta I$.
† Two specimens stacked to give one vector.
‡ Three specimens stacked in two's and then all three together to give four vectors.
§ B11Q possessed an anomalous cleaned direction of 353° and −32° after vecor subtraction and has therefore been excluded from the alternating field demagnetised mean. We do not know the reason for this.
‖ Cuts 10 and 14 were not measured because of extensive shattering incurred during the casting process. B14Q and D14Q have been used for dating purposes.
¶ Cut 18 also included specimen D19R which had exactly the same growth horizons.

the low intensities of the middle specimens it was felt that 50 Oe was an optimum demagnetising field. For most cuts the precision has increased on cleaning.

If speleothems possessed some kind of detrital remanent magnetism (DRM) the surface of deposition would be expected to have had an effect on the NRM directions. To test for this, specimens were prepared from the vertical sides of the Kingsdale stalagmite. They were taken from cuts 8 left-, 9 left-, 9 right- and 18 right-facing sides and contain, more or less, horizons spanning cuts 1 to 7,8,8 and 17 respectively. Their values (Table 1 and Fig. 2) are in accordance with other values for these layers, strongly suggesting that neither the D nor I values have been influenced by surface effects. This in turn points to a remanence of chemical origin.

The speleothem specimens have been dated by the $^{230}Th/^{234}U$ method assuming that no ^{230}Th was initially present and that the system had remained closed after initial deposition of the uranium. (Detrital thorium contained in the soluble fraction is detectable by the presence of ^{232}Th in the α-particle spectrum. Our method also takes into account the excess of ^{234}U over ^{238}U.) Radiometric data for TS (Table 2 and Fig. 2) show that it is young; the low uranium content renders the data imprecise. The stalagmite appears to have grown from about 12,500 yr BP, and was later redissolved down to an horizon which grew at about 10,000 yr BP, the remaining cap having been deposited more recently. Thus between 12.5 and 10 kyr BP it grew at 20 cm kyr^{-1}.

To test whether the stable primary magnetisation was syngenetic with its host growth horizon we have compared the modern field direction of the Kingsdale area with that for the upper cap of the Kingsdale stalagmite. (This is in contrast to many lake or marine cores where the tops are either missing or are severely disturbed. Note also that NRM direction errors, especially of inclination, may often go undetected in such cores.)

Table 2 Specimen dating and isotope activity ratios

Sample	Specimen	U (p.p.m.)	$^{230}Th/^{234}U$	$^{234}U/^{238}U^*$	$^{230}Th/^{232}Th$	Calculated age ± 1σ error (kyr BP)	Distance from top to 0.1 cm
Bear Jaw BJTL	1C	1.1	0.98 ± 0.04	1.00 ± 0.04	609	>350	0.9
Eagle cave ENF	A1	0.27	1.10 ± 0.05	1.26 ± 0.05	17	>350	0.9
Kingsdale TS	B5Q	0.15	0.092 ± 0.01	1.68 ± 0.085	9.9	10.4 ± 1.2	7.55
	B8Q	0.13	0.097 ± 0.015	1.70 ± 0.08	3.5	11.0 ± 1.8	13.45
	B14Q	0.12	0.14 ± 0.02	1.70 ± 0.08	>1000	16.3 ± 2.4	24.85
	D14Q	0.16	0.11 ± 0.01	1.77 ± 0.06	>1000	12.0 ± 1.7	24.9
	D20Q	0.16	0.09 ± 0.02	1.61 ± 0.06	14.5	10.7 ± 2.3	35.8
	E20Q	0.18	0.11 ± 0.01	1.61 ± 0.06	41	12.3 ± 1.4	36.0

* Low ^{232}Th count rates indicated the essential lack of detrital thorium in the Kingsdale TS sample.

The modern field direction is $D = 8°W$, $I = 69°$, while the three specimens of cut 1 which was only 1 cm in depth have a mean direction of $D = 6°W$ and $I = 67°$ with an α_{95} of 3.5°; this is probably an average of the past 50 yr or more.

Of nine other speleothems which we have analysed, two are too weakly magnetised to be measurable, two show large viscous instabilities, and the remainder have stable, measurable NRMs. The latter, like the Bear Jaw and Kingsdale samples, have NRMs apparently unaffected by surface forces such as roughness, water flow, crystal growth habit or the dip of the growth layers, and this again supports the idea of a chemical remanent magnetisation. Hysteresis curves obtained for several specimens show saturation fields of around 3,000 G and coercivities of remanence between 140 and 350 G; this indicates that magnetite is the major carrier of the remanence.[5]

Although the stable remanence of speleothems is often weak, our findings indicate that in many cases we will be able to produce good records of secular variation in D and I. We expect the uranium concentrations will be sufficient to allow us to assign accurate absolute ages to most of these records.

A disadvantage of this method is that speleothems are objects of great natural beauty which once removed from a cave cannot be replaced. It is better to investigate limestone quarries where caves are sometimes breached and where speleothems may in any case be destroyed.

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Growth rate of a clam from the Galapagos Rise hot spring field using natural radionuclide ratios

THE submarine hydrothermal springs on the Galapagos Rift are the sites of lush biological populations supported ultimately by sulphur-oxidising bacteria utilising hydrogen sulphide transported from depth by the springs[1]. Two types of bivalves, one a mytilid mussel and the other a vesicomyid, have been recovered from these thermal regions. The shells of these organisms are as much as 30 cm long. We are determining growth rates of these organisms using a method which measures natural radionuclide content[2]. In this report we present our first results on a clam collected in the February–March 1977 Alvin dive series.

A 22 cm long valve of an articulated but dead specimen of a vesicomyid clam from the active thermal spring area, called Clambake I[1], at a depth of about 2,500 m, was analysed for the radionuclides listed in Table 1 within 1 yr after collection. The valve was cut in half from the hinge to the growing edge. The analyses were made on aliquots of the pulverised and homogenised half with the other half remaining intact for growth structure analysis and other studies. The analytical procedures involve α-spectrometric techniques for the metallic radionuclides (except ^{226}Ra which was determined by scintillation counting of ^{222}Rn)[3], and liquid scintillation analysis for radiocarbon assay[4].

Using the data shown in Table 1 we can first set upper limits to the age of the clam by making limiting assumptions about the radiochemical composition of the water from which the clam deposited its shell. In all of these calculations we assume a constant mass deposition per unit time growth law. We do not yet know the exact growth law for these deep-sea clams but shallow water clams generally show the following pattern[5]: an initial slow growth followed by a quasi-linear mass increase with time and then a slowing down at old age. By assuming that at all times in the life of the Galapagos Rift vesicomyid it deposits shell at a constant mass rate we will be erring in the direction of calculating too old an age.

The lowest $\Delta^{14}C$ value for dissolved inorganic carbon at depth in the eastern South Pacific measured in the GEOSECS programme by Ostlund *et al.*[6] is −240. If the shell grew with a constant mass per unit time, then its age can be calculated by the equation: $A/A_0 = (1 - e^{-\lambda t})/\lambda t$, where A is the activity after time t and A_0 is the initial activity. The calculated age by this model is about 500 yr. The assumption that the water from which the shell grew is unmodified ambient Pacific deep water conflicts with the observations[1]. Indeed a significant amount of dissolved inorganic carbon is released by the vents to the bottom water. This carbon can only be lower in ^{14}C than the ambient dissolved carbon whether it is imprinted by mantle material or old calcium carbonate deposits on the ridge flanks.

The $^{210}Pb/^{226}Ra$ activity ratio in the shell is 3.8. Without independent information of the $^{210}Pb/^{226}Ra$ ratio of the bottom water in this region an age assignment cannot be made. We proceed as follows. If the ^{226}Ra activity is 60 d.p.m. per 100 kg (P. E. Biscaye, personal communication) and the ^{222}Rn activity is 6,000 d.p.m. per 100 kg (J. Dymond and J. Edmond, personal communication), then in steady state conditions, with the ^{210}Pb activity equalling the ^{222}Rn activity, the expected initial activity ratio of $^{210}Pb/^{226}Ra$ would be about 100 in the water. If no strong fractionation between ^{210}Pb and ^{226}Ra occurred during shell deposition[7] this would also be the initial ratio in the shell. A constant growth model would then yield an age of 830 yr. It is unlikely that the ^{210}Pb produced from the radon would persist in solution, however. Rather it will be scavenged by the precipitating ferromanganese oxides found everywhere in the region

Table 1 Radiochemical analyses of a vesicomyid clam from the Galapagos Rift (Clambake I)

	Activities (d.p.m. g^{-1})
^{226}Ra	0.0515 ± 0.0022
^{228}Ra	0.00200 ± 0.00029
^{228}Th	0.00135 ± 0.00030
^{210}Pb	0.195 ± 0.006
	Activity ratio
$^{234}U/^{238}U$	1.19 ± 0.08
$^{228}Th/^{228}Ra$	0.68 ± 0.18
$^{228}Ra/^{226}Ra$	0.039 ± 0.006
$^{210}Pb/^{226}Ra$	3.79 ± 0.20
$\delta^{14}C$	−213 ± 8
$\Delta^{14}C$	−263 ± 10

All measurements are corrected to time of collection. Sample is designated G3-2(L) obtained on drive 713 (approximately 0°47.6′ N, 86°09′ W).

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including on the shells of living mussels. As the estimated ^{210}Pb value in the water from which the shell is growing is diminished the calculated age of the clam will also become smaller.

The $^{228}Ra/^{226}Ra$ activity ratio of the shell provides a third method of setting an upper limit. This will, however, be more constraining than the previous two methods since two isotopes of the same element are involved and the limits on the ratio of the two isotopes in the water can be more accurately defined. An upper limit to the $^{228}Ra/^{226}Ra$ ratio in the water is set by the ratio of $^{232}Th/^{238}U$ in the basalt undergoing leaching. Mid-ocean ridge basalts have a Th/U weight ratio of around 2 (ref. 8). This corresponds, at secular equilibrium, to a $^{228}Ra/^{226}Ra$ activity ratio of about 0.65. If this were the ratio for the water from which the clam deposited its shell, the constant mass growth law age would be 140 yr.

The only direct method of arriving at the growth rate of the clam is by the use of the $^{228}Th/^{228}Ra$ activity ratio. ^{228}Ra is supplied to the water at the ocean bottom from the ocean floor rocks and sediments as the result of production by radioactive decay of ^{232}Th and diffusion. Once in the water ^{228}Ra decays with a half life of 5.7 yr to ^{228}Th which decays with a half life of 1.9 yr. As the shell grows it adds ^{228}Ra, presumably with little or no ^{228}Th, just as it adds ^{226}Ra. As the deposited shell material ages, ^{228}Ra decays within the shell to ^{228}Th which itself is subject to radioactive decay. Using our assumption that the rate of precipitation of calcium carbonate by the organism is constant with time during its life, the average $^{228}Th/^{228}Ra$ activity ratio of the whole shell will be a unique indicator of its age at time of capture. The relevant equation is:

$$\frac{A_T}{A_R} = \frac{\lambda_T}{\lambda_T - \lambda_R}\left[1 - \frac{\lambda_R(e^{-\lambda_T t} - 1)}{\lambda_T(e^{-\lambda_R t} - 1)}\right]$$

where R and T refer to ^{228}Ra and ^{228}Th respectively and A values are activities.

Figure 1 shows the plot of $^{228}Th/^{228}Ra$ activity ratio for the whole shell as a function of age of organism assuming constant rate of calcium carbonate deposition. From Table 1 the measured ratio in our clam is 0.68 ± 0.18, where the stated error is the 1σ counting error. This ratio corresponds to an age of 6.5 yr. At the 1σ level the uncertainty is -3, $+5$ yr.

This means that the growth rate of the long axis of this clam is about 4 cm yr^{-1} and the mass growth rate per animal (both valves) is about 20 g $CaCO_2$ per yr. We compare this with the growth rate we reported for an Atlantic Ocean deep-sea clam, *Tindaria callistiformis*[2], which reaches a length of 8.4 mm in about 100 yr for an average growth rate of 0.0084 cm yr^{-1}: about 500 times slower. Evidently the opportunistic nature of the clams living on the peculiar food source at the thermal vent permits a more rapid growth than the general ambient deep ocean floor environment. The same sort of opportunism has been reported for the deep ocean wood-boring clam, *Xylophaga* sp. by Turner[9]. She reported that 2 cm thick wooden planks, inserted in the North Atlantic ocean floor at a depth of 1,800 m by submersible and subsequently recovered, were almost completely devoured in 100 d by these clams.

Growth rates of clams on the deep ocean floor then appear to be primarily a function of the rate of food supply.

If we accept an age of 6.5 yr for this clam then we can interpret the radiochemical data used in establishing the upper age limits discussed above.

The $\Delta^{14}C$ of the clam is about 10% lower than the expected value from Pacific Deep Water. This can be ascribed to dilution of the deep water with old carbon supplied from the vents. Corliss *et al.*[1] determined the slope of the dissolved inorganic carbon to heat (via temperature) at 25×10^{-9} mol cal^{-1}. A temperature of 12 °C for the water in which the clam grew corresponds to an increase of total inorganic carbon of 250 μmol kg^{-1} above the Pacific Deep Ocean value of about 2,400 μmol kg^{-1} (ref. 10) (at a temperature of 2 °C). This is an approximately 10% increase which matches the observed ^{14}C decrease, if the vented carbon were totally devoid of ^{14}C.

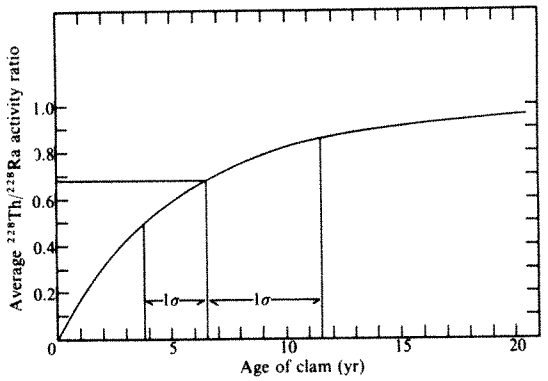


Fig. 1 The change in the average $^{228}Th/^{228}Ra$ activity ratio in a clam depositing a constant mass of calcium carbonate per unit time with a constant $^{228}Ra/Ca$ ratio and no initial ^{228}Th. A vesicomyid clam (Dive 713, Sample G3-2(L)) collected dead, but articulated, has an age at time of collection of 6.5 yr on the basis of this diagram.

Lead-210 appears to be sequestered by shallow water calcareous organisms more efficiently than radium by a factor of no more than two[7]. The $^{210}Pb/^{226}Ra$ ratio reported in Table 1 may then be assumed to be equal to or greater than the ratio in the ambient water in which the clam grew. As the shell is about 6.5 yr old, only a small amount of ^{210}Pb decay will have occurred, and the ratio provides a measure of the $^{210}Pb/^{226}Ra$ ratio in the water in which the clam grew. The deficiency of ^{210}Pb over that expected from the production from dissolved ^{222}Rn then provides a measure of the efficiency of the chemical removal process for ^{210}Pb from seawater in the region of the clam's habitat. The following material balance calculation can be made assuming a first order law for chemical removal as well as radioactive decay: Activity of ^{222}Rn (A_{Rn}) = Production rate of ^{210}Pb = Rate of radioactive decay of $^{210}Pb(A_{Pb})$ + Rate of chemical removal of ^{210}Pb. Therefore:

$$A_{Rn} = A_{Pb}\left(1 + \frac{\tau_{Pb}}{\tau_c}\right)$$

where τ_{Pb} (32 yr) and τ_c are the mean life of ^{210}Pb and the chemical removal residence time respectively. Referring to our calculation above involving ^{226}Ra and ^{210}Pb and using the same normalisation we obtain, as an upper limit, assuming equal fractionation of ^{210}Pb and ^{226}Ra:

$$\tau_c = \frac{32}{26 - 1} = 1.3 \quad \text{yr}$$

This implies that scavenging of ^{210}Pb is proceeding efficiently at least in the rock–water interface where the clam is growing. Corliss *et al.*[1] have already indicated how effectively seawater is stripped of Cu, Zn, Cd, Ni and U as the result of precipitation by H_2S within the "plumbing" of the thermal vent even before massive dilution with seawater occurs. Our results indicate that scavenging of ^{210}Pb, and possibly certain other elements that behave the same chemically, occurs even in the mixing zone between the hot water coming through the complex plumbing in the basalt and the ambient cold seawater of the ocean bottom. This is probably in the major part due to precipitation of ferromanganese oxides.

The initial $^{228}Ra/^{226}Ra$ value (0.057) in the clam is of the same order of magnitude as the ratio found in bottom water at GEOSECS station 263 from the South Pacific[11]. This concordance, however, may be largely fortuitous. If half of the radium in the bottom-most water in which the clam grew were derived from the leaching of basaltic rocks, the $^{228}Ra/^{226}Ra$ activity ratio of the bottom water would be about 0.34 which is markedly higher than the predicted value from the shell. In order to attain the measured value the length of time necessary to transfer the radium from its source to the surface would have to be 15 yr to

allow sufficient decay time for ^{228}Ra. On the other hand the high radon standing crop (6,000 d.p.m. per 100 kg) implies derivation from a secondary deposit of ^{226}Ra near the water–rock boundary. We cannot therefore identify the exact sources of the two radium isotopes based on the shell analysis.

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Eucalyptus fruits in stratigraphic context in Australia

THE eucalypts are the outstanding group of Leptospermeae (subfamily Leptospermoideae of Myrtaceae) and of the whole Australian flora[1]. There are over 500 species of eucalypts, nearly all restricted to Australia. The origin of this remarkably diverse and successful genus has been a matter for speculation. The comparitively well known Tertiary palynological record is of little assistance in answering these questions, as spores and pollen have insufficient properties to identify the genus. Hence only the macrofloral record is taxonomically significant. Although Tertiary macrofossils[4] are abundant, the fossil record of *Eucalyptus* and even Leptospermoideae is poorly known[2,3]. The first unequivocal fossil gumnuts (*Eucalyptus* fruits), of indefinite age, were identified only recently from silicified floras near Woomera[5], in South Australia's present arid zone. These specimens were collected without reference to the stratigraphy. However, we have now collected specimens from similar silicified outcrops near Stuart Creek (Fig. 1), where their probable age may be deduced by correlation of the sediments with Tertiary sequences elsewhere in South Australia. *Eucalyptus* can now be placed in stratigraphic context.

Although Tertiary stratigraphy in South Australia is in a state of flux[7–12], we have been able to restrict the age range of the fossils discussed here (Fig. 2) to two possibilities within the Tertiary. The alternative correlations presented here permit an Eocene–Oligocene age on the one hand, and Miocene age on the other. All that could have been said previously was that the gumnuts were Mesozoic or younger.

We investigated the site in October 1978, and Fig. 2 shows a selection of gumnuts from the fossiliferous sediments. Other fruits and leaves present in abundance will be described elsewhere. The flora was collected from a sandstone-conglomerate sequence (Fig. 3, section 1) which disconformably overlies Aptian–Albian shales[6]. The basal conglomerate has clasts of quartzite, kaolinised shale, cream and grey cryptocrystalline silcrete and polished chert, jasper and quartz. Overlying this unit are cross-bedded, mature fine- to medium-grained sandstones. On a nearby ridge (Fig. 3, section 2), similar but unfossiliferous siltstones, sandstones and conglomerates disconformably overlie Cretaceous beds; the upper units here are representative of Tertiary sandstones north and south of Stuart Creek. The plant fossils are in a different facies, but the two sections are thought to belong to the same sedimentary cycle.

The Tertiary of South Australia in the Lake Eyre and Pirie-Torrens Basins, abutting the northeastern and southeastern margins of the area under discussion, has usually been divided into two formations of characteristically distinct lithology. Extensive sheets of coarse, mature sands and silts (Eyre Formation[7]), determined palynologically as Palaeocene to Eocene, are disconformably overlain by the rather immature fine sediments

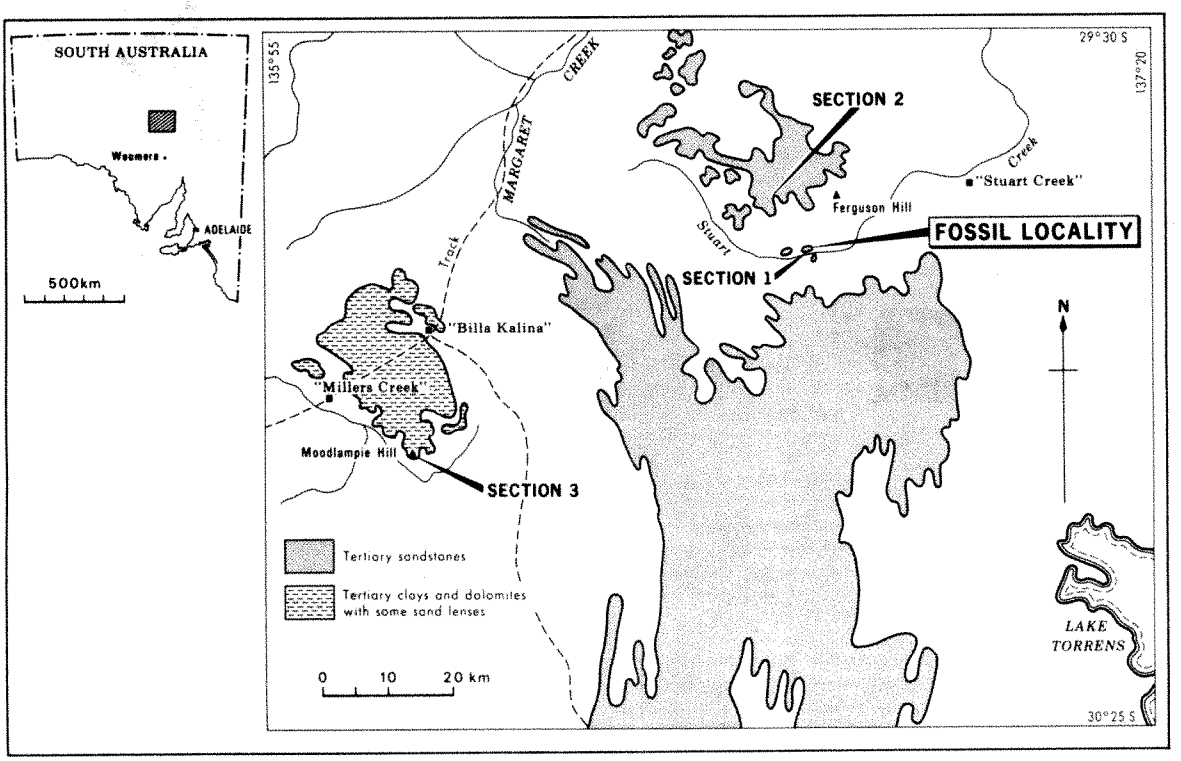


Fig. 1 Simplified Tertiary geology of the Billa Kalina homestead area, showing positions of stratigraphic sections shown in Fig. 3.

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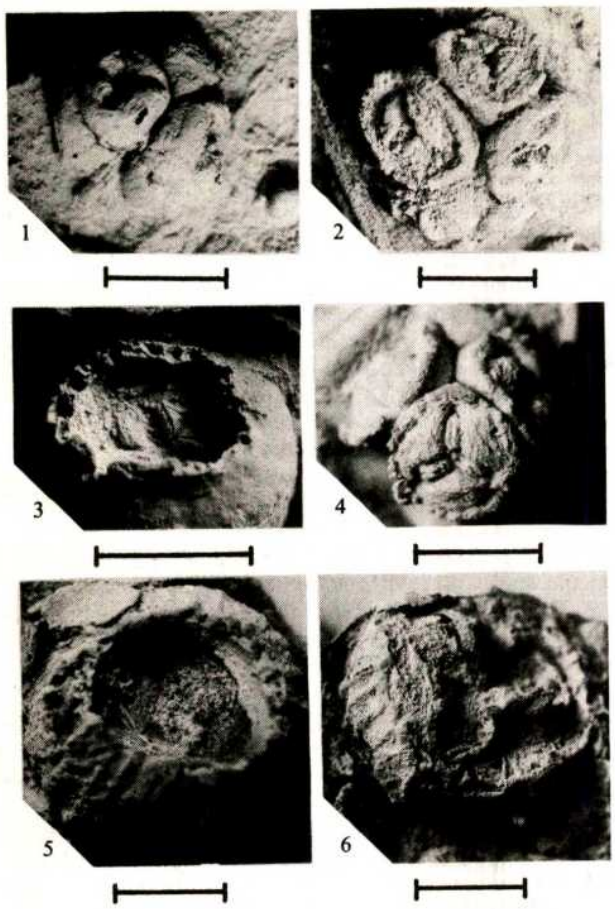


Fig. 2 Gumnut fossils from the Stuart Creek area. Scales represent 5 mm. Specimen 1: inflorescence of seven eucalypt-like capsules nearly sessile on a peduncle, each wedged against its neighbour but free at the summit which is flattened and exhibits three-valved loculicidal dehiscence. Specimens 2, 4: similar but another species, with raised rim, vertical disk and convex capsule dehiscent by three valves between which seed and chaff plugs sometimes remain. Specimens 3, 5: larger solitary fruits consistent with those of eucalypts, of maximum diameters much exceeding orifice diameter; with narrow raised rims, deep vertical disks and sunken capsules. One displays initial traces of three-valved dehiscence. Specimen 6: a ribbed eucalyptoid capsule with a flat summit and wide-gape three-valved dehiscence, the broken valve-tips apparently exerted.

of the almost equally extensive Medial Miocene, characterised by green clays and dolomites (Etadunna Formation[9]). The Medial Miocene age was also determined palynologically[7,10], from the basal Etadunna Formation and equivalents. Its upper strata contain vertebrates which are unlikely to be younger than Late Miocene (R. H. Tedford, personal communication 1973) so the unit is essentially Miocene.

The gumnut-bearing conglomerate contains rounded and sorted cryptocrystalline silcrete (siliceous duricrust) pebbles. Despite much mapping and drilling of Tertiary strata throughout South Australia and adjacent regions, no pre-Eocene silcrete of this type is known. Thus Tertiary sediments containing silcrete pebbles are probably Eocene or younger. However, the fossiliferous sediments are lithologically similar to the Eyre Formation in the adjacent basins. They could therefore represent the upper part of the Eyre Formation deposited contemporaneously with silicification, or a separate phase of sedimentation partly synchronous with or younger than this

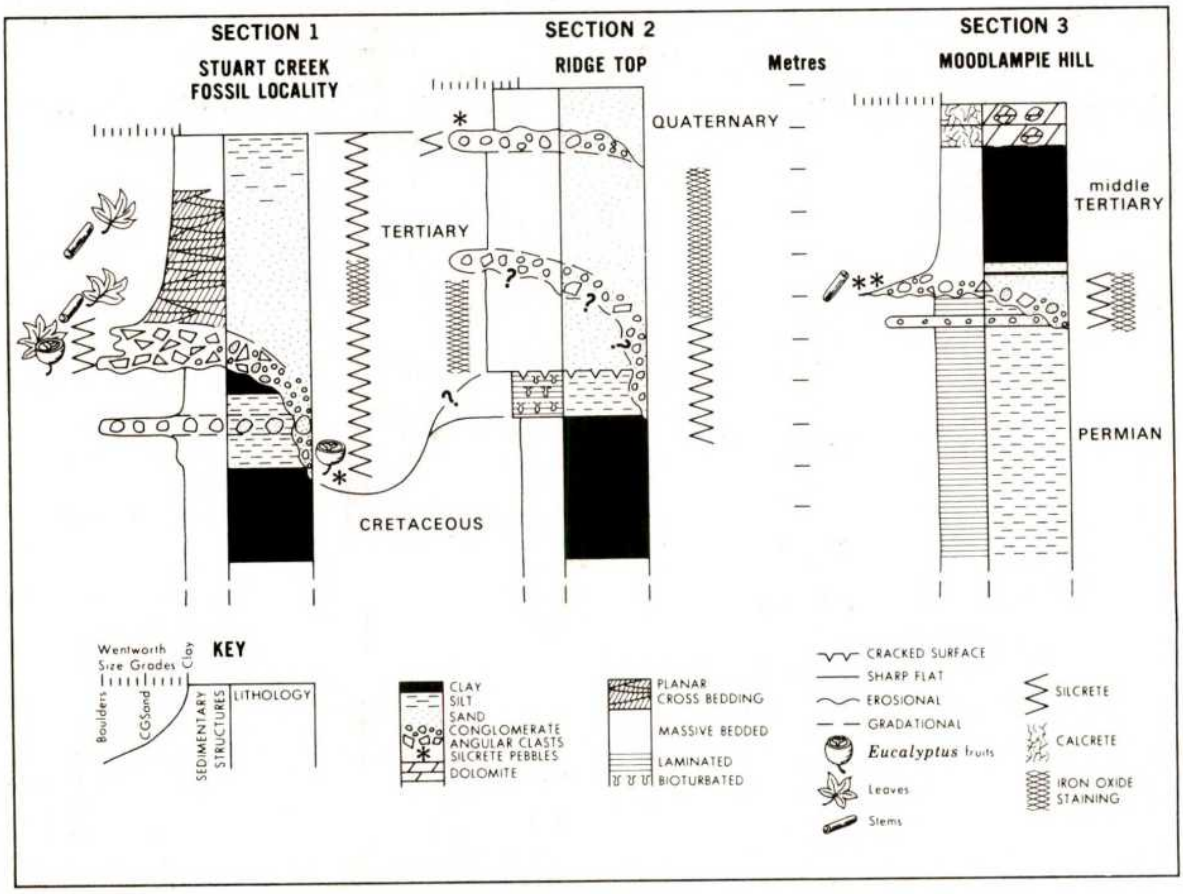


Fig. 3 Key stratigraphic sections. Tertiary sequences are disconformable on Cretaceous and Permian sediments and overlain by Pleistocene sand dunes in section 2.

Formation (of Eocene–Oligocene age) following a major interval of duricrust development.

On the other hand, at Moodlampie Hill (Fig. 1), conglomerate and sandstone intertongue with green clay and carbonate (Fig. 3, section 3) which we correlate with the Etadunna Formation of the Lake Eyre Basin. The conglomerate and sandstone, however, are similar to that at Stuart Creek, containing silcrete pebbles. A stratigraphic analysis of Tertiary sediments in the Billa Kalina area indicates that sediments at Moodlampie Hill and Stuart Creek may be facies equivalents (G.J.A. and R.B.F., in preparation). On this basis the *Eucalyptus*-bearing sediments would be Miocene. This concept presents problems in terms of currently accepted Tertiary stratigraphy[7,9,10], but is the simplest explanation in local terms. It opens the possibility of a late Tertiary age for at least part of similar extensive sandstone sheets elsewhere in the state, at present regarded as Eyre Formation.

Australian Palaeogene vegetations represented in southern and eastern near-coastal macro-floras were like those found today in equatorial and tropical wet regions, apparently being devoid of Leptospermeae[14,15]. Such conditions are supported by evidence from marine planktonic organisms, palynological data, and palaeotemperature measurements[16–19]. Kemp[16] has shown that in the Australian flora (excluding *Eucalyptus* which is not mentioned) there is no evidence of pronounced cooling until the Oligocene, and none of open vegetation reflecting seasonal aridity until the Miocene. Climatic zonation is apparently absent for the Eocene, but floral data suitable for reconstructing climatic zones are only available for this epoch, and even then derive mainly from southern Australia and the present coastal regions. By analogy with Quaternary climatic events one expects dry conditions and a zonal climatic distribution to have coincided with the periods of pronounced cooling in the late Eocene–Oligocene, and late Miocene. Hence xeromorphic vegetation and *Eucalyptus* in particular is unlikely to have radiated until at least the very late Eocene times. Xeromorphy, however, may have originated earlier, and there is some evidence for xeromorphy and cooling in the middle Eocene[16]. This is in keeping with the alternative ages suggested for the flora. In this context, the diversity of species in the silcrete floras will also be significant.

The gumnuts should have been readily preserved in the abundant coastal early Tertiary macrofloras, had they been even a minor component of the source litter. If they are Miocene or Oligocene, their absence from these Palaeocene–Eocene floras is readily explained, and their age is in accord with climatic interpretations[10]. If they are early to middle Eocene, then in Central Australia conditions must already have deteriorated from absolute mesicity, permitting upsurge of xeromorphic Leptospermeae so as to displace more mesic vegetation coastwards. This may imply drier conditions, suggesting rifting from Antarctica was sufficient to produce climatic changes influencing Australia, before the major cooling episode of the late Eocene to Oligocene.

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Long-term recognition of father's song by female zebra finches

RECENTLY, attention has been directed at the question of how sexual imprinting, or the development of mating preferences, affects assortative mating within polymorphic species[1–3] and regulates the extent of inbreeding and outbreeding in a population[4–6]. It may be selectively advantageous for a young organism to learn individual characteristics of parents and/or siblings to avoid subsequent mating with kin as well as to avoid hybridisation by not mating with individuals that differ greatly from rearing partners[4–6]. I report here that adult female zebra finches (*Taeniopygia guttata*) can recognise the song of their own father after a period of early exposure followed by more than 2 months of separation while attaining sexual maturity.

The zebra finch is an Australian grassfinch (family Estrildidae), the young of which are totally dependent on parental care on hatching. Hatchlings are fed by both parents and do not leave the nest until about the third week[7]. Between days 30 and 40, the young leave (or are driven off by) the parents and form mixed flocks with other young finches. Sexual maturity is reached by 90 d (ref. 8). Thus, in early development (that is, before 40 d of age), females are exposed to the song of their father, and thereafter to the developing songs of male siblings that typically bear some resemblance to the father's song[9,10]. Adult male zebra finch song is highly variable between individuals in acoustic structure but relatively stable within an individual, thus providing a possible basis for individual recognition[11].

Females were hatched in rooms containing 12–21 breeding cages (82.0 cm long, 39.0 cm high, 30.0 cm deep) with one adult zebra finch pair per cage. (No other species were housed in these rooms.) At 35 ± 3 d after hatching, females were separated from their parents and housed in a soundproof room with other young female zebra finches in $82.0 \times 39.0 \times 30.0$-cm cages, each containing three females. At day 100 ± 3, each female was given a single 30-min simultaneous auditory choice test involving the song of her father and either the song of another adult male zebra finch that differed considerably from her father's song in acoustic structure, or the song of another adult male zebra finch that was similar to her father's song in acoustic structure. All male zebra finch songs used in the playback tests were recorded when the male was singing to his mate. No sounds other than the male's song were on the test tapes. Examples of the songs are shown in Fig. 1. The purpose of the first type of test (father's song compared with dissimilar song) was to assess the general capability of a female to recognise and show a preference for her father's song over that of a randomly chosen conspecific male that was not a close relative. The second type of test (father's song compared with similar song) assessed the specificity with which a female could distinguish her father's song from one that

Table 1 Choices of female zebra finches in simultaneous auditory choice tests

Test	*n*	Percentage responding	No. showing preference	Latency (s) Mean s.d.	Duration (s) Mean s.d.
Father's song compared with dissimilar song	31	77	17 $P<0.05$ 7	527 ± 616 $P<0.003$ 1,242 ± 768	1,069 ± 746 $P<0.001$ 401 ± 661
Father's song compared with similar song	21	86	9 NS 4	637 ± 661 NS 865 ± 806	942 ± 696 $P<0.02$ 503 ± 600

Statistical comparisons of preference scores were assessed using the χ^2 one-sample test (d.f. = 1), and latency and duration scores were assessed using the Mann–Whitney *U* test. All probability values are two-tailed.
NS, Not significant.

was similar. (Because song structure can differ considerably among individual males, a song similar to that of the father could be obtained only by recording either the female's uncle and/or male siblings, as related males tend to have similar songs due to early vocal learning[9,10].) Each female was tested only once in either the first or second test.

The rectangular test apparatus, housed in an anechoic chamber, was divided into two approach zones at opposite ends separated by a neutral zone, each zone being 70.5 cm long. Two loudspeakers were located outside the apparatus adjacent to each approach zone. Behavioural measures recorded were (1) latency of approach from a centrally located start box in the neutral zone to each approach zone, and (2) total time spent in each approach zone. A preference was recorded when a bird spent twice as much time in one approach zone as in the other.

The results are shown in Table 1. Female zebra finches recognised and significantly preferred the song of their own father to the dissimilar song of another male zebra finch. When confronted with the perceptually more difficult test of the father's song compared with a similar song, females still recognised their father's song. Although the tendency of females actually to prefer their father's song in the latter test was not statistically significant, those females that did prefer their own father's song had significantly longer duration scores than those preferring the similar song.

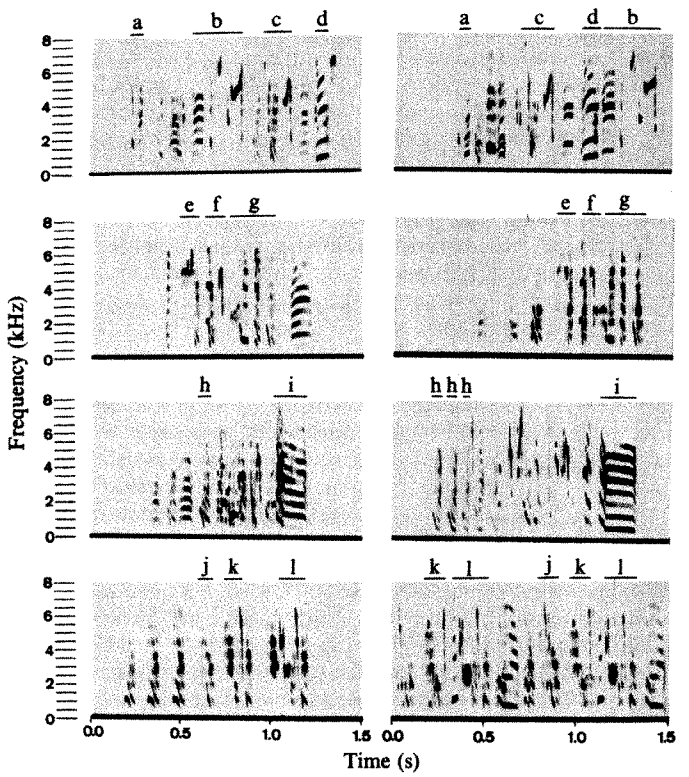


Fig. 1 Wide-band sound spectrograms of song phrases from eight different adult male zebra finches. Each pair (row) of sound spectrograms depicts two songs that are similar to one another and were uttered by two males that were closely related (either brothers or father and son). The common elements in these phrases are depicted above each spectrogram with a bar and a letter. The four spectrograms going down each of the columns are dissimilar from one another in acoustic structure and were uttered by unrelated males.

Thus, female zebra finches can recognise their father's song as a function of early exposure followed by a relatively long period of isolation from male song. These tests of approach behaviour do not necessarily reflect sexual motivation on the part of the female and therefore do not represent a demonstration of sexual imprinting as such. Rather, they demonstrate the extent to which females can recognise and discriminate their father's song from that of another conspecific male. Indeed, as females presumably do not mate with their fathers in nature, I would have predicted the outcome of these tests to be quite different had they been assessing sexual behaviour. Nevertheless, this study may have important ramifications regarding the study of sexual imprinting in general. The learning of both supra-individual (species) and individual characteristics during early exposure may, at the time of sexual maturity, influence an animal to (1) mate with its own species—a direct effect of learning supra-individual features—and at the same time (2) avoid mating with close kin—a direct effect of individual recognition. However, many studies[12–16] have shown that, in several species, individuals can identify conspecifics in advance of exposure to them and that embryonic and postnatal self-stimulation probably plays an important part in these and other instances of 'instinctive' preferences for species-typical stimuli[17,18]. Thus, it is conceivable that the role of imprinting in mate selection in nature may be of greater relevance to the learning of individual rather than supra-individual characteristics.

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Vertebrate and invertebrate predation intensity on freshwater zooplankton communities

PREDATION is widespread in the pelagic zone of lakes and both vertebrate and invertebrate predators are abundant and diverse in their feeding habits. Vertebrate predators are often size-selective and can virtually eliminate larger prey items[1–3]. These dramatic effects resulting from fish predation have led several workers to conclude that vertebrates contribute the largest percentage of the total predation intensity on freshwater zooplankton. Particular invertebrate predators, however, have also been shown to crop a substantial proportion of their prey populations[4–6]. I report here a study showing that invertebrate predation is more intense than vertebrate predation in the pelagic zone of Gull Lake, Michigan, with the first *in situ* evaluation of both types of predation on a single zooplankton prey assemblage. The significance of identifying major predation pathways in lakes is basic to many contemporary discussions of nutrient and energy flow as well as considerations of community structure and stability.

Gull Lake is a marl lake in southwestern Michigan which has undergone excessive nutrient enrichment[7–9]. The lake is 8.2 km^2 in area and 33 m deep. Following the development of a hypolimnetic oxygen deficit in 1966, there have been large changes in the zooplankton community. The present dominant species are *Bosmina longirostris, Cyclops bicuspidatus* and *Tropocyclops prasinus*. There are six species of *Daphnia* and moderate populations of *Diaptomus oregonensis* and *D. minutus*. Large copepods such as *Mesocyclops edax* and *Epischura lacustris* and one predaceous cladoceran (*Leptodora kindtii*) are present but rare. *Chaoborus* spp. are immature dipterans that live in the bottom sediments.

There are over 70 species of fish in Gull Lake; however, most of these are inshore or rare species. In 1952, smelt (*Osmerus mordax*) were introduced into the lake. By 1966, this species had become the dominant fish in the pelagic zone and it now represents 95–100% of all fishes taken with vertical gill nets in the central basin. This species schools by age class and is a stenotherm[10]. As eutrophication has progressed, smelt have become stressed, as their requirements for cold water and high oxygen concentration preclude their use of much of the anoxic hypolimnion and the warm epilimnion in summer. Immature fish are sometimes found in the upper few metres of Gull Lake but their appearances are highly seasonal. The population and predation dynamics of juvenile *Lepomis machrochirus* and *Labidesthes siccules* have also been studied[11].

Predation rates of fish were determined by multiplying densities of fish by the mean number of prey consumed per predator[12]. Densities of fish collected in 1973 are given in Table 1. Each fish was measured (length and weight), its age and sex determined and its stomach removed. Per cent stomach fullness was also recorded and all prey individuals were enumerated. To convert stomach content data to predation rates, values of stomach evacuation time and diel feeding periodicity were also determined (Fig. 1). While attempts to culture smelt were partially successful, it was not possible to obtain reliable feeding information in the laboratory and all results presented here are derived from field data.

Smelt have a definite seasonal pattern in their predation dynamics. In June, when the hypolimnion is well aerated, the fish consume mostly *Chaoborus* spp. larvae and some immature forms of *Tendipes* spp. and *Penteneura* spp. near the bottom. There is no evidence to suggest that smelt feed on the bottom of the lake in any season. In July, the fish are found higher in the hypolimnion and their feeding habits reflect their distribution patterns. Approximately 60% of the fish feed on *Daphnia* spp. and the rest consume insect larvae. In August, smelt consume more *Daphnia* spp. than insects. Generally, few copepods are eaten except in August. Burbidge found the same seasonal feeding pattern in 1965–66, except the feeding shift from insect larvae to crustacean zooplankton occurred later in the season[10]. His study included the year preceding the occurrence of the first oxygen deficit in the hypolimnion.

The determinations of *in situ* invertebrate predation were obtained using the fluorescence technique which is described in detail elsewhere[13,14]. Prey are stained with a fluorescent dye (Acridine Orange or Rhodamine B), placed in a grazing chamber and lowered into the lake. The chamber captures 9.8 litres of lake water including a natural assemblage of prey and predators. Simultaneously, the stained prey are released. Predation continues for 1 h, then all animals in the chamber are recovered and scored under a fluorescence microscope. A predator of a stained prey exhibits fluorescent appendages and/or gut tube. Invertebrate predators except *Chaoborus* spp. do not usually consume their prey whole; therefore, it is impossible to ascertain how much of a given prey was consumed, but many partially ingested prey are presumably eliminated from the lake. Mean predation rates, calculated as number of prey partially ingested per predator per time period, were determined in the summers of 1973 and 1974 and densities in 1972, 1973 and 1974.

The major cause of death for the Gull Lake zooplankton community (except insect larvae) is invertebrate predation

Table 1 Smelt densities (animals per m^3) for each sampling period, calculated from vertical gill net data

Depth (m)	SS	MT	SR	N
16 June 1973				
1–5	*	*	*	*
6–10	*	2	0.8	*
11–15	0.4	0.4	1.2	*
16–20	*	*	1	*
21–25	0.2	0.6	*	*
26–30	1	0.2	1	0.8
15 July 1973				
1–5	* *	*	*	*
6–10	0.2	1.8	0.2	*
11–15	0.8	4.4	0.4	*
16–20	0.8	8.0	2.2	*
21–25	*	2.8	0.8	*
26–30	0.2	*	4.2	*
15 August 1973				
1–5	*	*	0.2	*
6–10	1.4	0.6	0.2	*
11–15	0.2	2.2	0.4	*
16–20	1.4	0.2	*	*
21–25	*	0.2	*	*
26–30	*	*	*	*

SS, sunset; MT, midnight; SR, sunrise; N, noon. *, No fish captured. The gill nets extended the total depth of the central basin. Several mesh sizes were used; however, only the 1.3-cm mesh captured substantial quantities of fish and only these results are reported. The nets were sampled at four time periods during each diel period. In June, the fish were mostly in the 6–15-m range with some individuals near the bottom of the lake. In July, as the surface water warmed, the fish were usually located at 11–20 m with an indication of downward movement at sunrise. In August, there was a notable lack of smelt in the hypolimnion during the period of oxygen depletion. For all dates, few fish were collected at noon. Sonar records have confirmed that fish are absent in the daytime when they probably migrate inshore[10].

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Table 2 Comparison of % prey cropped by major vertebrate predator (*Osmerus mordax*) and small invertebrate predator (*Cyclops bicuspidatus*)

Prey	Depth (m)	June Density	June *Cyclops* (% cropped)	June Smelt (% cropped)	July Density	July *Cyclops* (% cropped)	July Smelt (% cropped)	August Density	August *Cyclops* (% cropped)	August Smelt (% cropped)
Daphnia	5	5,040	33	<0.1	10,200	22	1	31,100	6	0.3
	20	6,300	69	NC	16,800	8	11	7,250	11	<0.1
Cyclops	5	4,480	1	<0.1	25,200	5	NC	3,080	5	<0.1
	20	2,620	1	NC	15,700	5	<0.1	3,560	5	<0.1
Leptodora	5	10	*	NC	38	*	NC	8	*	63
	20	12	*	NC	38	*	2	14	*	100
Chaoborus	5	11	*	100	25	*	100	13	*	100
	20	14	*	100	7	*	100	15	*	100

Smelt as a population is less effective in cropping zooplankton prey than is *C. bicuspidatus*. Only night values and one invertebrate predator are included to enhance comparability. In addition, only prey species found in smelt stomachs are included. *C. bicuspidatus* consumes several other prey species and has a greater predation impact on the zooplankton community than indicated here[13]. Predation rates for *C. bicuspidatus* were averaged from several experiments in the field and laboratory and then multiplied by *in situ* predator–prey densities to determine number of prey consumed. This value was divided by *in situ* density to estimate % prey cropped, at the appropriate season, depth and time. Although *C. bicuspidatus* cannibalism occurred in all field experiments, there were not enough experiments with this species used as prey, thus, predation rates were difficult to calculate and % prey cropped values are rough estimates. *C. bicuspidatus* does not prey effectively on *Leptodora* and *Chaoborus* and these interactions were treated as not occurring. (*) NC, not consumed in this time–depth period. Densities of zooplankton in the water column were determined from samples by using water bottles or by making horizontal tows with a Clarke–Bumpus zooplankton sampler[7] and are given as organisms per m^3.

(Table 2). *Cyclops bicuspidatus* is an especially effective predator and, given its high densities, can substantially crop the rest of the community. This species possesses several adaptations that contribute to its efficiency[13]. First, the predator does not use vision to locate its prey. It feeds mostly by mechano- or chemoreception and perhaps selects prey more for their availability than for any other factor. Thus, this predator exhibits an effective density-dependent regulation on the other species. Second, it is highly cannibalistic at high densities, producing short self-damping loops in the ecological network (foodweb) which are stabilising to the ecosystem as well as producing effective regulation of its own population[13]. Third, several members of this species often consume a single prey simultaneously[13]. This group behaviour increases its predation efficiency and also has important ramifications for calculations of nutrient and energy flow. Fourth, *C. bicuspidatus* often nibbles on its prey and does not consume them completely, which makes accurate estimates of energy and nutrient flow difficult. With the fluorescence technique, the percentage of a prey individual consumed cannot be calculated as only the presence–absence and not amount of fluorescence is quantified[13].

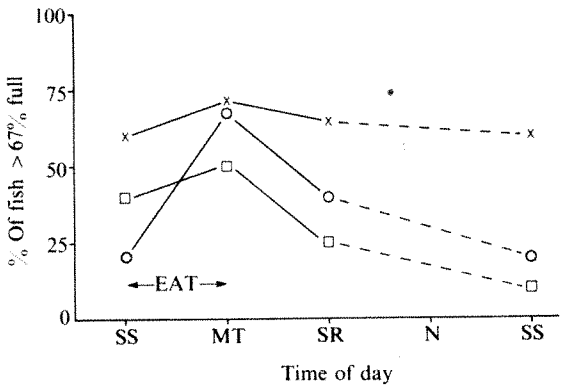


Fig. 1 Feeding periodicity based on stomach fullness shows that most smelt were full at midnight. From sunset to midnight, percentage of fish with stomachs at least 67% full increased markedly and then decreased until dawn to levels similar to those at sunset. Thus, smelt consume most of their prey from sunset to midnight. As fish are relatively empty at sunset and they apparently do not feed in the daytime, most of their feeding is concentrated in a 6-h period after sunset. Hourly stomach evacuation rates were calculated by subtracting the percentage of fish that were at least 67% full at dawn from those 67% full at midnight and dividing this value by six. For all dates (including several not shown) a mean value of 4.3% of the stomach contents was evacuated per hour or in a 6-h period ~25% of the stomach contents were evacuated.

Smelt consume a broad range of prey species and sizes and do not feed as a stereotyped size-selective fish as is suggested by contemporary dogma[15]. Although they may decrease populations of hypolimnetic zooplankton such as *Daphnia pulex* and *Chaoborus* spp., smelt probably have little effect on the myriad of interactions among most zooplankton species[13,16]. The net effect of smelt on several prey species may be positive as the smelt's high predation rate on insect larvae such as *Chaoborus* spp. (which are voracious zooplankton predators) may indirectly benefit the other prey more than the fish's direct predation harms them. *Chaoborus* spp. populations had decreased in 1973 to 70% of their 1966 densities. These animals are meroplanktonic and they find refuge against smelt predation in the bottom sediments. For Gull Lake, and undoubtedly many other lakes, invertebrate predators constitute the main predation pressure on a wide variety of zooplanktonic prey species. Because there have been so many technical problems in assessing invertebrate predation in the field before the development of the fluorescence technique, many workers have tended to over-emphasise the relative importance of fish predation[1,3,15]. Fish are definitely more effective predators on a per individual basis but invertebrate predators are disproportionately more abundant than fish. Thus, on a per population basis, invertebrate predators constitute the greatest danger to most zooplankton prey.

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Autoradiographic localisation of benzodiazepine receptors in the brains of humans and animals

THE benzodiazepine (BZ) drugs are used as anxiolytics, hypnotics, anticonvulsants and muscle relaxants. They are the most widely prescribed of all psychotropic drugs and seem to exert their action by binding to a specific receptor site on brain membranes[1,2]. This receptor appears to interact with neurotransmission mediated by γ-aminobutyric acid (GABA)[3–8]. Biochemical studies of BZ receptor distributions in brain reveal large regional variations[9,10]. An increase in resolution of localisation of BZ receptors would provide greater insight into mechanisms of actions of BZs. In this study, we report for the first time the light microscopic autoradiographic localisation of BZ receptors in mammalian central nervous system (CNS) and, to our knowledge, the first receptor autoradiography in human brain tissue. We observed striking variations in receptor densities in different areas which, in some cases, were species dependent.

The recently developed overall autoradiographic method[11] is based on *in vitro* labelling of receptors, since *in vivo* labelling results in low ratios of specific to nonspecific binding[12,13] and because it is desirable to use human postmortem tissues. Previously, *in vitro* labelling of receptors in brain sections and subsequent coating by nuclear emulsion for autoradiography has been feasible with irreversibly, or nearly irreversibly, binding ligands[14,15]. However, this approach has been unsatisfactory for most or all diffusible ligands[11,16]. Our method largely circumvents this problem by apposing a dry layer of emulsion against labelled tissue.

Briefly, brain tissues are placed on brass chucks and frozen in liquid nitrogen. Sections (4–6 μm) are cut in a cryostat microtome and thaw-mounted onto gelatin-coated slides. The slides are then dipped into 0.17 M Tris-HCl (pH 7.4 at 4 °C) containing 1 nM ^{3}H-flunitrazepam (87 Ci mmol^{-1}, NEN) to label the receptors. Some solutions contain 1 μM clonazepam to produce sections with nonspecific binding only. The incubations are performed at 4 °C for 40 min at which time the slide-mounted sections are washed for 2 min in buffer alone to reduce nonspecific binding. Routinely, we observed ratios of specific to nonspecific binding of at least 20 : 1 in preliminary biochemical studies. The binding was saturable with a K_D of 2.5 nM and was blocked only by other BZ drugs. The slides are then rapidly dried (for approximately 30 s) under a stream of cold air and flexible, emulsion (Kodak NTB3 emulsion, 1 : 1 with H_2O) coated coverslips are attached in the dark by glue to the slide at the end away from the tissue sections. After exposure for 2 weeks at 4 °C, the coverslips are gently bent away from the tissue sections, the latent autoradiograms developed, and the tissues fixed and stained with pyronine Y as previously described[11]. The assemblies are reapposed and then examined under the microscope.

Male Sprague–Dawley rats (180–250 g) and mice (20–50 g) (Jackson Laboratories) were used in animal studies. Human postmortem tissue samples were obtained at autopsy from a 50-y-old male (3 h post mortem; sudden death with no drug history) and a 22-y-old male (4 h post mortem; stab wound with no recent drug history) in cooperation with the Medical Examiners Office in Baltimore, Maryland.

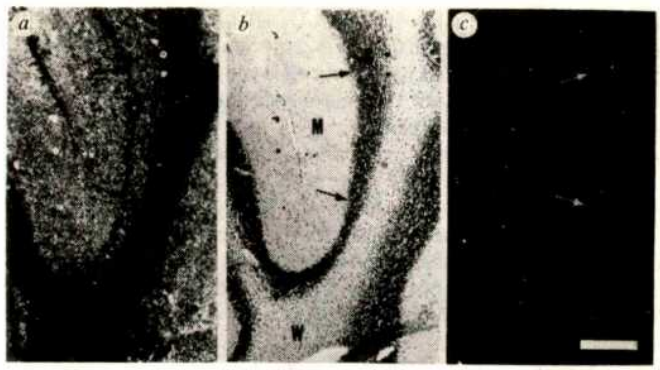


Fig. 1 Benzodiazepine receptors in human cerebellum. The darkfield photomicrograph in *a* shows the autoradiographic grain distribution over the tissue in *b*. Note the high density of receptors in the molecular layer (M) and in the granule cell layer (G), the reduced density in the Purkinje cell layer (indicated by arrows), and the absence of receptors in the white matter (W). The darkfield photomicrograph in *c* shows the blockade of receptor binding in an adjacent 4 μm section (brightfield not shown) due to the addition of 1 μM clonazepam. The conditions for labelling the receptors are outlined in the text. Sections were incubated with 1 nM ^{3}H-flunitrazepam in Tris-HCl (*p*H 7.4, 0.17 M) for 40 min at 4 °C, and washed for 2 min at 4 °C in the same buffer. The emulsion was exposed for 2 weeks at 2–4 °C. In tissues from two humans, actual grain densities (grains per 1000 μm^2 ± s.d., $n = 6$) for the cerebellar layers were as follows: molecular, 73 ± 9 and 79 ± 3; Purkinje cell, 67 ± 8 and 60 ± 18; granule cell, 77 ± 3 and 73 ± 8; and white matter 13 ± 7 and 8 ± 3. The corresponding values for rat cerebellum were 84 ± 17, 53 ± 5, 35 ± 4, and 6 ± 1. Grain densities in the presence of 1 μM clonazepam were 5–11 grains per 1,000 μm^2. The grain densities were counted in at least two preparations in six areas using a calibrated eyepiece grid. Scale bar, 500 μm

In human tissues, we examined the autoradiographic distribution of BZ receptors in cerebellar and cerebral cortical areas including the superior frontal, the precentral and the calcarine areas of the cortex. In all of these areas there was a striking absence of BZ receptors in the subcortical white matter. In the cerebellum, there was a high density of receptors in association with both the granule cell layer and the molecular layer. There was a slight reduction in receptor density in the Purkinje cell layer (Fig. 1; see also Table 1). In the cerebral cortical areas, there were variations in autoradiographic grain densities. The receptor density in the calcarine cortex, for example, was highest in Brodmann[17] layers III, IVA and IVC (Fig. 2, Table 1). This striking variation emphasises the value of this technique in the study of the neuroanatomical aspects of receptor localisation.

The absence of BZ receptors in white matter and the regional variations in receptor density were also observed in brain tissues from rats and mice. In the cerebellum there was a similar high density of BZ receptors in the molecular layer but, compared with the human tissues, there was a markedly reduced level of BZ receptors in the granule cell layer (see Table 1). In the rat and mouse cerebral cortex, there were also variations in the different cortical layers. In the rat, the autoradiographic grain densities in the caudate-putamen and septum were lower than in the cortex and fairly evenly distributed over these structures, although still of a fairly high density. In these same tissue sections we observed an elevation of BZ receptors in and near the islands of Calleja in the ventral forebrain (Table 1).

A striking regional variation in BZ receptors was observed in the rat cervical spinal cord. As in other areas, there were no receptors in the white matter areas, but high levels of receptors were found in laminae II, III, IV, and X while there was a much

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lower receptor density in lamina IX which contains the motor neurones. Finally, we observed BZ receptors highly localised to the inner plexiform and ganglion cell layers of the rat retina. The grains were also present to a lesser extent in the nerve fibre and inner nuclear layers (Table 1).

While one cannot rule out some glial localisation of receptors, the striking absence of BZ receptors in white matter and its restriction to grey matter areas are consistent with the notion that the receptors are localised to neurones. The striking variations of receptor densities within grey matter areas suggest that the binding site is not simply a common constituent of all neuronal membranes. Earlier suggestions that the receptors are localised to glial cells[19] are difficult to reconcile with the observed interactions with the neurotransmitter GABA[3-8] and recent reports that these receptors are reduced in *nervous* mice[19-22]. In addition, we did not observe binding of ^{3}H-flunitrazepam to blood vessels of any size, although the ependymal cell layer around the fourth ventricle showed significant binding of ^{3}H-flunitrazepam which was not displaced by 1 μM clonazepam and was not apparent with controls for positive chemography.

There have been many biochemical and electrophysiological studies showing an interaction of BZ receptors with GABA receptors[3-8]. Our autoradiographic localisations of GABA receptors have not revealed a simple correlation between BZ receptors and GABA receptors labelled with ^{3}H-muscimol in the cerebellum. We did not observe a high density of ^{3}H-flunitrazepam binding sites in and around the Purkinje cells as was observed previously with ^{3}H-muscimol[23]. In fact, our initial studies with ^{3}H-muscimol and ^{3}H-GABA in the rat reveal higher receptor levels in the granule cell layer than in the molecular layer (J. M. Palacios *et al.*, in preparation). When GABA (200 μM) was present during the labelling of the BZ receptor in the rat cerebellum, autoradiographic studies showed that the increases in ^{3}H-flunitrazepam binding occurred in the same proportion in all layers. Our observed high density of BZ receptors in the dorsal horn of the spinal cord does partially overlap with the distribution of glutamic acid decarboxylase (GAD) immunoreactivity in these areas[24]. These overall results are compatible with the observed interaction between BZ and GABA receptors but further suggest that BZ receptors are more widely distributed for additional functions and that only a subpopulation of GABA receptors interact with benzodiazepine receptors.

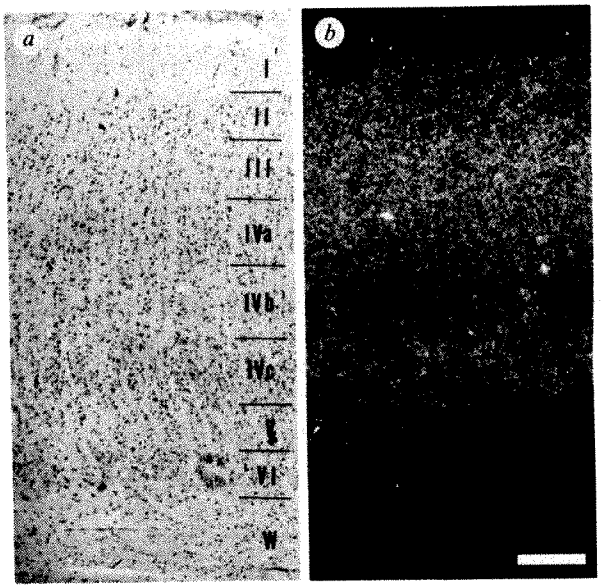


Fig. 2 Benzodiazepine receptors in human calcarine cortex. The darkfield photomicrograph in *b* shows the autoradiographic grain distribution over the tissue in *a* where the cortical layers (I–VI) and the subcortical white matter (W) are labelled. The receptor density varied in the different layers (for example elevated in Brodmann[17] layers III, IVA, and IVC) and was relatively negligible in the white matter. Adjacent sections labelled in the presence of 1 μM clonazepam showed a loss of the autoradiographic grains with a resultant grain density similar to that shown in *a* and *b* for white matter (not shown). Experimental details are given in the text and in the legend to Fig. 1. Scale bar, 250 μm.

Table 1 Relative density of benzodiazepine receptors in selected brain regions

Region	Relative grain density
Rat:	
Cerebellum	
Molecular layer	+ + +
Purkinje cell layer	+ +
Granule cell layer	+ +
White matter	0
Striatum	+
Cerebral cortex (frontal)	+ + to + + +
Corpus callosum	0
Islands of Calleja	+ + + to + + + +
Spinal cord	
Laminae I, VIII, IX	+ to + +
Laminae II, X	+ + +
Laminae III, IV, V, VII	+ + to + + +
Retina	
Inner plexiform layer	+
Ganglion cell layer	+
Human:	
Cerebellum	
Molecular layer	+ + +
Purkinje cell layer	+ +
Granule cell layer	+ + +
Calcarine cortex	
Layers I, II, IVb, V, VI	+ +
Layers III, IVa, IVc	+ + +

Tissues were processed and examined as described in text and Fig. 1.

The wide distribution of BZ receptors through the CNS contrasts with the much more restricted localisations of other receptors, such as the opiate receptors[25]. The cholinergic muscarinic receptors are similarly widespread but are more localised to telencephalic areas than brainstem areas[26]. Interestingly, high densities of muscarinic receptors were demonstrated in and around the islands of Calleja[26] which is similar to what we have observed in this study.

The species differences in receptor distribution in the cerebellum and possibly in other areas suggest that that extrapolation of results from pharmacological studies with animals to humans must be done with caution. Administration of benzodiazepines has striking clinical effects as mentioned above. While the widespread distribution of BZ receptors in the CNS suggests caution in correlating anatomy and drug action, it is tempting to associate anxiolytic action roughly with receptors in cortical areas[27] and muscle relaxant actions with spinal cord receptors.

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Central remyelination restores secure conduction

ONE of the outstanding characteristics of multiple sclerosis and other clinical and experimental diseases associated with demyelination, is the tendency to remit. Although there is good evidence that an important cause of functional loss in such conditions is conduction block produced by the selective destruction of the myelin sheath[1], the mechanisms underlying remission are unknown[2]. It has long been recognised that in certain experimental lesions demyelinated central axons can be remyelinated by the normal myelin-forming cell, the oligodendrocyte[3]. The new sheaths are, however, abnormally thin and short and it is not known whether they are capable of restoring conduction. A previous investigation was inconclusive[4]. Because of the physiological and clinical importance of this problem, we have now examined conduction serially through the lesion induced by the direct microinjection of lysophosphatidyl choline (LPC) into the cat spinal cord[5]. This lesion has the advantage that the phases of active demyelination and remyelination are well separated, thus permitting a reasonable correlation of the morphological with the physiological changes. We have found that secure conduction is restored with remyelination.

Pairs of platinum or stainless steel stimulating electrodes were permanently implanted at two extra-dural sites over the dorsal columns (usually L1 and L3) in five cats. Recordings were made from the saphenous nerves with stainless steel needles which were accurately repositioned at each session with the aid of tattoo marks. For the recording sessions the cats were anaesthetised with Saffan (Glaxo), paralysed with gallamine triethiodide, intubated and artificially respired to maintain the end-tidal CO_2 level at 4%. Rectal temperature was maintained at 37 ± 0.5 °C. Differential recordings were made with unity-gain cathode followers and Devices pre-amplifiers. The stimulus thresholds were re-determined at each recording session and remained fairly constant over many months. The responses to stimuli at 4 × threshold were averaged ($n = 64$; Biomac 1000) from each nerve after stimulation at each site. Occlusion of the response from the rostral stimulating site as the stimulus strength at the caudal site was increased established that the fibres contributing to the caudally evoked response included those stimulated at the rostral site. The refractory period of transmission[6] (RPT) was determined from the averaged response to the second of two stimuli at 4 × threshold at a range of interstimulus intervals[7]. Recordings were made for 1–2 months before and up to 4 months after inducing the lesion. When the responses were stable at successive sessions, injections of 1.5–2.0-μl LPC (0.8%) were made into the dorsal columns. At the end of the experiment the lesions were studied by light and electron microscopy following glutaraldehyde (4%) fixation by perfusion or immersion.

The sequence of morphological events in the LPC lesion has already been reported[5] and is summarized in Fig. 1. At 3 and 7 days there was extensive demyelination and at these times the amplitudes of the compound action potentials elicited from the rostral stimulating site were markedly reduced in contrast to the control response, which remained unchanged. The conclusion that conduction in many fibres was blocked by demyelination was confirmed by the observation of a large positivity in antidromic recordings made acutely at the lesion site after 7 days.

Remyelination had begun by 14 days, and there was a coincident increase in the number of fibres contributing to the rostrally evoked response (Fig. 1). Over the succeeding weeks the thickness of the new sheaths and the response amplitude increased in step until approximately 81 days, when both stabilised. These later recordings were similar in form to those made before the injection but had a reduced amplitude attributable to the loss of some fibres by Wallerian-type degeneration. There was little change in the latency of the long-term responses. The control response remained constant throughout.

A measure of the security of conduction was obtained from the RPT. In normal fibres, the maximum observed RPT was 1.2 ms. At 3 and 7 days, the range of RPTs of the few fibres still conducting was normal, in keeping with the presence of some histologically unaffected fibres. However, at 14 days, when the compound action potential had increased in size, the majority of fibres had prolonged RPTs (up to 4 ms). Thereafter, the RPT decreased progressively and was normal by 81 days. The RPTs of the control response remained normal throughout.

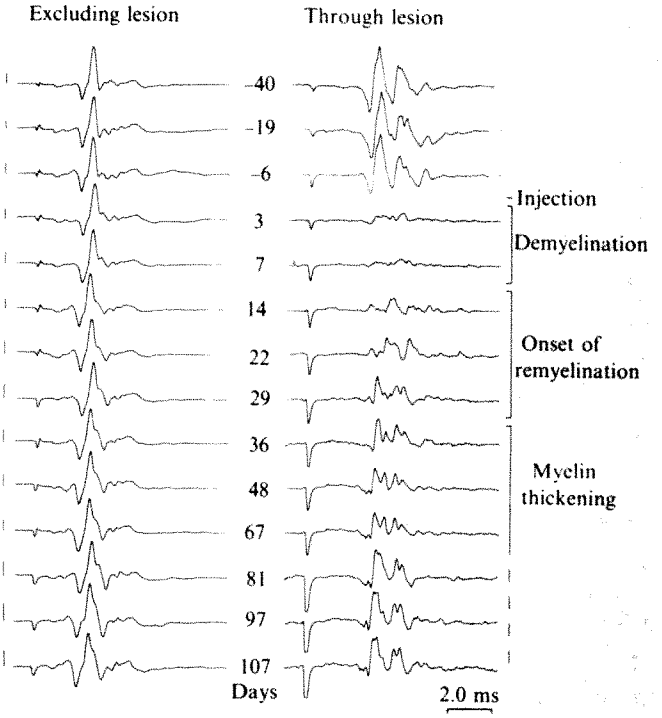


Fig. 1 Saphenous nerve compound action potentials recorded at different times during the development of a focal demyelinating and remyelinating lesion in the dorsal column. To compensate for variables at the recording site the gain on each control response has been adjusted so that their peak-to-peak amplitudes are equal. The corresponding rostrally evoked response has been adjusted by the same factor; each was recorded at 3.3 times the gain of the control. Vertical calibration bar for each recording, 10 μV.

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These experiments have established that conduction begins to return to previously blocked fibres at about the time remyelination begins. Conduction is at first insecure but as the sheaths thicken the RPTs progressively return to normal despite the fact that the internodes remain both thinner and shorter than normal (ref. 5 and W. F. B. and Murray, unpublished observations). The nodal and paranodal morphology is, with few exceptions, normal (W.F.B., unpublished observations). The observation that conduction returns at the same time as new myelin sheaths are formed suggests that it is saltatory in nature. This interpretation is consistent with the return of the RPT to normal. The techniques now available cannot establish whether there is a preceding phase of continuous conduction as in demyelinated[8] and amyelinated[9] peripheral axons.

The restoration of conduction by remyelination is probably of only limited importance to the remissions and delayed evoked potentials[10] seen in multiple sclerosis, as remyelination is limited in this disease[11]. However, it will probably be more important in mediating the recovery seen after demyelination induced by certain compressive lesions of the spinal cord which, at least experimentally, are associated with the formation of new myelin sheaths with similar characteristics to those found in the LPC lesions[12,13]. Although the reason for the striking differences in the extent of remyelination between different types of lesion is unknown[14], the observation of restoration of secure conduction by remyelination suggests that further investigation of techniques for promoting central remyelination would be well worthwhile[15].

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The optical mechanism of the eye of *Limulus*

THE compound eyes of many marine and some terrestrial arthropods have smooth surfaces with no convex curvature to the facets. This means that image formation by ordinary spherical refraction is impossible. Nevertheless, apposition eyes of this kind form images behind each surface facet[1,2] (Fig. 1*b*) and the question has repeatedly arisen as to how these images are produced. Exner chose the king crab, *Limulus*, as providing the definitive example of such an eye, and from a mixture of observation and theory came up with the idea of a lens-cylinder,

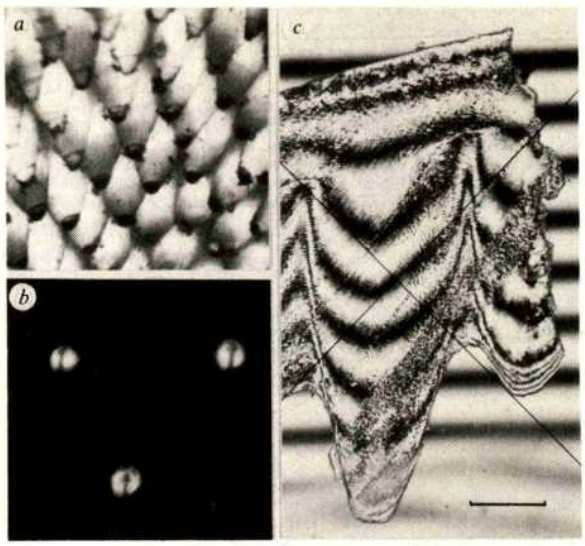


Fig. 1 *a*, Inner surface of the cornea of *Limulus polyphemus* showing the cleaned tips of the crystalline cones. *b*, Images photographed at the cone tips. The object was a black arrowhead on a light circular background whose diameter subtended 8.9° at the eye. The preparation was immersed in seawater. Average separation of the cone tips is 255 μm. *c*, Appearance of a thin parallel-sided frozen section through the centre of a cone using shearing interference optics and a fringe-field eyepeice (Vickers). The whole fringe pattern is visible on the right, with the zero-order (black) fringe at the top. This fringe reappears in the specimen under the crosswires, and it can be seen that in the cone centre it is displaced by just over 4 wavelengths ($\lambda = 542$ nm) and that away from the cone axis this displacement decreases roughly parabolically to about 2 wavelengths. The refractive index (n) at any point can be found from the path difference (PD):

$$PD = m\lambda = t(n - 1.334), \text{ or } n = m\lambda/t + 1.334$$

where m is the number of wavelengths by which the appropriate fringe is displaced and t is the section thickness (13.0 μm). The tip of the cone overlaps the microscope's second image, and the last fringe is lost.

a flat-ended optical device in which the refractive index decreases from the central axis to the periphery in a roughly parabolic manner[1]. This arrangement will form an image (Fig. 2*a*), and devices of this kind have actually been manufactured[3,4]. Exner's theory has long been considered to be the correct account of image formation in this type of eye[5]. However, Levi-Setti, Park and Winston[2] have recently produced a fundamentally different explanation of image formation in *Limulus* eyes (Fig. 2*b*). This was based on the idea that each optical element, or crystalline cone, concentrated light by reflection, not refraction, and that it was the shape of the crystalline cone's reflective surface, and not its internal refractive index gradient, that led to the image-forming properties of the structure. This principle had already been used in another optical device, the 'ideal light collector' (ref. 6) which was invented as a tool for concentrating faint radiation. In this report I discuss the two suggested optical mechanisms for the eye of *Limulus*, and present evidence from interference microscopy that Exner's theory is probably the correct one.

One difference between the refracting (Fig. 2*a*) and reflecting (Fig. 2*b*) explanations is that the former predicts an inverted image and the latter an erect one. (It is, in fact, a design feature of the ideal light collector that the parabolic profile on the left focuses light onto the lower tip of the right profile, thereby excluding light making larger angles with the axis than that shown in the diagram[6].) The images seen at the tips of the cones in *Limulus*, are, in fact, inverted (Fig. 1*b*).

A second difference is that in the refracting mechanism there should be a relatively gradual refractive index change from centre to periphery of each cone, whereas in the reflecting

mechanism this should be abrupt, so that total internal reflection can occur at the interface. The exact form of the gradient in a refracting lens-cylinder was not known to Exner[1], but was worked out later by Fletcher, Murphy and Young[7]. Their solution, for a structure of length F and central refractive index n_0 is

$$n = n_0 \operatorname{sech}(\pi r/2F) \quad (1)$$

where n is the refractive index at a radial distance r from the axis of the cone. Some of these curves, for different values of F, are plotted in Fig. 3*a*.

It is possible to determine which prediction is correct by interference microscopy. An interference microscope splits light from a single source into two beams, one of which passes through the specimen and the other through the bathing medium. These beams are recombined in the objective, and where the optical path-length through the specimen differs from that in the medium, interference occurs, resulting in colours in white light, or distinct dark and light fringes in monochromatic light. In the microscope I used, the interference version of a Vickers M41, there are two distinct ways of viewing the specimen. It can be seen against a uniform background with a constant path difference across the field of view, in which case optical inhomogeneities within the specimen show up as a series of fringes representing 'contour lines' of refractive index, provided the specimen is of uniform thickness. Alternatively, a fringe-field eyepiece incorporating a quartz wedge can be used; this produces a series of visible fringes in the field of view, and the effect of a specimen is to displace these in such a way that the refractive index profile through the section is depicted directly as the pattern of fringe distortion. The first method is capable of giving accurate local measurements of refractive index, but the latter is much more useful for demonstrating the shape of the whole refractive index profile. From their studies Levi-Setti *et al.*[2] and Carricaburu[8] concluded that the cone itself had a high and fairly uniform refractive index, but was surrounded by a lower refractive index, less homogeneous matrix, and that the transition from cone to matrix was abrupt enough for total internal reflection to occur.

Figure 1*c* shows otherwise. This shows a frozen section, 13.0 μm thick (the exact thickness was determined from path difference measurements in two media of different refractive index, water and air in this case) taken along the axis of a cone from an excised eye, kept in seawater containing 1% formaldehyde as a preservative. The eye used for sectioning retained the same image-forming properties as freshly excised material, and so was presumably optically identical. The photograph shows a series of fringes that have a roughly parabolic appearance, curving only gently in the centre of the cone, but increasingly more steeply into a trough that represents the centre of the inter-cone matrix. There is no sudden boundary, and when the fringe pattern is translated into a refractive index profile, this is found to coincide very closely with that of an Exner lens cylinder[1,7] 600 μm long (the actual length of the inhomogeneous

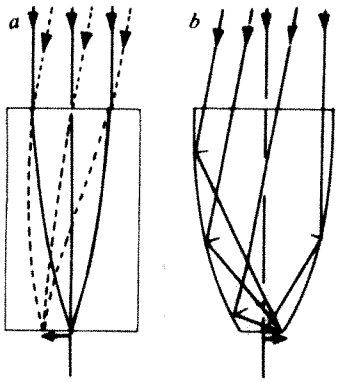


Fig. 2 Optical mechanism of *Limulus* crystalline cones. *a*, The refracting mechanism of Exner[1]; *b*, total internal reflection mechanism of Levi-Setti, Park and Winston[2]. Scale bar, 100 μm.

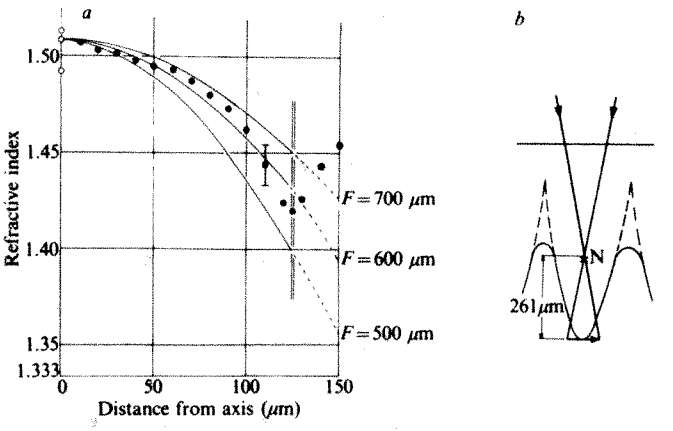


Fig. 3 *a*, Refractive index gradient from centre to periphery of a single cone. The filled circles are means of six measurements made on each side of the three principal fringes in Fig. 1*c*. The range of the measurements is shown for the most variable point by the vertical bar. Open circles on the ordinate are central refractive indices of three different cones. The continuous curves are three solutions of equation (1), with $n_0 = 1.51$, and three values for F. Excluding the outer cornea, 100 μm thick, which does not distort the fringes and is optically homogeneous, the axial length of the 'lens-cylinder' part of the cone (F) is about 580 μm. The theoretical curves (dashes) continue to fall indefinitely, but the points begin to rise again at the double vertical line as one cone ends and the next beings. *b*, The location of the nodal point (N) in a cone, based on image magnification (Fig. 1*b*), is indicated by the intersection of image-forming rays. The small cross indicates its position as predicted from equation (2).

portion of the cone is 580 μm) with a central refractive index of 1.51 (Fig. 3*a*). Note that equation (1) contains no arbitrary constants; the fit of theory and data thus means that the profile shown in Figs 1*c* and 3*a* must give an image at the cone tip.

By way of confirmation, the focal length of the cone (f, defined as image size/angle in radians subtended by an object at infinity) also coincides with that predicted by Fletcher *et al.*[7] They give

$$f = 2F/\pi n_0 \quad (2)$$

which for $F = 600$ μm and $n_0 = 1.51$ gives a value for f of 253 μm. The measured value, obtained directly from the images shown in Fig. 1*b*, was 261 μm. The receptor clusters, situated just beneath the cone tip, have a diameter of about 35 μm, so that for $f = 261$ μm each cluster should subtend an angle of 7.7°, which is almost identical to the inter-ommatidial angle in the centre of the eye.

These three observations, the inverted image, the existence of the refractive index profile required for a refracting lens-cylinder and the finding that the focal length is just what this model would predict, must be taken to mean that Exner's enlightened guess was quite correct (Fig. 2*a*) and that the reflection model (Fig. 2*b*) is not the way in which the images are produced.

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GTP hydrolysis in intact rod outer segments and the transmitter cycle in visual excitation

THE light-sensitive dark current of vertebrate retinal rods is the main metabolic load on the receptor cells, turning over the cytoplasmic Na^+ every 1–6 min (refs 1–4) and the cytoplasmic ATP as often as every 20 s (refs 5, 6). However, a second process in the rod outer segment (ROS), the transmitter cycle, probably demands metabolic free energy during visual excitation. Photons absorbed in the intracellular rod disks reduce the dark current traversing the nearby plasma membrane by altering the cytoplasmic level of a diffusible intracellular transmitter substance[7–10] that may be free Ca^{2+} (refs 11–14). As tens to thousands of transmitter particles must be released and removed in a single photon response[15–17], the transmitter cycle should cause fast metabolic events in ROSs even when they are isolated from those parts of the receptor cells that sustain the dark current. We report here that light causes ROSs to destroy GTP and form GDP at a rate sufficient to meet the predicted requirement of the transmitter cycle. Briefly, ROSs with intact plasma membranes and normal cytoplasmic nucleotide content were isolated within a few seconds from live frog retinas; aliquots of the ROS suspension were exposed to flashes of light, and the cytoplasmic levels of ROS nucleotides monitored as a function of time. The experimental procedure permits changes in cytoplasmic nucleotides in ROS to be distinguished from those involving nucleotides in the suspending medium or bound to rod disk membranes that are not enclosed within an intact plasma membrane.

Rana pipiens or *Rana catesbiana* were dark adapted for 4 h and their retinas removed by dissection in oxygenated Ringer solution containing 10 mM glucose. All operations were done under IR illumination with the aid of image converters. Four retinas were placed in a plastic syringe barrel of internal diameter 1.44 cm in 1.5 ml of frog Ringer solution containing 0.9 mM Ca^{2+}, 10 mM glucose and 0.5 mM phosphate. Potato apyrase (Sigma), 1 U ml^{-1}, was added to split extra cytoplasmic nucleoside di- and triphosphates to monophosphates. *p*H was buffered to 7.1 with PIPES and the retinas were slowly tumbled with a stream of O_2 bubbles. After 3 min, a cylindrical Kel-F spindle 0.89 cm in diameter was dropped axially into the syringe barrel. The spindle turned at 3,900 r.p.m., agitating the retinas in a hydrodynamic shear for 10 s. The spindle was withdrawn and the contents of the syringe expelled with a rubber piston through a coarse platinum mesh and a nylon mesh with 37-μm holes on a side. The filtrate contained ROSs, rod cell nuclei and a few erythrocytes (Fig. 1*a*). When a sample of the suspension was made 10 μM in didansyl cystine (DDC) and examined under 365 nm excitation, many non-fluorescent outer segments with intact plasma membranes were seen (Fig. 1*b*)[18]. When such suspensions were fractionated on metrizamide density gradients, the DDC-staining ROS held less than 10% as much acid-extractable nucleotides per unit particle volume as did the unstained ROS (W. E. R. and W. A. H. in preparation). As 20–35% of the ROS in the suspensions were DDC-excluding the contribution of nucleotides bound to DDC-staining rods to our measurements could usually be neglected.

ROSs were separated from their suspending medium by centrifugal filtration through silicone oil[19]. Aliquots (100-μl) of the rod suspension were layered into 1.5 ml Eppendorf centrifuge tubes previously loaded with 0.1 ml of 0.6 M $HClO_4$ brought to density 1.06 with glucose and overlaid with 0.4 ml of a mixture of Dow–Corning types 555 and 200 silicone fluids of density 1.04 at 25 °C. At selected times a loaded tube was placed in an Eppendorf centrifuge and its contents exposed to a 2-ms flash of 520–590 nm light which reached the rod suspension through the translucent polyethylene tube cap. After a timed delay the centrifuge was run for 10 s at 16,000*g*. The ROSs and other particles in the upper aqueous layer passed into the lower $HClO_4$ layer and became packed as a button. Control experiments with ROSs suspended in Ringer solution containing tritiated water showed that the total volume transferred between the aqueous layers was about three times that of the particles. Particle transfer between the upper and lower aqueous layers was complete within less than 4 s of the start of centrifugation. The lower aqueous layers containing soluble constituents of the cellular particles were neutralised with KOH and



Fig. 1 Photomicrographs of bullfrog ROS suspensions prepared as described. Many rod cell nuclei (n) and a few erythrocytes (e) are present. The nucleoside phosphates are more than 10 times as abundant in ROSs that do not stain with DDC than in those that do. *a*, Dark field, 546 nm illumination; *b*, DDC fluorescence, excited at 365 nm and observed at wavelengths greater than 450 nm (see ref. 18).

K_3PO_4 and analysed by high-pressure liquid chromatography on Partisil 10-SAX (Whatman) with a 40-min gradient of KH_2PO_4, *p*H 3.5, running from 0.05 M to 0.75 M. Nucleotide yields were expressed as mol per l of non-DDC-staining rod water. The detection limit for A and G nucleotides was about 60 μM.

The only nucleotides detected in the extracts were mono-, di-, and triphosphates of adenosine and guanosine, and a trace of UTP. Figure 2*a* shows the adenine and guanine nucleotides of a suspension that was sampled over a 5-min period in darkness after collection from the live retinas. There was a slow loss of all forms of A and G that could be shown in control experiments to

be due to leakage from ROSs into the suspending medium. Ouabain (50 μM) added to the ROS suspension had no detectable effect on the loss of ATP or ADP in darkness: no ATPase activity seemed directed by ROSs towards their own internal ATP. Guanosine phosphates were usually more abundant than those of adenosine. The total nucleoside phosphate content of ROS cytoplasm (including bound forms released during the 5–10-min extraction period in $HClO_4$) exceeded 5 mM, as previously reported[5]. The triphosphate:diphosphate ratios of the adenine and guanine systems differed widely, that of the G system being much lower even with stringent precautions against exposure of the ROSs to light. The ATP : ADP ratio was very sensitive to the oxygenation of the retinas before separation of the ROSs, dropping from 4–7 to about 1 if the O_2 bubbles were stopped for 1 min before the ROSs were sheared from the retinas.

When ROSs were illuminated with flashes that bleached 1% of their photopigment, there was little effect on the levels of ATP or ADP (Fig. 2*b*). The extractable GDP fell within 4 s by 1.0 ± 0.1 mM ($n = 7$). Then about two-thirds of the extractable GTP disappeared in 20 s and an equal amount of GDP was formed. After 30 s there was a small increase in GMP in some experiments. After allowing for leakage of nucleotides from the ROS, there was little light-induced conversion of ATP into ADP or AMP in the first 20 s after the bleaching light flash. The light-induced changes in nucleotide levels were unaffected by lowering the free Ca^{2+} activity to 1 μM or adding 1 mM of the cyclic nucleotide phosphodiesterase inhibitor, isobutylmethylxanthine, to the ROS suspension.

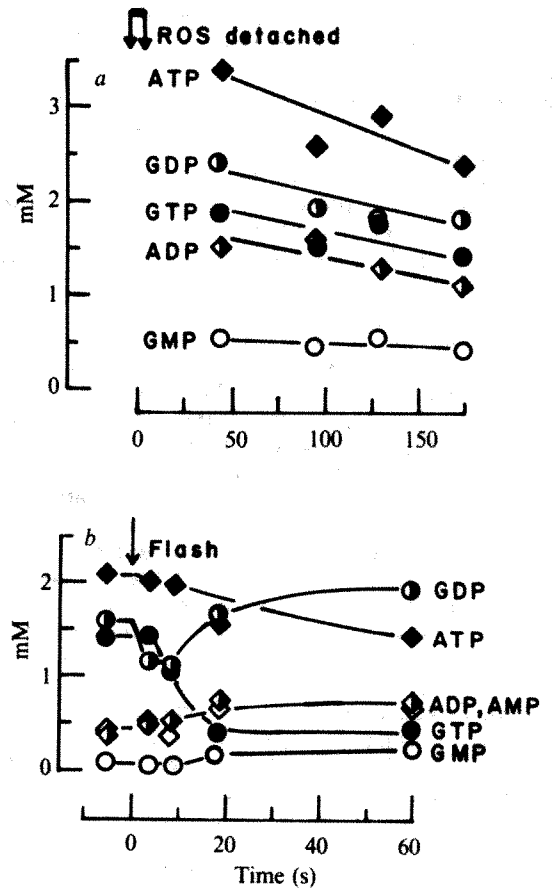


Fig. 2 Nucleoside phosphate levels in intact isolated frog ROSs: *a*, in darkness with time scale beginning the instant that separation from retinas began; *b*, after a 2-ms flash of green light that bleached 1% of the rhodopsin chromophores, time scale beginning at the flash. Light had little effect on ATP or ADP levels but produced a very fast disappearance of GDP followed by a conversion of GTP to GDP with a half time of about 10 s. The values have been corrected for the small slow loss of all nucleotides that occurs in the dark during the 5–10-min course of the experiments.

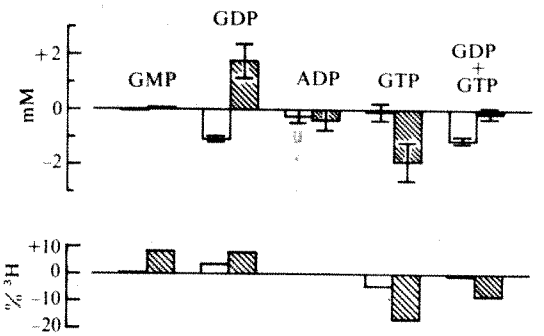


Fig. 3 Changes in guanine nucleotide levels in frog ROSs following a 2-ms 560-nm light flash that bleached 1% of the rhodopsin. Data from four experiments similar to that of Fig. 2*b*. Aliquots of ROS suspension were extracted after centrifugal filtration through silicone oil (see text) before (*a*), 4 s (*b*) and 64 s (*c*) after the bleaching flash. The observed nucleotide level in each aliquot was first corrected for leakage (Fig. 2*a*) by normalising all values to those that would have been obtained if the cytoplasmic ATP + ADP were exactly 5 mM. Open bars, change in nucleotides 4 s after flash. Hatched bars, change in nucleotides between 4 s and 64 s after light flash. Upper row, total extractable nucleotides. Mean of four experiments. Error bars, s.e.m.; lower row, ^{3}H-labelled nucleotides from ROS of two retinas incubated for 1 h in labelled guanosine in stirred, oxygenated glucose Ringer solution. At 4 s total extractable GDP dropped by 1 mM (process (3)), and both total and ^{3}H-labelled GTP dropped slightly between 4 s and 64 s. Total GTP was quantitatively replaced by GDP, while the decline in ^{3}H-labelled GTP was accompanied by the appearance of roughly equal amounts of GDP and GMP. There was no significant change in ATP in the 60 s following the flash.

Do all the guanine nucleotides occur in one light-sensitive pool? The experiments summarised in Fig. 3 suggest that they do not. The upper line of bars in the figure shows net changes in the total extractable guanine nucleotides in four experiments similar to that of Fig. 2*b*. In the first 4 s after the flash, the usual large drop in GDP was seen. Between 4 s and 64 s, GTP dropped and GDP rose by similar amounts. There was no significant change in the GMP level. The lower row of bars denotes changes in the acid-extractable tritiated nucleotides from ROSs of two retinas that had been incubated for 1 h in darkness in stirred, oxygenated glucose Ringer solution containing 20 μCi ml^{-1} of [8-^{3}H]guanosine (NEN). The specific activity of GTP recovered from the ROSs was about 1% of that of the guanosine. The GTP : GDP ratio for the labelled nucleotides was 3.8, whereas that for the total extractable GTP and GDP was 0.96. The ATP : ADP ratio was 7.6. Thus, the exchangeable guanine nucleotides had a phosphate potential much closer to that of the adenine nucleotides. This would be the case if about 74% of the extractable GDP in the ROS were bound to inexchangeable sites. The flash caused a small increase in [8-^{3}H]GDP at 4 s matched by an equal loss of tritiated GTP.

Control experiments on quick-frozen frog retinas showed that the labelled nucleotides were in the layer of outer segments and sedimented with intact ROSs. Thus, the guanine nucleotide pool of frog ROSs must consist of at least two parts: an exchangeable part comprising as much as 25% of the total, apparently following two parallel light-induced pathways:

$$\text{GTP} \rightarrow [\text{Cyclic GMP}] \rightarrow \text{GMP} \quad (1)$$

$$\text{GTP} \rightarrow \text{GDP} \quad (2)$$

and an inexchangeable part that shows process (2) and an additional reaction:

$$\text{GDP (acid soluble)} \rightarrow \text{GDP(acid inextractable)} \quad (3)$$

Process (1) is to be expected, as rod disks contain both guanyl cyclase[23-25] and cyclic nucleotide phosphodiesterase[26-29]. Because the main product of GTP metabolism in illuminated ROSs is GDP, not GMP, either process (2) is the main biochemical pathway for destruction of GTP, or GMP formed by process (1) must be rapidly phosphorylated to GDP.

Process (3) is a surprise. It is hard to reconcile with the protein inventory in the ROS. The binding sites are not likely to be on rhodopsin molecules because only one GDP is bound for every 2–2.5 rhodopsins and because in ROSs whose plasma membranes exclude DDC, at least 90% of the GDP will leak out on hydrodynamic shearing that renders the ROSs leaky to DDC. Thus, the binding sites must be able to leak out of the outer segments along with their bound GDP. The GDP binding resembles that shown by tubulin[30]. We have found tubulin in DDC-negative ROSs by SDS gel electrophoresis, but the abundance of tubulin monomers is less than 1 per 10 rhodopsin chromophores (W.E.R and W.A.H., unpublished). The light-induced binding reaction shows a large chemical gain: bleaching of 1 rhodopsin in 100 causes 1 GDP to become inextractable for every 2 photopigment chromophores in the ROS.

The light-induced conversion of GTP to GDP is much faster than that observed in isolated ROS disk membranes[20-22,31]. In intact ROSs at least 100 GTP molecules are converted to GDP for each photon absorbed by photopigment molecules during the 20 s following a flash, whereas less than 30 GTP molecules are split by ROS disks in a similar time for each photoisomerised rhodopsin chromophore[20,21]. As only 1 rhodopsin in 1,000 must be photoisomerised to activate the disk GTPase fully, the GTPase activity (if that is what process (2) represents) in the intact ROS must be more than 20 times that previously seen. It would be sufficient to drive a mechanism that removes 1,000 transmitter particles previously released in a photon response[15].

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Potentiation of Ca^{2+}-dependent K^+ activation by theophylline is independent of cyclic nucleotide elevation

THE action potential in many nerve cells is followed by an after-hyperpolarisation (AH). In several types of neurone, this AH consists of two components: a brief voltage-sensitive K^+ conductance, and a longer Ca^{2+}-sensitive K^+ conductance (refs 1–5, see ref. 6 for review). In sympathetic neurones of the bullfrog, theophylline potentiates this Ca^{2+}-sensitive K^+ conductance[4]. As theophylline is a well known inhibitor of phosphodiesterase[7], the enzyme which catabolises cyclic nucleotides, we examined the role of cyclic nucleotide metabolism in the regulation of the spike AH. We used both electrophysiological and biochemical methods to investigate whether potentiation of the spike AH by theophylline is correlated with cyclic nucleotide elevation in bullfrog sympathetic neurones. Our results indicate that the potentiation of this Ca^{2+}-dependent K^+ conductance is apparently independent of cyclic AMP and cyclic GMP levels, and suggest that this potentiation by theophylline is not due to inhibition of phosphodiesterase.

The spike AH and other membrane potentials generated in sympathetic neurones were recorded by the sucrose gap technique, as reported previously[4]. Figure 1 shows the spike AH recorded following procedures which would be expected to increase cyclic nucleotide concentration. Theophylline (5 mM) increased the duration of the spike AH (Fig. 1*a*). Two other phosphodiesterase inhibitors, papaverine (0.1 mM)[8-10] and diazepam (0.1 mM)[11-13], however, had little effect on the duration of the spike AH (Fig. 1*b*, 1*c*). Stimulation of the cholinergic preganglionic nerve fibres which synaptically innervate the neurones in these sympathetic ganglia increases the content of both cyclic GMP and cyclic AMP[13,14]. However, neither the administration of the cholinergic agonist methacholine nor preganglionic stimulation prolonged the duration of the spike AH (Fig. 1*d*, 1*e*).

Figure 1*f* and *g* shows that the application of dibuturyl cyclic AMP (3 mM) or dibutyryl cyclic GMP (3 mM) also did not significantly alter the spike AH. As shown in Fig. 1*h*, theophylline (5 mM) increased the duration of the AH in the presence of papaverine (0.1 mM). This observation raised the question of whether the increase in AH duration by theophylline is due to inhibition of phosphodiesterase, as prior inhibition of this enzyme by papaverine might prevent any physiological action of theophylline that is mediated by phosphodiesterase inhibition. To evaluate this question, and to confirm that the experiments shown in Fig. 1 did alter cyclic nucleotide content, we measured both cyclic AMP and cyclic GMP concentrations by radioimmunoassay[15].

Figure 2 shows the change in cyclic AMP and cyclic GMP concentrations which were produced by the various experimental procedures shown in Fig. 1. The content of both cyclic AMP (Fig. 2*a*) and cyclic GMP (Fig. 2*b*) was elevated by the administration of methacholine and by each of the three phosphodiesterase inhibitors, theophylline, papaverine and diazepam. The interaction of papaverine and theophylline on cyclic nucleotide content is shown in Fig. 2*c* and *d*. If the potentiation of the spike AH induced by theophylline in the presence of papaverine results from further inhibition of phosphodiesterase, one would expect these two substances to have an additive effect on cyclic nucleotide levels. However, no additive effect was observed (Fig. 2*c*, *d*). In the presence of papaverine, theophylline reduced the concentrations of both cyclic AMP (Fig. 2*c*) and cyclic GMP (Fig. 2*d*) towards the control concentration seen before papaverine was added. Although the reason for this theophylline-induced decrease in cyclic nucleotide content is

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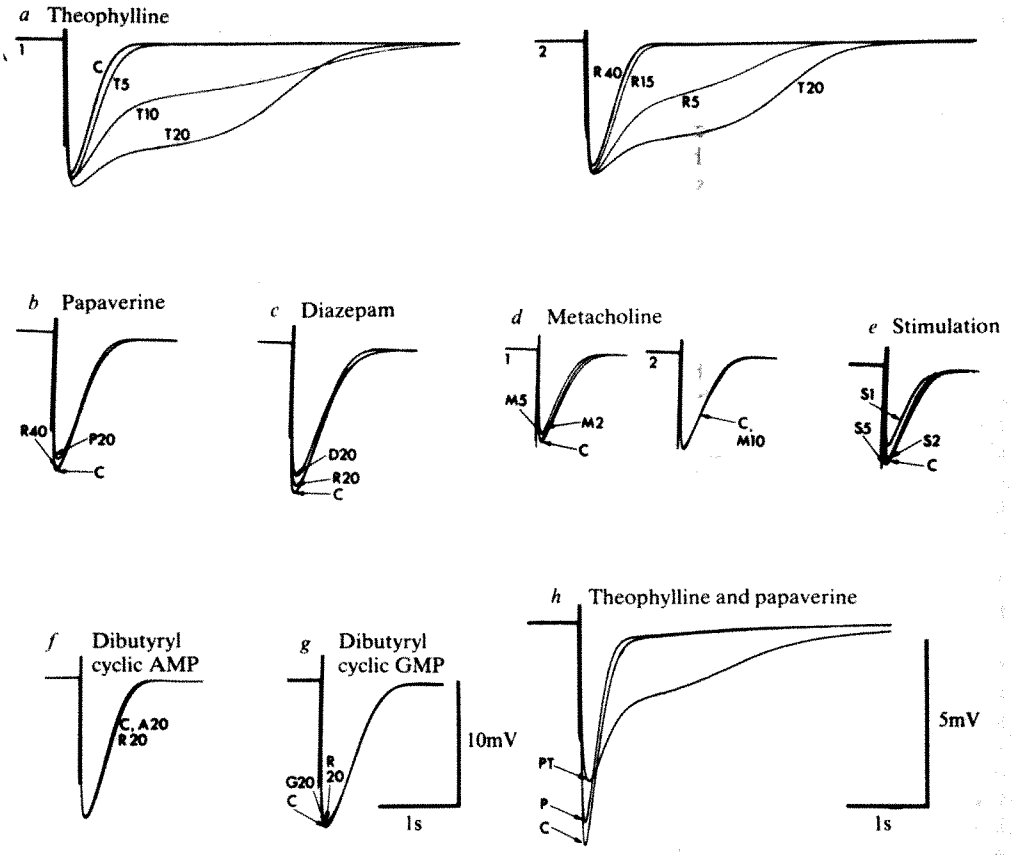


Fig. 1 Action potential after-hyperpolarisation (AH) recorded in conditions expected to increase cyclic nucleotide concentration. *a1*, Effect of theophylline on spike AH. Control AH before theophylline added (C), and superimposed records of spike AH after superfusion with Ringer's solution containing 5 mM theophylline for 5 min (T5), 10 min (T10) and 20 min (T20). Note prolongation of AH duration. *a2*, Recovery from theophylline effect. Spike AH after 20 min in 5 mM theophylline (T20), and superimposed recovery records of AH recorded 5 min (R5), 15 min (R15) and 40 min (R40) after beginning theophylline washout. *b*, Effect of papaverine on spike AH. Control spike AH before papaverine added (C), and superimposed records of AH after 20 min superfusion with 0.1 mM papaverine (P20), and recovery recorded 40 min after beginning papaverine washout (R40). *c*, Effect of diazepam on spike AH. Control AH before diazepam (C), and superimposed records of AH recorded after superfusion with 0.1 mM diazepam for 20 min (D20), and recovery recorded 20 min after beginning diazepam washout (R20). *d*, Effect of methacholine on spike AH; in the presence of 70 μM (−)tubocurarine. *1*, Control AH before methacholine (C), and superimposed records of AH recorded after superfusion with 1 mM methacholine for 1 min; records taken 2 min (M2) and 5 min (M5) after methacholine administration. *d2*, Control record (C) from same experiment as *d1* with superimposed record 10 min after administration of 1 mM methacholine for 1 min (M10). *e*, Effect of synaptic stimulation on spike AH. Control AH before stimulation (C), and superimposed records of AH recorded 1 min (S1), 2 min (S2) and 5 min (S5) after preganglionic stimulation. The sympathetic chain was stimulated between the eighth and ninth ganglia supramaximally with 1-ms pulses for 2 min at 10 Hz (see ref. 14). *f*, Effect of dibutyryl cyclic AMP on spike AH. Control AH before dibutyryl cyclic AMP (C), and superimposed records of AH recorded after superfusion with 3 mM dibutyryl cyclic AMP for 20 min (A20) and recovery record 20 min after beginning dibutyryl cyclic AMP washout (R20). *g*, Effect of dibutyryl cyclic GMP on spike AH. Control AH before dibutyryl cyclic GMP (C), and superimposed records of AH recorded after superfusion with 3 mM dibutyryl cyclic GMP for 20 min (G20) and recovery record 20 min after beginning dibutyryl cyclic GMP washout (R20). *h*, Effect of theophylline on spike AH in the presence of papaverine. Control AH before addition of either drug (C), and superimposed record of AH recorded after superfusion with 0.1 mM papaverine (P). The third superimposed trace was recorded 20 min after the addition of 5 mM theophylline to the Ringer's solution containing papaverine (PT). Note the increased duration of the spike AH after the addition of theophylline. The experiments were carried out on the ninth or tenth paravertebral sympathetic ganglion of the bullfrog by the sucrose gap method. Action potentials were elicited by antidromic stimulation once per minute, as reported previously[4]. The depolarising phase of the action potential was obscured by the stimulus artefact. The spikes were truncated for the purpose of illustration. All records illustrated were obtained using a Brush 280 pen recorder. The composition of the Ringer's solution was (mM): NaCl 100; KCl 2; CaCl 1.8; Tris-HCl 16 (*p*H 7.2); glucose 1 g l^{-1}.

not clear, it may have important implications for our understanding of cyclic nucleotide metabolism.

Our data show that although theophylline, papaverine, diazepam, methacholine and preganglionic stimulation increase the concentrations of both cyclic AMP and cyclic GMP, only theophylline prolongs the duration of the spike AH. This indicates that the duration of the spike AH does not seem to be associated with the elevation of cyclic AMP and cyclic GMP. Consistent with this is the observation that the administration of dibutyryl cyclic AMP or dibutyryl cyclic GMP did not significantly affect the spike AH. The observation that theophylline reduced cyclic nucleotide content in the presence of papaverine but potentiated the spike AH, indicates that the potentiation of this Ca^{2+}-dependent K^+ conductance is independent of cyclic nucleotide elevation. This observation also suggests that the potentiation of the AH by theophylline is not due to phosphodiesterase inhibition. This interpretation has additional significance in view of the fact that the potentiation of various responses in neurones by theophylline has often been cited as evidence for cyclic nucleotide mediation of such phenomena[16–18].

In the sympathetic neurones studied here, potentiation of the spike AH by theophylline is dependent on the presence of extracellular Ca^{2+} ions[4]. During an action potential, in addition to an influx of Na^+ ions there is an influx of Ca^{2+} ions into these neurones[19]. It is likely that this Ca^{2+} influx activates the K^+ conductance which prolongs the duration of the spike AH. Busis and F.F.W.[4] suggested that theophylline may potentiate the spike AH by increasing the stimulus-induced entry of Ca^{2+} ions. Recently, Klein and Kandel[20] reported that phosphodiesterase inhibitors prolong the duration of a Ca^{2+} spike in a sensory neurone of *Aplysia*. They showed that the prolongation of this Ca^{2+} conductance could also be produced by synaptic stimulation and suggested that cyclic AMP was mediating this effect of the neurotransmitter, serotonin. Cyclic nucleotides have also been reported to prolong a Ca^{2+}-dependent K^+ activation in spinal motoneurones[21]. Our data, on the other hand, indicate that the prolongation of the Ca^{2+}-dependent K^+ activation in sympathetic neurones is independent of cyclic nucleotide elevation. It thus seems that various mechanisms may be regulating Ca^{2+}-dependent conductance changes in different types of neurone. Such a diversity is further illustrated by the recent observation that Ca^{2+}-dependent conductance changes can be reduced rather than enhanced by neurotransmitter action[22,23].

The AH following an action potential produces the absolute and relative refractory periods which prevent spike reactivation after short time intervals and thus limit spike firing frequency. An understanding of the cellular mechanisms controlling the

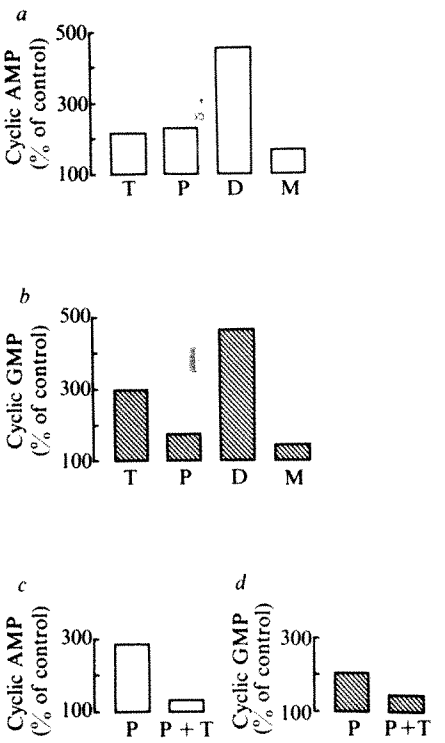


Fig. 2 Effect of phosphodiesterase inhibitors and methacholine on cyclic nucleotide concentration in bullfrog sympathetic ganglia. *a*, Increase in cyclic AMP content produced by theophylline (T), papaverine (P), diazepam (D) and methacholine (M) illustrated as per cent of control. For each experiment, $n = 5$ or more. The changes in cyclic AMP concentrations were as follows, expressed as pmol per mg protein (mean ± s.e.m.). Incubation of ganglia with 5 mM theophylline for 20 min increased cyclic AMP concentration from 10.53 ± 1.63 to 23.26 ± 2.42 ($P < 0.005$). Incubation with 0.1 mM papaverine for 20 min increased cyclic AMP from 17.83 ± 1.61 to 41.9 ± 1.37 ($P < 0.001$). Incubation with 0.1 mM diazepam for 20 min increased cyclic AMP from 20.5 ± 2.9 to 93.17 ± 32.3 ($P < 0.05$). Incubation with 1 mM methacholine for 2 min increased cyclic AMP from 40.05 ± 6.69 to 70.08 ± 12.21 ($P < 0.1$) determined 5 min after M administration, in the presence of 70 μM (−)tubocurarine. *b*, Increase in cyclic GMP content produced by theophylline, papaverine, diazepam and methacholine illustrated as per cent of control. For each experiment, $n = 5$ or more. The changes in cyclic GMP concentrations were as follows, expressed as pmol per mg protein (mean ± s.e.m.). Incubation of ganglia with 5 mM theophylline for 20 min increased cyclic GMP concentration from 0.97 ± 0.12 to 2.92 ± 0.4 ($P < 0.001$). Incubation with 0.1 mM papaverine for 20 min increased cyclic GMP from 0.98 ± 0.07 to 1.78 ± 0.18 ($P < 0.005$). Incubation with 0.1 mM diazepam for 20 min increased cyclic GMP from 0.29 ± 0.03 to 1.38 ± 0.29 ($P < 0.005$). Incubation with 1 mM methacholine for 2 min increased cyclic GMP from 0.58 ± 0.13 to 0.84 ± 0.12 ($P < 0.4$) determined 5 min after M administration, in the presence of 70 μM (−)tubocurarine. *c*, Effect of theophylline on the % increase in cyclic AMP produced by papaverine. For each experiment, $n = 5$ or more. Incubation of ganglia with 0.1 mM papaverine for 40 min increased cyclic AMP concentration from 17.83 ± 1.61 to 51.5 ± 6.51 ($P < 0.001$). However, when ganglia were incubated with 0.1 mM papaverine for 20 min followed by 0.1 mM papaverine plus 5 mM theophylline for 20 min, cyclic AMP increased to only 24.3 ± 1.9 ($P < 0.05$). *d*, Effect of theophylline on the % increase in cyclic GMP produced by papaverine. For each experiment, $n = 5$ or more. Incubation of ganglia with 0.1 mM papaverine increased cyclic GMP concentration from 0.98 ± 0.07 to 2.02 ± 0.17 ($P < 0.001$). However, when ganglia were incubated with 0.1 mM papaverine for 20 min followed by 0.1 mM papaverine plus 5 mM theophylline for 20 min, cyclic GMP increased to only 1.5 ± 0.19 ($P < 0.05$). Cyclic nucleotide content was determined as follows. After dissection, ganglia were equilibrated in oxygenated Ringer's solution for 1–2 h. After test procedures, ganglia were plunged into liquid nitrogen. Samples were fixed in 0.5 ml 0.1 M HCl in 90% methanol at −20 °C for 2 h. Following addition of 1 ml water, samples were extracted at 80 °C for 30 min and centrifuged. The supernatants were transferred, lyophilised, resuspended in water and assayed by radioimmunoassay using the method of Harper and Brooker[15]. Protein content was determined by the method of Lowry[24].

duration of the spike AH is thus important for determining the factors involved in regulating excitability and the frequency of spike firing. Further investigation of the potentiation of the spike AH by theophylline may lead to a greater understanding of the molecular mechanisms involved in the control of Ca^{2+}-sensitive K^+ conductances.

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Whitefly have the highest contraction frequencies yet recorded in non-fibrillar flight muscles

ASYNCHRONOUS (myogenic) insect flight muscles have evolved independently at least seven times[1]. Although such muscles do not necessarily contract at particularly high frequencies, potentially they allow far greater wing stroke rates than synchronous muscle which, because of the delays involved in muscle activation, has been believed to be incapable of operating at frequencies above ~100 Hz (ref. 2), unless power output is small and much of the muscle volume occupied by sarcoplasmic reticulum[3]. Asynchronous muscle has so far always been shown to be of the 'fibrillar' histological type, and this correspondence has been assumed to be invariable[1]. Predictably, very small insects, for which a high stroke frequency is believed to be essential[2], almost always have fibrillar flight muscle. However, a few groups, notably the hemipterous family Aleyrodidae (whitefly), have muscle of the 'close-packed' type, hitherto supposed to operate only synchronously[1]. We have measured cinematographically the wing-beat frequencies of the whitefly *Trialeurodes vaporariorum* (Westwood), and report here that they are well above the usually assumed limit for synchronous functioning. Either this limit, or the assumption that all asynchronous muscle is of fibrillar type, must therefore be incorrect.

The studies of Greenwalt[4] indicate that, albeit with many deviations, insect wing-beat frequencies vary approximately as the reciprocal of the wings' linear dimensions. Weis-Fogh has confirmed that within many families and superfamilies this is

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almost exactly true, and has discussed the mechanical and aerodynamic reasons for it[5].

The smallest flying insects which lack fibrillar muscle are probably some Nepticulidae (Lepidoptera), with wing length 2 mm or less, and Aleyrodidae (Hemiptera), whose wings are often shorter than 1.5 mm, and of which *Trialeurodes vaporariorum* undoubtedly has close-packed flight muscle[1]. Insects of this size might be expected to have wing-beat frequencies well above the assumed limit of 100 Hz for synchronous flight muscle. Various small Diptera with wings between 2 and 7 mm long show frequencies between 150 and 600 Hz (ref. 4), and psyllid, mirid and cimicid Hemiptera within the same size range have been timed at between 100 and 150 Hz (ref. 1). All these have fibrillar flight muscle. We have filmed *T. vaporariorum* in free flight, using a Hycam high-speed cine camera (Hadland), operating at approximately 3,000 frames per s. The exact framing rate was measured using a timing light generator operating a neon timing light within the camera which marked the film at precisely 1,000 Hz.

The flapping frequencies of nine individuals were measured during short flights. They varied between 143 and 181 Hz, that is between 40% and 80% higher than the previously supposed maximum contraction frequency for synchronous flight muscle.

The highest wing-beat frequencies previously recorded for insects using close-packed muscle are 90–95 Hz in some sphingid moths[2]. *Trialeurodes*, by contrast, falls in the same frequency range as many medium-to-large Diptera and Hymenoptera[4] and rather above those of most recorded Coleoptera[4] and Heteroptera[1]. Published figures for Aphididae, which like Aleyrodidae are Homoptera but which have fibrillar muscle, all fall below 100 Hz (ref. 1). Why asynchrony should have evolved in aphids but not in the usually smaller and more rapidly beating whitefly is unclear.

For insects of their size the wing-beat frequency of *Trialeurodes* is still strikingly low. Two factors may contribute to this. First, Pringle (ref. 2, text and subsequent discussion) has suggested that Aleyrodidae may be able to limit their frequency through their unusually low wing loading, which would reduce the minimum power necessary for flight. Second, Weis-Fogh[5] explains the relatively low frequency of *Drosophila*—195–240 Hz in *Drosophila virilis*[6]—by their use of the 'clap and fling' mechanism[7] for generating extra lift. *Trialeurodes* performs a dorsal clap and fling in each wing beat; also the wing loading, which we have calculated by dividing the mean weight of 800 individuals by the mean wing area of a sample of 15, is exceptionally low. Its value, 0.047 kg m^{-2}, may be compared with 0.35 kg m^{-2} for the larger *D. virilis*[6] and 0.086 kg m^{-2} for the smaller *Encarsia formosa* (recalculated from Weis-Fogh[7] using Ellington's figure for wing area[8]). Such a low wing loading will undoubtedly have contributed to a low power requirement, which may be a prerequisite for high-frequency synchronous muscle—if this is what *Trialeurodes* is using.

Contraction frequencies up to 180 Hz in close-packed flight muscle imply one of two possibilities. First, the previously assumed upper frequency limit for synchronous flight muscle contraction may be far too low. Alternatively, the asynchronous mechanism may not, after all, be restricted to fibrillar muscle. This certainly cannot be ruled out; many different kinds of muscle are now known to display the delayed stretch activation which is required for asynchronous operation. The crucial information now required is the stimulation frequency of the muscles. Without this it is impracticable to judge which explanation is correct.

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Antiserum specific for motor nerve terminals in skeletal muscle

OUR understanding of synaptic function and development has benefited from the availability of neurotoxins that bind specifically to acetylcholine receptors and acetylcholine esterase at the neuromuscular junction[1–4]. It would be of additional value to have molecular probes for other components of this accessible and well-studied synapse, such as the nerve terminal. Antibodies to the synaptic vesicles that are concentrated in the motor nerve terminal would constitute one such marker; unfortunately, these vesicles are present in such small amounts that their purification has not yet been achieved. It is, however, possible to purify cholinergic synaptic vesicles from the electric organ of the elasmobranchs, *Narcine brasiliensis*[5] and *Torpedo marmorata*[6], and to obtain antisera to them (S.S.C. and R.B.K., in preparation). We report here that antibodies in anti-*Narcine* vesicle sera bind specifically to motor nerve terminals in skeletal muscle.

We have used the technique of indirect immunofluorescence to detect binding of antibodies to nerve terminals. Unfixed tissue (rat diaphragm except where noted) was frozen in liquid N_2 and sectioned in a cryostat. Sections were incubated with rabbit antiserum and then with a mixture of fluorescein-conjugated second antibody (goat anti-rabbit IgG) and rhodamine-conjugated α-bungarotoxin[3,7]. Appropriate filter combinations enabled us to excite and view fluorescein or rhodamine selectively, and thus to compare the distribution of immunohistochemical stain with that of α-bungarotoxin, which binds tightly and specifically to the acetylcholine receptors that are clustered in the postsynaptic membrane of the neuromuscular junction[1,2].

Our main results are shown in Fig. 1. Unfractionated antisera to whole *Narcine* synaptic vesicles[5] stained a variety of membranes in rat skeletal muscle, including those of blood vessels, nerves and muscle fibres (Fig. 1*a*). Most intensely stained, however, were small patches in the muscle fibres' surface (Fig. 1*a*), which could be identified as neuromuscular junctions because they also stained with rhodamine-α-bungarotoxin (Fig. 1*b*). Adsorption of antiserum with vesicle-free membranes from *Narcine* electric organ (S.S.C. and R.B.K., in preparation) removed antibodies that stained nonsynaptic membranes, but spared antibodies that stained the neuromuscular junction (Fig. 1*c* and *d*). This adsorbed antiserum also stained neuromuscular junctions in frog and chick skeletal muscle, and the innervated face of *Narcine* electroplaques. Adsorption of serum with sonicated *Narcine* vesicles reduced staining markedly. Preimmune serum did not stain neuromuscular junctions detectably. Thus, antibodies in anti-vesicle serum cross-react with components of mammalian, amphibian and avian neuromuscular junctions.

Two observations indicate that the neuromuscular components to which the antibodies bound were associated with nerve terminals. First, antibodies stained structures that resembled nerve terminals in size, shape and position[8,9]: comparison of the distribution of fluorescein-antibody and rhodamine-toxin showed that antibodies stained 1–3 μm wide structures (Fig. 1*c*, inset) that were tangential to toxin-stained gutters of postsynaptic membrane (Fig. 1*d*, inset). Second, antibodies did not

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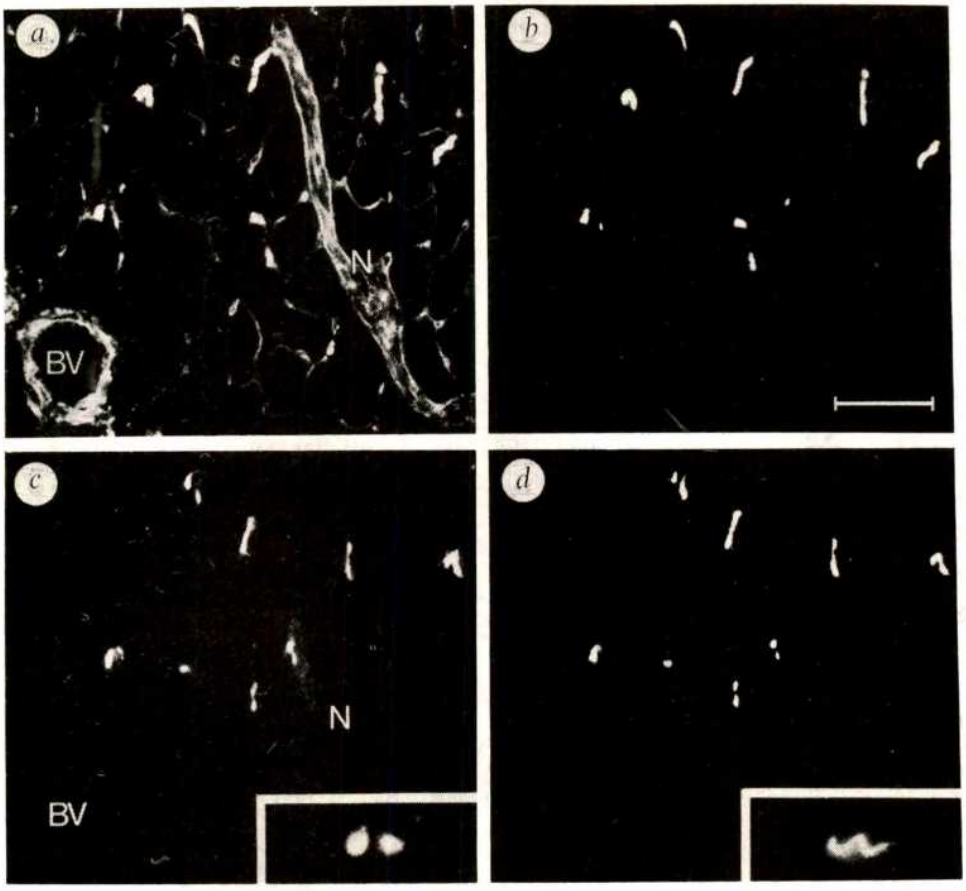


Fig. 1 *a*, *b*, Cross-section (4 μm) of frozen, unfixed rat diaphragm were incubated for 30 min at room temperature with rabbit antiserum to *Narcine* synaptic vesicles (1:60 dilution in phosphate-buffered saline containing 1% BSA). They were then washed and stained for 30 min at room temperature with a mixture of fluorescein-'second antibody' (fluorescein-goat anti-rabbit IgG; Cappel Laboratories; 1:50 dilution) and rhodamine-α-bungarotoxin (10 nM; a gift of P. Ravdin and D. Berg, University of California, San Diego). The section was photographed with filters appropriate to show either fluorescein (*a*) or rhodamine (*b*). (Immunohistochemical methods are detailed elsewhere[11]). Fluorescein fluorescence reveals distribution of anti-vesicle antibodies and rhodamine the distribution of acetylcholine receptors[3,7] and thus location of neuromuscular junctions. The coincidence of fluorescein and rhodamine shows that antibodies stain the neuromuscular junction; blood vessels (BV), nerves (N), and the muscle fibres' surface also stain. *c*, *d*, Section adjacent to that shown in *a* and *b*, incubated with anti-vesicle serum (1:60 dilution) that had been adsorbed with non-synaptic membrane from *Narcine* (5 μg membrane protein/μl antiserum, 16 h, 4 °C), and then stained with fluorescein-second antibody and rhodamine-α-bungarotoxin: *c*, fluorescein; *d*, rhodamine. Neuromuscular junctions stain, but extrasynaptic portions of the muscle fibre surface do not. Blood vessel and nerve were stained too faintly to be visible in the micrograph, but their positions are marked. Insets to *c* and *d* show a single neuromuscular junction (from the section in *c* and *d*), photographed to show fluorescein (inset to *c*) or rhodamine (inset to *d*). Antibody stains nerve terminals that abut the toxin-stained postsynaptic membrane. The scale bar in *b* is 50 μm and applies to *a*–*d*; 10 μm for insets.

stain endplates in muscles that were denervated 1 week before they were frozen and sectioned. At this time, postsynaptic receptors and Schwann cells remain, but nerve terminals have disappeared[10]. There is, therefore, little doubt that antibodies bind to antigens in the motor nerve terminal or on its surface.

The antigens detected by the anti-vesicle serum are distributed non-uniformly in the motoneurone, as would be expected if they were associated with synaptic vesicles. Although nerve terminals stained intensely, axons in the nerve or in intramuscular nerve branches, cut either longitudinally or in cross-section, did not stain well (Fig. 1*c*), nor did the cytoplasm of motoneurone somata or proximal dendrites, seen in frozen sections of rat spinal cord. (The synapse-rich gray matter of the spinal cord did stain, however, raising the possibility that nerve terminals other than motor nerve terminals contain antigen; experiments to examine this possibility are in progress.)

In conclusion, antibodies directed against *Narcine* synaptic vesicles recognise motor nerve terminals in mammalian, amphibian and avian skeletal muscle, and distinguish terminal from preterminal portions of motor axons. These antibodies should facilitate studies of the function, development, and molecular composition of nerve terminals at the neuromuscular junction.

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T-cell-derived helper factor allows Lyt 123 thymocytes to differentiate into cytotoxic T lymphocytes

IT is generally accepted that the diversity of T-cell responsiveness is generated in the thymus[1]. It is also known that except for a few Lyt 1 cells all thymocytes express the Lyt 123 phenotype[2,3]. Surprisingly, thymocytes are poorly responsive *in vitro*[4], and only the medullary thymocytes, comprising 5–10% of the total thymic cell population, show an *in vitro* responsiveness comparable with that of peripheral T cells[5]. Cortical thymocytes, comprising 90–95% of all thymocytes, have previously been considered to be immature and immunologically incompetent[4]. The result reported here show that thymocytes are able to generate alloantigen-, virus- and hapten-specific cytotoxic T lymphocytes (CTL), provided a T-cell-derived helper factor is added to the cultures. Furthermore, through the use of cell separation techniques we conclude that in the presence of the helper factor the great majority of Lyt 123^+ thymocytes have the capacity to differentiate into either alloreactive or H-2-restricted CTL.

The tissue culture system for the induction of alloreactive, trinitrophenyl (TNP)-specific and Sendai virus-specific CTL as well as the preparation of TNP-conjugated or Sendai virus-infected targets has been described elsewhere[6–8]. The target cells used in the 3-h ^{51}Cr-assay were *in vitro* propagated P815 ($H\text{-}2^d$) mastocytoma cells, LS cells ($H\text{-}2^k$) (Flow) and EL4 ($H\text{-}2^b$) tumour cells. Peanut agglutinin (PNA)-receptor-positive cells were detected using fluorescein isothiocyanate (FITC) labelled PNA (Industrie Biologique Française)[9,10]. The preparation of semi-purified T-cell-derived helper factor has been described in detail elsewhere[11]. Briefly, CBA mouse-derived splenic T cells were cultured in serum-free medium for 24 h in the presence of 1 $\mu g\,ml^{-1}$ concanavalin A (Pharmacia). The culture supernatant was concentrated 20-fold, eluted from a Sephadex G-100 column and the fractions with 'secondary CTL-inducing activity'[11] were pooled and concentrated 20-fold.

As can be seen in Table 1, CBA mouse ($H\text{-}2^k$)-derived splenic responder T cells are able to mount *in vitro* an efficient CTL response towards $H\text{-}2^d$ alloantigens. Similarly efficient H-2-restricted CTL responses can be generated which are specific for TNP-conjugated or Sendai virus-coated syngeneic targets. In contrast, CBA mouse-derived thymocytes used as responder cells responded only poorly, if at all. However, the low responsiveness of thymocytes was converted to high responsiveness when an optimal concentration of T-cell-derived helper factor was added to the cultures (Table 1). The augmented CTL responsiveness of thymocytes was observed for the induction of both alloreactive CTL and H-2-restricted TNP- or Sendai virus-specific CTL.

These results support two broad conclusions. First, thymocytes are immunocompetent at the level of alloreactive and H-2-restricted CTL precursors. Second, a rate-limiting step during the *in vitro* induction of thymocyte-derived CTL can be bypassed by supplementing the cultures with T-cell-derived helper factor.

Thymocytes can be separated into two classes of cells. Medullary thymocytes, comprising 5–10% of thymocytes, are immunocompetent, have high H-2 and low Thy-1 density[4] and are PNA-receptor negative[10] and cortisone resistant[5]. In contrast, cortical thymocytes, which represent 90–95% of thymocytes, are PNA-receptor positive[9,10], have low H-2 and high Thy-1 density[4] and are believed to be immunologically incompetent[4]. In view of the observed ability of thymocytes to generate CTL in the presence of T-cell-derived helper factor, we tested whether the use of cell separation techniques would allow us to define a particular subpopulation which responded in the culture conditions used.

CBA mouse-derived thymocytes were subject to 1*g* velocity sedimentation using the Bont–De Koningh separation apparatus[12]. Viable cells of the individual fractions obtained were tested for PNA-positive cells and for their CTL responsiveness to $H\text{-}2^d$ stimulator cells, TNP-conjugated syngeneic stimulator cells and Sendai virus-coated syngeneic cells.

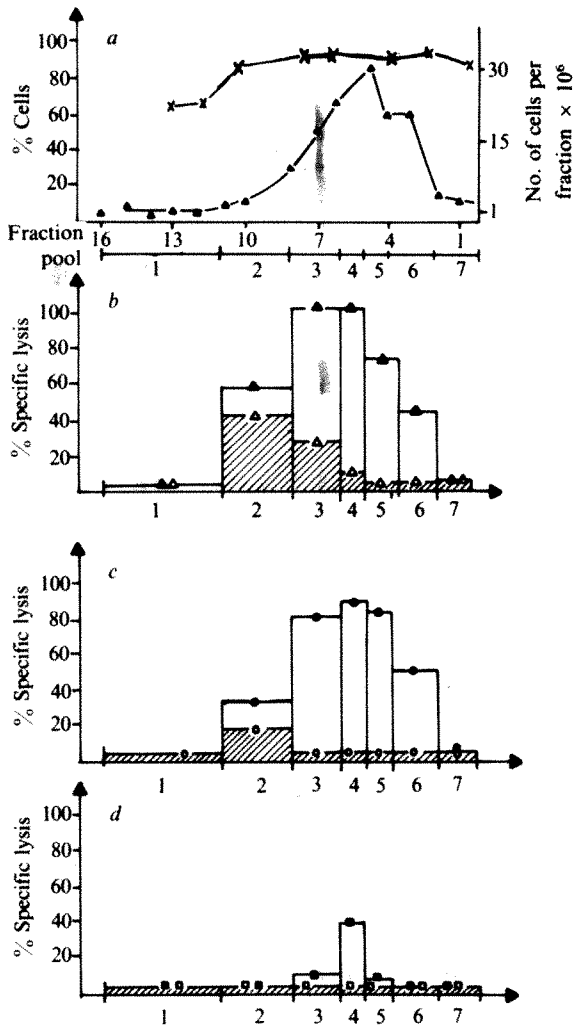


Fig. 1 CTL responsiveness pattern of thymocytes fractionated according to cell size. *a*, CBA mouse-derived thymocytes (2×10^8) were subjected to 1*g* velocity sedimentation using the Bont–De Koningh apparatus[12]. One of the advantages of this apparatus is the short sedimentation time required to separate the cells to be fractionated. This is achieved by turning the sedimentation vessel from its vertical position into the horizontal position. The number of viable cells per individual fraction obtained is given. Note that fraction 1 contains the very small cells whereas fraction 15 contains the very large cells. × Indicates the % of cells binding FITC-conjugated PNA. *b*, Fractionated thymocytes (3×10^6) were cultured with 1×10^6 irradiated (1,000 rad) BALB/c stimulator cells in TC-24 Linbro culture plates in the absence (△) or presence (▲) of 50 μl of semi-purified[11] T-cell-derived helper factor. After 5 d the lytic activity generated was tested in a 3-h ^{51}Cr-assay against P815 target cells at a ratio of attacker to target cells of 20:1. Background lysis of P815 targets was 9%. *c*, Fractionated thymocytes (3×10^6) were cultured with TNP-conjugated CBA spleens in the absence (○) or presence (●) of 50 μl semi-purified T-helper factor. After 5 d the lytic activity generated was assayed against TNP-conjugated LS cells at a ratio of CTL to target of 20:1. Background lysis was 12%. *d*, Fractionated CBA thymocytes (3×10^6) were cultured with 1×10^6 Sendai virus-coated syngeneic spleen cells in the absence (□) or presence (■) of 50 μl T-cell-derived helper factor. After 5 d the lytic activity generated was tested against Sendai virus-coated LS target cells at a ratio of CTL to target of 20:1. Background lysis was 17%. A similar responsiveness pattern was noted with fractionated BALB/c ($H\text{-}2^d$) and C57 BL/6 ($H\text{-}2^b$) thymocytes, the only variable being that in the absence of T-helper factor no response was obtained.

Figure 1 shows the results of a representative experiment. As can be seen from the cell distribution profile, thymocytes could be separated on the basis of cell size. PNA-negative cells were found only in the medium-to-large sized cell fractions. Cells collected in fractions 1–8 were PNA positive. With regard to

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Table 1 Effect of T-cell-derived helper factor on the capacity of CBA mouse thymocytes to mount alloreactive and H-2-restricted CTL responses

Responder cells ($H\text{-}2^k$)	X-ray irradiated splenic stimulator cells	Semi-purified helper factor added (μl per culture)	^{51}Cr-labelled target cells	% Specific lysis (ratio of CTL to target cells) 10:1	2:1	0.5:1
CBA thymocytes	BALB/c ($H\text{-}2^d$)	—	P815	7	3	0
	BALB/c	75	P815	90	77	44
	BALB/c	25	P815	61	49	17
CBA thymocytes	CBA-TNP	—	LS-TNP	2	1	0
	CBA-TNP	75	LS-TNP	67	45	9
	CBA-TNP	25	LS-TNP	26	9	2
CBA thymocytes	CBA-Sendai virus	—	LS-Sendai virus	0	0	0
	CBA-Sendai virus	75	LS-Sendai virus	32	12	4
	CBA-Sendai virus	25	LS-Sendai virus	17	9	2
CBA spleen	BALB/c spleen	—	P815	98	57	32
	CBA-TNP	—	LS-TNP	63	27	9
	CBA-Sendai virus	—	LS-Sendai virus	44	18	6

CBA mouse-derived responder cells (4×10^6) were cultured in the presence of the respective X-irradiated (1,000 rad) splenic stimulator cells (1×10^6) in 2 ml of medium in Linbro FB-24 Tc multidish culture trays (Linbro). Cultures contained 5% fetal calf serum, 10 mM HEPES, 2×10^{-5} M 2-mercaptoethanol with or without T-cell-derived helper factor[11]. After 5 d incubation at 37 °C, the cells were collected and set up in a standard 3-h ^{51}Cr-release assay against the target cells listed. The preparation of target cells using 10 mM 2,4,6-trinotribenzene sulphonic acid (Sigma) of β-propiolactone-inactivated Sendai virus suspension (Lot no. 138-1, Connaught) has been described elsewhere[8]. Background lysis of the target cells did not exceed 17%; % specific lysis represents the mean of triplicate determinations. In no case did the standard deviation exceed 5%.

cytolytic activity, medium-to-large sized thymocytes were able to mount CTL responses when stimulated *in vitro* with allogeneic stimulator cells, but were less responsive when stimulated with TNP-conjugated cells, and were unresponsive to stimulation with Sendai virus-coated syngeneic cells (Fig. 1). The unexpected finding, however, was that the apparent immunoincompetence of the large cell pool of medium-to-small sized thymocytes was converted to a state of high responsiveness when the T-cell-derived helper factor was added to the cultures. Interestingly, in the presence of this factor medium-to-small sized thymocytes not only mount efficient CTL responses towards alloantigens, but are also able to generate H-2-restricted CTL responses for the hapten TNP. Even Sendai virus-specific CTL could be induced from thymocytes of pool 4. The data in Fig. 1 also show that the very large and very small thymocytes were unable to generate CTL. In this context it is noteworthy that medium-to-small thymocytes were all PNA positive. This marker has recently been attributed to cortical thymocytes[10], known to express the Lyt 123 phenotype[3]. We therefore conclude that in the conditions described cortical thymocytes can differentiate into alloreactive as well as H-2-restricted CTL.

The experiments described formally demonstrate that depending on the 'antigen' used, thymic Lyt 123 T cells have the potential to give rise to H-2-restricted TNP or virus-specific CTL, or to non-restricted alloreactive CTL. The results also suggest that within cortical thymocytes both alloreactive and H-2-restricted CTL precursors express the Lyt 123 phenotype, and that at least at the level of CTL precursor cells, cortical thymocytes already express an antigen receptor diversity comparable with that of peripheral T cells. Obviously, at the level of CTL precursors cortical thymocytes are immunocompetent, and have the potential to respond efficiently to either alloantigens or foreign antigens. Thus, if the cortex of the thymus represents the site for the generation of the T-cell repertoire, immunocompetent cortical thymocytes are the result of these events. It should be stressed that *in vitro* the expression of the immunocompetence of cortical thymocytes occurs only in the presence of a helper factor previously shown to be derived from activated Lyt 1 T cells[11]. In fact, *in vitro* the rate-limiting step for the differentiation of Lyr 123 thymocytes into CTL represents the presence of the T-cell derived helper factor (Fig. 1).

Although the mechanism of this T–T-cell interaction is unknown, our experiments raise the question as to why within peripheral T cells of adult mice alloreactive T cells develop from the Lyt 23 T-cell subset[2]. Because, within the thymus, Lyt 123 T cells apparently represent the precursor pool of alloreactive CTL, a shift from Lyt 123 to the Lyt 23 T cell must take place. Recently, Burakoff *et al.*[13] provided convincing evidence that in mice within 3 weeks of birth the phenotype of peripheral alloreactive CTL precursors changes from Lyt 123 to Lyt 23. As the Lyt 23 phenotype is characteristic of H-2-restricted CTL, alloreactive CTL and memory T cells (refs 14–16 and unpublished results), and as H-2-restricted CTL specific for 'foreign antigens' to cross-react with alloantigen[17–19], we postulate that alloreactive peripheral Lyt 23 CTL precursors represent memory cells of virus-specific H-2-restricted CTL which by chance cross-react with alloantigen. A prediction of this view is that H-2-restricted versus non-restricted cytolysis is not a function of CTL, but a result of the respective target antigen recognised. In accordance with this we recently defined an *in vitro* propagated monoclonal CTL line ($H\text{-}2^k$) specific for allogeneic K^d targets. These monoclonal CTL also lyse (unpublished results) Sendai virus-infected syngeneic cells in an H-2-restricted manner.

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Lymphomas with cytotoxic activity

TUMOURS of antibody-secreting B lymphocytes (myeloma tumours) have contributed greatly to our understanding of antibody structure and function. T-lymphocyte tumours that retain effector activities might, if they exist, prove to be similarly useful for analysing T cells and their key components. The recent finding that a T lymphoma is capable of crawling on other cells, like antigen-activated normal T lymphocytes[1], prompted

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Table 1 Con A-dependent cytotoxicity of some mouse lymphomas

Lymphoma	Origin: Strain (H-2 haplotype)	Origin: Tissue*	Origin: Induction†	Ref.	Cytotoxicity (% specific lysis)
	Positive control: BALB/c anti-EL-4				27.3±0.1
TB-1	AKR (k)	LN	Spont.	3	14.4±1.2
TB-2	AKR (k)	Spl	Spont.	3	17.8±1.5
TB-3	AKR (k)	LN	Spont.	3	20.7±0.7
Ib	C58 (k)	Spl	Spont.	14	0.1±1.2
UM-1	C58 (k)	Spl	Spont.	‡	0.4±0.1
UM-2	C58 (k)	Spl	Spont.	‡	1.0±0.2
CAM-1	BALB/c (d)	Spl	Spont.	‡	0.2±1.3
A13	BALB.B (b)	LN, Spl	MuLV	15	−0.1±0.8
10B89	B10×BALB.B (b)	LN, Spl	MuLV	15	1.5±1.6
DC75	B10.D2 ×BALB/c (d)	LN, Spl	MuLV	15	0.3±0.9
F39	SWR/J (s)	LN, Spl	MuLV	‡	0.3±0.3
PF6-MELN	SWR/J (s)	LN	MuLV	‡	0.2±0.6
L1210	DBA/2 (d)	LN, Spl	MCA	16	0.2±1.0
P288	DBA/2 (d)	LN	MCA	17	0.2±1.2
P388	DBA/2 (d)	LN	MCA	18	1.0±0.8
L5178Y	DBA/2 (d)	Spl	MCA	19	0.2±1.3
Lymphoma 4	DBA/2 (d)			20	−0.5±0.8
SL-2	DBA/2 (d)		Spont.	21	0.8±0.1
P1534	DBA/2 (d)	Thymus	Spont.	22	0.9±0.4
R1	C58 (k)	Thymus	Spont.	23	−0.4±1.2
EL-4	C57Bl/6 (b)	Thymus	DMBA	24	−0.7±1.6
AKR-3T	AKR (k)	Thymus	Spont.	‡	−1.0±0.3
RLδ1-11	BALB/c (d)	Thymus	X-ray	25	1.0±0.4
L691-6	C57L (b)	Thymus	X-ray	26	0.3±1.4

Per cent specific target cell lysis is defined as 100(ER − SR)/(100 − SR), where ER (experimental release) and SR (spontaneous release) are percentages of ^{51}Cr release from target cells in, respectively, the presence and absence of Con A. Values are mean ± s.d. of determinations at lymphoma to target cell ratios of 13 and 25. The presence of lymphoma cells slightly (1 or 2%) decreased the spontaneous release of ^{51}Cr from target cells (in the absence of Con A). The target cells, MOPC-167 cells (H-2^d), were labelled by incubation in medium containing $Na_2{}^{51}CrO_4$, and cytotoxicity was assayed as described[13]. Cytotoxic cells used as positive controls for the Con A-dependent cytolysis were peritoneal exudate cells taken from BALB/c (H-2^d) mice that had had two intraperitoneal injections of EL-4 (H-2^b) cells[1], that is, H-2^d anti-H-2^b. TB-1 and TB-3 arose as spontaneous lymph node lymphomas in old (>14 month) thymectomised AKR mice; TB-2 arose as a spontaneous spleen lymphoma in a 14–16-month-old AKR mouse with an atrophic thymus[3]. These three lymphomas were adapted to grow in culture by Durda, Boos and Gottlieb[27]. Ib and UM-2, and CAM-1 are maintained in C58 and BALB/c mice, respectively, by intraperitoneal passage of cells from enlarged spleens. The seven DBA/2 tumours are maintained in ascites tumours in DBA/2 or CD2 F_1 mice. All other lymphomas are maintained in culture.

* Source from which the lymphoma was derived, not necessarily the tissue in which neoplastic transformation took place. LN = lymph node; Spl = spleen.

† Spont. = spontaneous; MuLV = murine leukaemia virus; DMBA = dimethyl benzanthracene; MCA = methylcholanthrene.

‡ UM-1 and UM-2 were isolated by L. Pease and W. H. Murphy, CAM-1 by T. W. Chang, E. Celis and H. N. Eisen, F39 and PF6-MELN by P. Fahnestock, N. H. Hopkins and P. D. Gottlieb, and AKR-3T by N. Rosenberg.

us to investigate whether some lymphocytic tumour cells can, like normal cytotoxic T lymphocytes, lyse target cells. We report here that they can.

Because it was unlikely that any particular cytotoxic T lymphoma would be specific for any of the few target cells available to us, we included concanavalin A (Con A) in the assay mixture of lymphoma cells and target cells. In the presence of Con A cytotoxic T cells lyse target cells nonspecifically, evidently because Con A links killer and target cells and activates the killer cell's lethal mechanism[2]. Initially we used cells of a myeloma (MOPC-167) of BALB/c mice as targets.

In a typical assay, 1×10^4 ^{51}Cr-labelled MOPC-167 cells and 0 to 50×10^4 lymphoma cells were mixed in a total volume of 200 μl of a standard cell culture medium (RPMI-1640 with 10% fetal calf serum), with or without Con A (5 μg ml^{-1}). The cells were incubated at 37 °C for 4 h, and the ^{51}Cr released into the medium by the target cells was measured. A positive control was included in each assay: either peritoneal exudate lymphocytes from BALB/c mice that had been immunised against allogeneic EL-4 lymphoma cells of C57Bl/6 mice[1], or one of the lymphomas with cytotoxic ('killer cell') activity (see below).

None of the 24 lymphomas tested was cytotoxic in the absence of Con A, but three (TB-1, TB-2 and TB-3) had cytotoxic activity in the presence of Con A (Table 1). Representative titrations are shown in Fig. 1. The three active lymphomas also caused Con A-dependent lysis of other target cells (P815, a mastocytoma of the DBA/2 strain, and EL-4), and their cytotoxic activity was facilitated just as well by phytohaemagglutinin as by Con A (data not shown).

The surface antigens of TB-1, TB-2 and TB-3 were previously studied with *in vivo*-passaged tumours and with TB-1 as cultured cells[3]. In addition to Thy-1 and Lyt, characteristic T-cell markers[4], these tumours were observed, through the use of immunofluorescence, intermittently to have surface (but not intracellular) immunoglobulins, Ia, and Fc receptor, considered characteristics of B cells[5]. We verified that virtually all cells of the cytotoxic cultured lines used in the present study could be lysed specifically by anti-Thy-1.1 antibody[6] (plus complement); we are re-investigating whether they synthesise immunoglobulin. The Lyt antigens and the crawling activity of all the tumours listed in Table 1 will be reported elsewhere (T.W.C., E. Celis, P. D. Gottlieb, F. Solomon and H.N.E., in preparation). Tentatively, it seems that all the killer cells have crawling activity, but that some crawlers are not cytotoxic[1].

Previous studies indicate that lysis of target cells in the presence of Con A or phytohaemagglutinin is due to cytotoxic T cells, not to other activated T cells[7], and that the same lytic mechanisms are involved in Con A-dependent and antigen-dependent target cell lysis[8]. Other evidence, however, indicates that the T cells engaged in Con A-dependent killing may lack specific receptors for antigen on target cells, while retaining the killing apparatus[9]. If specific receptors are absent, it may be possible to introduce them by fusing the immortal killer lymphomas with normal cytotoxic T lymphocytes of known specificity (or their precursors), just as myeloma cells have been fused with normal B lymphocytes to yield hybridomas that produce specific antibodies[10]. Cytotoxic T lymphomas are potentially useful for studying the cytolytic mechanism of killer lymphocytes and the properties of their antigen-recognition molecules.

Most mouse T lymphomas arise in the thymus, but the three cytotoxic lymphomas of Table 1 originated in the spleen or lymph nodes[3]. It may be more useful to look for additional killer T lymphomas among the rare lymphomas that seem to arise in relatively mature T-cell populations of spleen and lymph nodes than among the more common lymphomas that arise in the thymus. Lymphomas seem to appear with relatively high frequency in spleen and lymph nodes in old (>12 months) mice of the C58, B10.Y and HRS/J strains (H. Meier and W. B. Murphy, personal communications). Normal cytotoxic T cells, maintained in culture for long periods, are being evaluated as alternative sources of homogeneous killer cells. However, these normal cells require restimulation of T-cell factors for growth and for generation of cytolytic activity[11,12], and the quantity of cells that can be obtained is limited.

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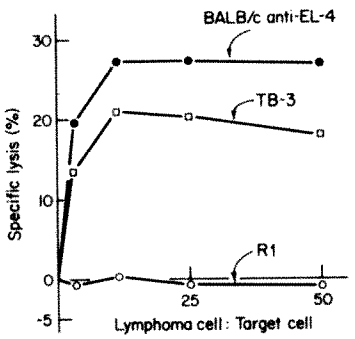


Fig. 1 Con A-dependent lysis of target cells by normal cytotoxic T lymphocytes and two lymphomas. The targets, immune normal lymphocytes, and lymphomas are described in Table 1.

lines: P. Gottlieb (TB-1, TB-2, TB-3, F87N, PF6-MELN, A13, 10B89, DC75), W. Murphy (Ib, UM-1, UM-2), M. Bevan (R1), H. Winn (EL-4), N. Rosenberg (AKR-3T, L691-6), E. Siden (RLσ1-11), and B. Benacerraf (SL-2); Arthur D. Little Co. provided P288, P388, L1210, P1534, L5178Y and Lymphoma 4. This work was supported by grants CA-15472 and CA-14051 from the NCI, DHEW, and by grant IM-161 from the American Cancer Society.

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The role of glucocorticoid receptor and gene expression in the anti-inflammatory action of dexamethasone

GLUCOCORTICOIDS are potent anti-inflammatory agents, but the molecular mechanisms involved in this activity are not yet understood. The biological actions of various steroid hormones have been shown to be mediated by functional proteins synthesised following translocation of a hormone-cytoplasmic receptor complex into the nucleus[1,2]. It is still not clear, however, whether the anti-inflammatory effects of glucocorticoids are mediate via binding to a cytoplasmic receptor in target cells and subsequent activation of gene expression[3], although glucocorticoid receptors have been demonstrated in HTC cells, liver[5], thymocytes[6] and lymphocytes[7]. The results reported here suggest that a glucocorticoid receptor and the induction of gene expression are involved in the anti-inflammatory action of dexamethasone.

Experiments were undertaken to determine (1) whether progesterone, fluoxymesterone and 17α-methyltestosterone (designated by Samuels and Tomkins[8] as 'anti-inducers' on the basis of their antagonism of corticoid induction of tyrosine aminotransferase in hepatoma tissue culture cells) interfere with anti-inflammatory action of dexamethasone and (2) whether RNA and protein synthesis is essential for the manifestation of the anti-inflammatory action of dexamethasone. Serotonin-induced footpad oedema in mice was used as a model of inflammation, since the mechanism of the vascular permeability increase in serotonin-induced inflammation has been well characterised[9].

An inflammatory response was induced by injecting serotonin (0.3 μg in 5 μl of *p*H 7.4 Tyrode's solution) in the subplantar region of the right foot of 6-week-old male ddY mice (25–30 g). The left foot was injected with Tyrode's solution only. Inflammatory response (oedema) was evaluated by measuring the difference in footpad thickness between right and left feet with a dial guage calliper 12 min after serotonin injection. The serotonin dose used was chosen on the basis of a dose-response curve obtained in preliminary experiments. Dexamethasone dissolved in ethanol/0.9% NaCl (1:39, v/v) was injected subcutaneously (s.c.) in the abdomen. Anti-inducer steroids were injected s.c. at a separate site on the abdomen.

Preliminary experiments suggested the existence of a time lag for manifestation of the anti-inflammatory effect of dexamethasone. To determine the appropriate dose for dexamethsone as inhibitor of serotonin-induced inflammation, dexamethasone was given at 0.15, 0.5 and 1.5 mg per kg body weight. There was significant inhibition of inflammation at 0.5 and 1.5 mg per kg (65 and 78% inhibition respectively). Using a dexamethasone dose of 0.5 mg per kg the inflammatory lesion was induced at various times after dexamethasone treatment. Dexamethasone did not inhibit serotonin-induced inflammation within 1 h after administration, but the anti-inflammatory effect was apparent at 2 h, reached a maximum at 3 h and persisted for 6 h or longer. Based on these results, the inflammatory lesion was provoked in all of the following experiments 2.5–6 h after injection of dexamethasone.

In the first series of experiments for anti-inducer compounds (Table 1), the test compounds were given 1 h before dexamethasone and footpad inflammation was provoked 3 h after dexamethasone injection. A single treatment with dexamethasone caused a marked reduction of the inflammatory response. Progesterone, fluoxymesterone and 17α-methyltestosterone, when applied before dexamethasone treatment, brought about a marked inhibition of the anti-inflammatory effect of dexamethasone. The degree of the inhibition was dose-dependent, and complete blockade of the anti-inflammatory action was observed with all three anti-inducer steroids at doses of 200 mg per kg. Treatment with these non-corticoid anti-inducer steroids alone exerted no appreciable influence on the serotonin-induced inflammatory response. On the other hand, androstenedione, was classified as an inactive compound by Tomkins *et al.*[8] exerted no detectable influence on either the inflammatory response to serotonin or the anti-inflammatory action of dexamethasone (Table 1, series 1).

Similarity between the ability of the anti-inducer steroids to block anti-inflammatory action of dexamethasone and their ability to inhibit both glucocorticoid induction of tyrosine aminotransferase and glucocorticoid binding with the specific receptor in HTC cells suggests that similar mechanisms may be involved in these processes. If binding of glucocorticoid to specific receptor, and subsequent transfer of the corticoid–receptor complex into the nucleus are essential components of anti-inflammatory corticoid effects, the application of an inhibitor of corticoid–receptor binding after the corticoid–receptor complex has been transferred into nucleus would not block the final effects of the corticoid. To examine such possibilities, the second series of experiments was performed. An anti-inducer steroid 17α-methyltestosterone was given 1 h after dexamethasone treatment and serotonin was injected 3 h after dexamethasone to induce inflammation. The results are presented in Table 1 (series 2). Administration of the anti-inducer steroid at this late stage exerted no detectable influence on the anti-inflammatory action of dexamethasone. Thus 17α-methyltestosterone inhibits glucocorticoid action by blocking exclusively an early step in the series of reactions which leads to the anti-inflammatory effect. Antagonism of some steroidal compounds to various biological effects of the corticoids has been described in HTC cells[4,8], skeletal muscle[10] and thymocytes[6], and discussed in relation to their ability to

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Table 1 Effect of progesterone, fluoxymesterone, 17α-methyltestosterone and androstenedione on anti-inflammatory action of dexamethasone in mice

									Time of treatment or measurement (h)
Series 1									
Progesterone (mg per kg)	0	0			100	100	200	200	−1
Dexamethasone (mg per kg)	0	0.5			0.5	0	0.5	0	0
Oedema (mm)	0.38 ± 0.034	0.13 ± 0.009*			0.25 ± 0.013*†	0.36 ± 0.032†	0.32 ± 0.021†	0.32 ± 0.034†	3
Fluoxymesterone (mg per kg)	0	0			100	100	200	200	−1
Dexamethasone (mg per kg)	0	0.5			0.5	0	0.5	0	0
Oedema (mm)	0.35 ± 0.024	0.10 ± 0.006*			0.25 ± 0.011†	0.31 ± 0.033†	0.34 ± 0.029†	0.37 ± 0.031†	3
17α-methyltestosterone (mg per kg)	0	0	50	50	100	100	200	200	−1
Dexamethasone (mg per kg)	0	0.5	0.5	0	0.5	0	0.5	0	0
Oedema (mm)	0.39 ± 0.037	0.15 ± 0.019*	0.19 ± 0.035*	0.39 ± 0.022†	0.29 ± 0.041†	0.33 ± 0.031†	0.33 ± 0.049†	0.31 ± 0.036†	3
Androstenedione (mg per kg)	0	0			100	100	200	200	−1
Dexamethasone (mg per kg)	0	0.5			0.5	0	0.5	0	0
Oedema (mm)	0.41 ± 0.028	0.15 ± 0.013*			0.16 ± 0.020*	0.36 ± 0.024†	0.15 ± 0.019*	0.34 ± 0.015†	3
Series 2									
17α-methyltestosterone (mg per kg)	0	0			100	100			1
Dexamethasone (mg per kg)	0	0.5			0.5	0			0
Oedema (mm)	0.41 ± 0.041	0.09 ± 0.007*			0.10 ± 0.011*	0.36 ± 0.031†			3

In series 1 experiments, non-corticoidal steroids were given s.c. 1 h before s.c. injection of dexamethasone. Serotonin-footpad-oedema was provoked 3 h after the dexamethasone treatment. In series 2, 17α-methyltestosterone was injected s.c. 1 h after application of dexamethasone. Serotonin-footpad oedema was induced 3 h after the dexamethasone treatment. The oedema was expressed in terms of the increase in the footpad thickness and the values represent means of seven or eight mice ± s.e.m.

*Statistically significant differences with respect to control group, $P < 0.01$.

†Significance differences with respect to dexamethasone group, $P < 0.01$.

Table 2 Effect of cycloheximide and actinomycin D on anti-inflammatory action of dexamethasone in mice

							Time of treatment or measurement (h)
Series 1							
Dexamethasone (mg per kg)	0	0.5	0.5	0			0
Cycloheximide (mg per kg)	0+0	0+0	3+3	3+3			0 and 1.5
Oedema (mm)	0.47 ± 0.026	0.17 ± 0.016*	0.40 ± 0.036†	0.49 ± 0.042†			3
Dexamethasone (mg per kg)	0	0.5	0.5	0			0
Cycloheximide (mg per kg)	0+0	0+0	6+6	6+6			0 and 1.5
Oedema (mm)	0.45 ± 0.058	0.16 ± 0.025*	0.43 ± 0.038†	0.38 ± 0.041†			3
Dexamethasone (mg per kg)	0	0.5	0.5	0	0.5	0	0
Actinomycin D (mg per kg)	0+0	0+0	1+1	1+1	2+2	2+2	0 and 1.5
Oedema (mm)	0.38 ± 0.033	0.14 ± 0.024*	0.38 ± 0.034†	0.39 ± 0.072†	0.38 ± 0.012†	0.45 ± 0.029†	3
Series 2							
Dexamethasone (mg per kg)	0	0.5	0.5	0			0
Cycloheximide (mg per kg)	0+0	0+0	3+3	3+3			3 and 4.5
Oedema (mm)	0.36 ± 0.043	0.06 ± 0.012*	0.13 ± 0.021*	0.39 ± 0.055†			6
Series 3							
Dexamethasone (mg per kg)	0	0.5	0.5	0			0
Cycloheximide (mg per kg)	0	0	3	3			1
Oedema (mm)	0.47 ± 0.058	0.15 ± 0.015*	0.44 ± 0.036†	0.41 ± 0.036†			2.5

In series 1, cycloheximide and actinomycin D were given s.c., twice, simultaneously and 1.5 h after s.c. injection of dexamethasone. Serotonin-footpad oedema was provoked 3 h after the injection of dexamethasone. In series 2, cycloheximide was applied, twice, at 3 and 4.5 h after the application of dexamethasone and the serotonin oedema was induced 6 h after the dexamethasone injection. In series 3, cycloheximide was given 1 h after the dexamethasone treatment and the serotonin oedema was induced 2.5 h after the dexamethasone injection. Amounts of cycloheximide and actinomycin D used were sufficient to inhibit RNA and protein synthesis[11–13].

*Statistically significant differences with respect to control group, $P < 0.01$.

†Statistically significant differences with respect to dexamethasone group, $P < 0.01$.

compete with glucocorticoids in binding with the specific receptor in target cells. Our demonstration of antagonism by 'anti-inducer steroids' of the anti-inflammatory action of the corticoid suggests a role for corticoid receptor in the anti-inflammatory action of the corticoid. It will be difficult, however, to directly demonstrate antagonism at the receptor site because it is not clear which are the primary target cells in the anti-inflammatory action of the corticoid.

To test for the requirement for RNA and protein synthesis, actinomycin D or cycloheximide[11–13] was given twice, simultaneously with dexamethasone and 1.5 h later, in an attempt to inhibit RNA or protein synthesis for 3 h consecutively after the application of dexamethasone. The results summarised in Table 2 (series 1) clearly indicate that the anti-inflammatory effect of dexamethasone is completely blocked by actinomycin D or cycloheximide, suggesting a requirement for synthesis of new

RNA and protein. To establish whether uninterrupted protein synthesis throughout experimental periods is essential, mice were injected with cycloheximide 3 and 4.5 h after application of dexamethasone in an attempt to block protein synthesis for the period from 3 to 6 h after dexamethasone treatment. The inflammatory lesion was provoked at 6 h after the dexamethasone treatment. Cycloheximide given at this stage did not block the anti-inflammatory action of the steroid (Table 2, series 2). Further experiments were performed to characterise the mechanism of the effect of cycloheximide injected 1 h after application of dexamethasone: When serotonin footpad oedema was induced 1.5 h after application of cycloheximide there was complete blockade of the anti-exudative effect of dexamethasone (Table 2, series 3).

For several of the metabolic effects of glucocorticoids a latency period of 0.5–2 h has been reported[14]. These time lags probably represent time required for events subsequent to entering of the corticoid into target cells to bring about production of specific protein through gene expression. A similar time lag has been reported[15] for the inhibitory effect of dexamethasone against experimental anaphylaxis and histamine-induced inflammatory reactions in rat and mice. In agreement with these observations, a latency period of 1–2 h was observed in the present experiments with respect to manifestation of the anti-inflammatory action of dexamethasone. When actinomycin D or cycloheximide was applied simultaneously (Table 2, series 1) with dexamethasone or during the latency period (Table 2, series 3), the anti-inflammatory effect of the steroid was abolished. Therefore, both RNA and protein synthesis appear to be essential for manifestation of the anti-inflammatory activity of dexamethasone. However, as the anti-inflammatory effect of the steroid was not affected by cycloheximide applied 3 h after administration of the steroid (Table 2, series 2), it is not essential that protein synthesis proceeds without interruption over the entire period. It is likely, therefore, that new RNA and protein synthesised under the influence of dexamethasone play an essential role in the manifestation of the anti-inflammatory activity.

During preparation of this paper Flower and Blackwell reported evidence of induction in the guinea pig lung by glucocorticoids of biosynthesis of a protein (or peptide) inhibitor which prevents protaglandin generation[16]. It is possible that similar mechanisms might underlie the inhibitory action of dexamethasone against serotonin-induced inflammatory response, since serotonin stimulates some tissues to generation of rabbit aorta contracting substances (a mixture of prostaglandin endoperoxides and thromboxane A_2)[17]. However, the finding that indomethacin does not affect serotonin-induced paw oedema in rats[18] suggests that serotonin provokes an inflammatory response independently of the prostaglandin and thromboxane generation system. We also confirmed that indomethacin was inert in inhibiting mice footpad oedemata induced by various phlogistic agents such as serotonin, bradykinin and PGE_1, whereas dexamethasone suppressed all of them. It is therefore likely that dexamethasone displays anti-inflammatory action, apart from phospholipase inhibition[16], through direct suppression of endothelial cell contraction induced by chemical mediators such as serotonin[9].

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A new proposal regarding the subunit composition of $(Na^+ + K^+)$ATPase

THREE types of high affinity ligands are available for studies with $(Na^+ + K^+)$ATPase: nucleotide substrates, vanadate and ouabain. Of these, the last is unique in apparently being absolutely specific for $(Na^+ + K^+)$ATPase. One of the generally accepted facts regarding $(Na^+ + K^+)$ATPase is that there are equal numbers of measurable (high affinity) binding sites for ATP and ouabain per molecule of enzyme[1–5]. There is a majority opinion that direct binding experiments yield binding capacities for these ligands of 1 mol site per 250–300 kg protein in the purest preparations available[3,6–8]. From these data, from determinations of the peptide composition and from estimates of the apparent molecular weight of solubilised enzyme the idea of $(Na^+ + K^+)$ATPase as a dimer (with respect to the large, so-called catalytic, peptide) has emerged[9–14]. Such a dimer would have one high affinity binding site for nucleotide and one for ouabain. The present report on binding site determinations on mammalian kidney microsomes before and after detergent treatment casts serious doubts on the general validity of the above simple relationship between sites and peptide composition.

The essential points of this report are represented in Figs 1 and 2. Figures 1 and 2*b* show that, in agreement with what has been outlined above, detergent-treated kidney microsomes have an equal number of high affinity nucleotide-binding sites and of ATP-supported ouabain-binding sites and P_i-supported ouabain-binding sites. The number of vanadate-binding sites happens also to be the same, contrary to the findings of Cantley *et al.*[5].

If one turns to the source of these enzyme preparations, that is, crude microsomal fraction from pig kidney outer medulla, it can be seen that this simple stoichiometry no longer holds (Table 1 and Fig. 2*a*). The microsomes have equal capacities for binding nucleotide and vanadate and for ATP-supported ouabain binding, but capacities exactly twice as large for both P_i-supported ouabain binding and for vanadate-supported ouabain binding.

The complexity of the situation is further stressed by the curvature of the isotherms for nucleotide binding, vanadate binding, and ATP-supported ouabain binding (Fig. 2*a*). Such curvature is diagnostic of an inhomogeneity in the site populations[21,22]. On the assumption that the observed inhomogeneity is due to a mixture of only two populations of sites, non-linear regression analysis of the data[23] strongly suggests that for all three ligands the two populations are of identical size (Table 1). This result is also mathematically compatible with a system of two inherently identical, negatively cooperating sites. The excellent fit of the calculated isotherms to the experimental data, as seen in Fig. 2*a*, makes it unnecessary to consider more complicated models at this point.

Obviously, some changes have occurred in the microsomal enzyme during or subsequent to detergent treatment. Figure 2*b* shows the results of binding studies made in the presence of those amounts of SDS added to the same microsomal

Table 1 Binding capacities for various ligands and K^+-dependent *p*-nitrophenyl phosphatase activities of microsomal $(Na^+ + K^+)$ATPase from pig kidney outer medulla before and after SDS treatment

Enzyme preparation	Relative ligand binding capacities*				Relative *p*-nitrophenyl phosphatase activity†	Mol *p*-nitrophenyl phosphate split per min per mol P_i-supported ouabain-binding site
	P_i-supported ouabain-binding capacity	Vanadate-supported ouabain-binding capacity	Nucleotide-binding capacity, vanadate-binding capacity, and ouabain-binding capacity (ATP-supported) Total	Subpopulations		
Microsomes	4	4.0	2.0	1.07 & 0.93	1	0.39×10^3
		4.0	2.0	0.96 & 1.04		0.41×10^3
Microsomes + SDS	4.1	—	4.0	None apparent	3.9	1.53×10^3
	3.9		4.0		3.9	1.59×10^3
Purified enzyme preparations						1.49×10^3
						1.52×10^3

The table shows values obtained with two separate microsome preparations before and after addition of SDS.
* Values are expressed relative to P_i-supported ouabain-binding capacity of untreated microsomes. This we define as being equal to 4.
† Relative to that of untreated microsomes.

preparation of Fig. 2*a* so as to yield maximal *p*-nitrophenyl phosphatase activity. It can be seen (see also Table 1) that the SDS-treated microsomal enzyme has equal binding capacities for each of the ligands tested; that the P_i-supported ouabain-binding capacity has not changed; that *p*-nitrophenyl phosphatase activity has quadrupled; and that the molar activity of the phosphatase is identical to that of enzyme purified through SDS treatment. In other words, the enzyme has now been converted to the same state as found in purified (and pure) preparations. And pure preparations of $(Na^+ + K^+)$ATPase contain one ouabain-binding site and one nucleotide-binding site per two large, catalytic peptides. As the P_i-supported ouabain-binding capacity of the enzyme remains unchanged with SDS treatment, it must be concluded that untreated microsomes have one P_i-supported ouabain-binding site per two large peptides.

As microsomes have one nucleotide-binding site and one vanadate-binding site per two P_i-supported ouabain-binding sites, two conclusions are possible at this stage. (1) In microsomes there exists a mixture of exactly equal amounts of two types of enzyme, each containing two large peptides and one P_i-supported ouabain-binding site. One nucleotide-binding site and one vanadate-binding site must then be distributed between the two types of enzyme. (2) Microsomal $(Na^+ + K^+)$ATPase contains four large peptides per molecule, with two P_i-supported ouabain-binding sites, one nucleotide-binding site and one vanadate-binding site. The fact that protein with one vanadate-binding site has two vanadate-supported ouabain-binding sites (Fig. 2*a* and Table 1) makes the choice of an ATPase molecule with four large peptides obvious at this point. However, in microsomes the nucleotide-binding sites, the vanadate-binding sites and the ATP-supported ouabain-binding sites all seem to be distributed among two populations of identical size (Table 1). It therefore becomes necessary once again to make a choice between two possibilities. (1) In microsomes there are identical amounts of two different enzymes, each with one nucleotide-binding site, one vanadate-binding site and two P_i-supported ouabain-binding sites per molecule. (2) Microsomal $(Na^+ + K^+)$ATPase has four P_i-supported ouabain-binding sites, two nucleotide-binding sites and two vanadate-binding sites per molecule.

A choice can be made on the basis of the following arguments. Treatment of microsomes with SDS converts the curved binding isotherms of Fig. 2*a* into straight lines (Fig. 2*b*). This suggests that the inhomogeneity of the microsomal site populations was a consequence of interactions between pairs of inherently identical sites (for example, negative cooperativity)[24,25], and that treatment with SDS has disrupted the interaction.

We will disregard the possibility that it is due to chance alone that in microsomes there are equal amounts of two types of nucleotide-binding site—vanadate-binding sites and ATP-supported ouabain-binding site—and exactly twice as many P_i-supported ouabain-binding sites and vanadate-supported ouabain-binding sites; and that SDS treatment exactly doubles the number of the former sites and quadruples the *p*-nitrophenyl phosphatase activity. Thus, for example, we deem it unlikely that one can reproducibly prepare microsomes containing appropriate proportions of inside-out, right-side-out and open vesicles with peculiar permeabilities. We must therefore choose the second possibility mentioned above, and conclude that native $(Na^+ + K^+)$ATPase as found in pig kidney microsomal preparations from the outer medulla seems to be a molecule containing eight large, catalytic peptides with four

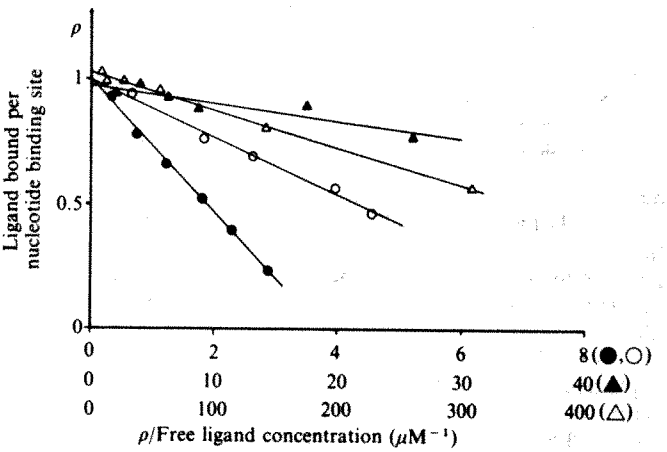


Fig. 1 Scatchard plots of data of equilibrium binding of ^{14}C-ATP (○), ^{14}C-ADP (●), ^{48}V-vanadate (▲) and of ^{3}H-ouabain (as supported by P_i) (△) to a purified preparation of $(Na^+ + K^+)$ATPase from pig kidney outer medulla. The intercepts on the ordinate, calculated by linear regression analysis, give a measure of the total ligand capacities. The numerical value of each slope is equal to the dissociation constant of the ligand in question. The enzyme was prepared as described by Jørgensen[15], except that ATP was omitted from the SDS activation medium. $(Na^+ + K^+)$ATPase activity (assayed according to Nørby[16]): 12.7 μmol substrate split per min per mg protein. K^+-dependent *p*-nitrophenyl phosphatase activity (assayed according to Skou[17]): 2.3 μmol substrate split per min per mg protein. Nucleotide binding was measured by the method of Nørby and Jensen[18]. P_i-supported ouabain binding was carried out according to Hansen[19] in the presence of 3 mM Mg^{2+}, 3 mM P_i and 40 mM Tris-HCl (*p*H 7.25, 37 °C). A similar filtration method was used for determinations of vanadate binding, after incubation of enzyme with varying concentrations of ^{48}V-vanadate for 60 min at 25 °C with 5 mM Mg^{2+}, 10 mM K^+ and 40 mM Tris-HCl (*p*H 7.25). ^{48}V-vanadate was from the Radiochemical Centre, Amersham and was diluted with orthovanadate obtained by dissolving $NaVO_3$ in NaOH.

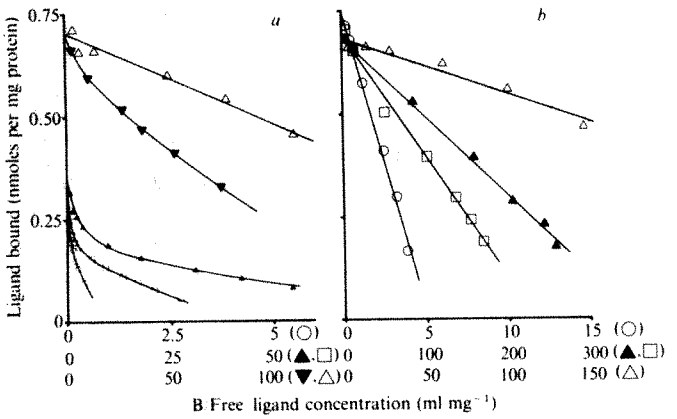


Fig. 2 Scatchard plots of data of equilibrium binding of ^{14}C-ATP (○), ^{48}V-vanadate (▲), ^{3}H-ouabain as supported by P_i (△), by vanadate (▼) and by ATP (□) to pig kidney microsomal ($Na^+ + K^+$)ATPase prepared according to the first steps of Jørgensen's procedure[15]. Total ligand binding capacities can be obtained from intercepts of the curved plots with the ordinate. Non-linear regression as described in the text was used for an analysis of these curves. For the straight line plots, see text to Fig. 1. *a*, Microsomes before SDS treatment. ($Na^+ + K^+$)ATPase activity: 1.18 μmol ATP split per min per mg protein. K^+-*p*-nitrophenyl phosphatase activity: 0.31 μmol substrate split per min per mg protein. *b*, Same microsomal preparation after SDS treatment. Microsomes suspended at a concentration of about 4 mg protein ml^{-1} in 250 mM sucrose, 30 mM imidazole and 0.06 mM EDTA (*p*H 7.2, 20 °C), were treated for 20 h at room temperature with 0.06% SDS (stable, maximal K^+-*p*-nitrophenyl phosphatase activity was obtained in these conditions). ($Na^+ + K^+$)ATPase activity: 6.30 μmol ATP split per min per mg protein. K^+-*p*-nitrophenyl phosphatase activity: 1.19 μmol substrate split per min per mg protein. Binding of ligands was carried out in the presence of the added SDS. ATP-supported ouabain binding took place in the presence of 3 mM Mg^{2+}, 120 mM Na^+, 3 mM ATP and 40 mM Tris-HCl (*p*H 7.25, 37 °C). Vanadate-supported ouabain binding took place in the presence of 3 mM Mg^{2+}, 40 mM Tris-HCl and 10 μM vanadate[20]. Other procedures were those listed in Fig. 1.

ouabain-binding sites and four nucleotide-binding sites. Of the latter, only two are available for binding substrate at any one time, that is, there is total negative cooperativity between two pairs of such sites. The nucleotide-binding sites and ouabain-binding sites would be distributed most reasonably on four identical subunits containing two large peptides each.

The result of treatment of microsomes with SDS could be explained as a relaxation of two different and coexistent interactions: the total negative cooperativity between pairs of sites and another, less overwhelming, interaction within pairs of sites. The conversion of one molecule of enzyme into four more-or-less independent subunits would be reasonable grounds for the quadrupling of *p*-nitrophenyl phosphatase activity that accompanies SDS treatment.

If the effects of SDS described in the present report are not due to a trivial artefact, and if they can be duplicated (by whatever physiological mechanism) *in vivo*, the proposed changes in interaction between four functional subunits of ($Na^+ + K^+$)ATPase would allow for a wide range in the regulation of the activity of the enzyme and, therefore, of the sodium pump.

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Semi-synthesis of human insulin by trypsin-catalysed replacement of Ala-B30 by Thr in porcine insulin

HUMAN insulin differs from porcine insulin by a single amino acid (Thr instead of Ala) at the C-terminal residue of the B-chain. Inouye *et al.*[1] have established a procedure for the semi-synthesis of human insulin, in which trypsin is used as a catalyst for the coupling of desoctapeptide-(B23–B30)-insulin (DOI) with a synthetic octapeptide corresponding to positions B23–B30 of human insulin. DOI was prepared by tryptic digestion of porcine insulin. The enzymatic method has many advantages, in particular, better yields and simple operation, over the chemical methods originally used by Ruttenberg[2] and Obermeier and Geiger[3]. Here we report a more simple and economical method for the semi-synthesis, in which trypsin can catalyse the coupling between desalanine-B30-insulin (DAI) and Thr-OBu^t to generate human insulin in good yield.

DAI was obtained by digestion of porcine insulin with carboxypeptidase A (E/S = 1/100, in weight) for 8 h at 25 °C in the presence of 0.1 M NH_4HCO_3 (*p*H 8.3)[4]. Trypsin-catalysed coupling of DAI and Thr-OBu^t was determined quantitatively using a reverse-phase liquid chromatography (LC) system (for conditions, see Fig. 1), in which the ratio of [Thr-OBu^t-B30]-insulin produced to the remaining DAI was determined from their peak areas and intensity factors. A preliminary experiment indicated that the coupling occurred with a large excess of the amine component (Thr-OBu^t) in the presence of high concentrations of organic co-solvents. The significance of these co-solvents in coupling has been described previously[1,5,6]. To our surprise, there was no splitting of the Arg(B22)–Gly(B23) bond in DAI or the product; this was expected to be a problem, and has previously been thought to occur through the hydrolytic activity of trypsin.

Preparative-scale coupling was carried out as follows. 0.7 ml of 0.5 M Tris buffer containing trypsin (recrystallised three times; 4.1 mg, 0.1 mM), final *p*H 6.5, was added to a 1.05 ml solution of DAI (100 mg, 10 mM) and Thr-OBu^t (205 mg, 0.5 M) in a mixture of dimethylformamide and ethanol (1/1 by volume), and the reaction mixture was kept at 37 °C for 20 h. LC showed that 73% of the DAI had been converted into the product. The reaction mixture was applied to a column (3.2 × 41 cm) of Sephadex G-50 (super fine) and eluted with 0.5 M

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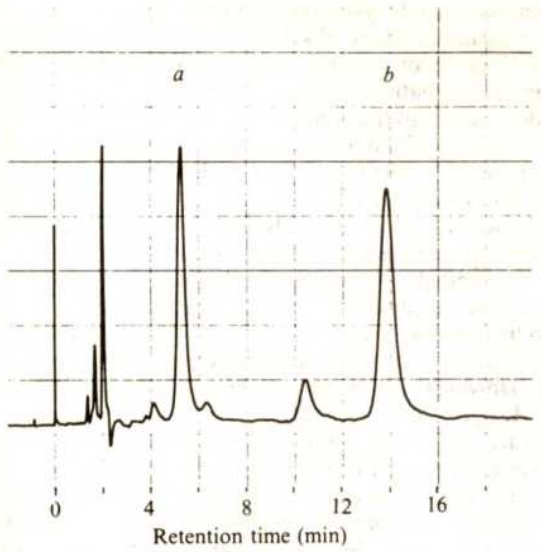


Fig. 1 Liquid chromatogram of porcine DAI (*a*) and [Thr-OBu^t-B30]-porcine insulin (*b*): column, Nucleosil 5 C_{18}, 0.4×20 cm; eluant, 30.5% CH_3CN in 5 mM phosphate buffer (*p*H 3.0) containing 5 mM n-$BuSO_3Na$ and 50 mM Na_2SO_4; detection at 220 nm.

acetic acid. Three fractions corresponding to trypsin, insulin or its derivatives, and Thr-OBu^t were successively eluted. The fraction corresponding to insulin or its derivatives was lyophilised (89 mg), and then applied to a column of DEAE-Sephadex A-25 (2×20 cm) which had been equilibrated with 0.01 M Tris buffer (*p*H 7.4) and 7 M urea. A linear Na^+ gradient (to 0.3 M NaCl) was begun at 4 °C according to the method of Bromer and Chance[7]. The first peak (35 mg) corresponded to [Thr-OBu^t-B30]-insulin and the second one (27 mg) to DAI and/or intact insulin (Figs 1, 2).

[Thr-OBu^t-B30]-insulin was treated for deprotection with trifluoroacetic acid in the presence of anisole. The yield was 41% (32 mg) based on the assumption that the purity of DAI used as the starting material was 78%, which had been deduced from its amino acid composition. This semi-synthetic human insulin was homogeneous according to LC[1] and polyacrylamide gel electrophoresis (Fig. 2). Amino acid analysis (theoretical values in parentheses) gave: Lys 1.00 (1), His 1.89 (2), Arg 0.87 (1), Asp 3.32 (3), Thr 3.16 (3), Ser 2.99 (3), Glu 7.35 (7), Pro 1.29 (1), Gly 4.39 (4), Ala 1.26 (1), $CySO_3H$ 5.38 (6), Val 3.93 (4), Ile 1.61 (2), Leu 6.51 (6), Tyr 3.68 (4), and Phe 3.19 (3). In the bioassay for hypoglycaemic activity, no significant difference was observed between the semi-synthetic human insulin and porcine insulin.

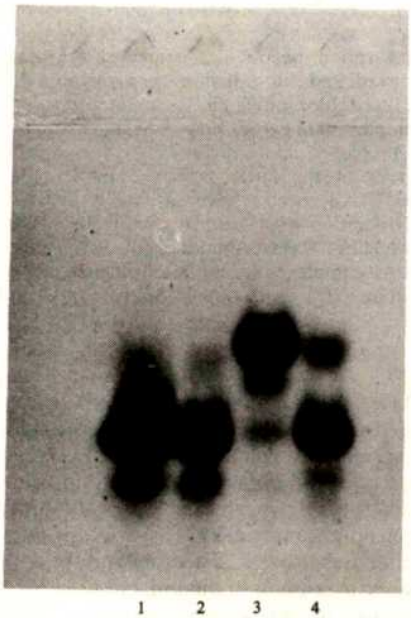


Fig. 2 Polyacrylamide gel electrophoresis of insulins (*p*H 8.0, 10% gel): 1, porcine insulin; 2, porcine DAI; 3, [Thr-OBu^t-B30]-porcine insulin; 4, semi-synthetic human insulin.

A similar preparative-scale experiment was carried out using DAI (5 mM) prepared from bovine insulin in the above conditions except that the enzyme concentration was 1 mM. LC showed that 80% of DAI had been converted into the product. The product was isolated by the above method, and pure semi-synthetic [Thr-B30]-bovine insulin was obtained in 60% yield.

The present study indicates that an enzymatic coupling system is a useful means of semi-synthesis of biologically active products, with either one or both component(s) starting from natural materials of very large molecular weight. Unlike conditions for chemical methods, for enzymatic methods functional groups in the starting materials may not need to be blocked. We must also stress that the hydrolytic activity of proteases used as a coupler is minimal in conditions suitable for synthesis, that is, in the presence of high concentrations of organic solvent and the amine or carboxyl component to be coupled.

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Chlorophyllin–apomyoglobin complexes

CHLOROPHYLL (Chl), *in vivo* as well as in protein complexes purified from photosynthetic organisms, absorbs light at longer wavelengths than does Chl as an isolated molecule in an organic solvent[1]. These bathochromic absorbance shifts are substantial, up to 40 nm (800 cm^{-1}) for Chl *a* in green plants and 100 nm (1,400 cm^{-1}) for bacteriochlorophyll (Bchl) *a* in photosynthetic bacteria. An important question is whether these shifts are caused largely by Chl–Chl interactions or by interactions between Chl and the proteins to which all Chl is bound *in vivo*[2–4]. This has been difficult to answer because no native Chl–protein complex yet found contains only one pigment molecule. We report here, substituting the water-soluble Chl derivative, chlorophyllin (Chln) *a*, for haem in myoglobin, and irradiating the resultant complex with red light, the first model Chl–protein complex that verifiably contains a single pigment molecule and that also exhibits a 1,000 cm^{-1} bathochromic shift in absorbance.

For protein-free model Chl dimers in which bathochromic absorbance shifts are also observed, explanations of the shift energy have been based in part on the resonance (exciton) interaction between the electronic transitions of the two closely

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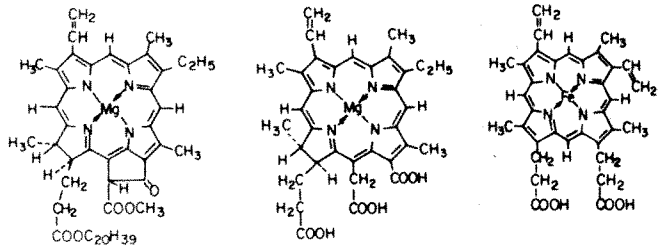


Fig. 1 Structure of (left to right): Chlorophyll *a*, Mg chlorin e_6 (chlorophyllin *a*), and Fe protoporphyrin IX (haem).

spaced Chl molecules[5]. A recent theoretical analysis attributes nearly half the shift energy to the exciton interaction[6]. But for two different Bchl–protein complexes about whose structure something is known[7,8], exciton interactions account in one case[7] for only a quarter of the shift[9] and in the other[8,10] for none of it. In choosing myoglobin as our model protein, we were guided by specific findings on these two complexes. The Bchl *a*-protein from *Prosthecochloris aestuarii* is the only native complex whose structure has been determined by X-ray crystallography[7]. The Mg atoms of all 21 of its Bchl molecules are 5-coordinate; for 5/7 of the Bchls the fifth ligand of the Mg is histidine[11]. The other is a 22,000 molecular weight purple bacterial complex (from *Rhodopseudomonas sphaeroides* R-26) that contains only two pigment molecules[8]. In this remarkable complex, the centroid of the CD spectrum is bathochromically shifted by 1,300 cm^{-1} (relative to Bchl *a* in ether[12]), occurring at a slightly longer wavelength than that of the absorbance peak. Theoretically, in an aggregate that contains only two chromophores, the centroid of the conservative CD band, which results from the exciton interaction, occurs at the wavelength where absorbance would be maximal in the absence of that interaction[13]. Thus, analysis of the spectra of this two-pigment complex shows that in it the exciton interaction causes a small hypsochromic shift in absorbance—the entire 1,300-cm^{-1} shift is unexplained.

In light of these considerations, apomyoglobin is almost ideally suitable as a model protein. Myoglobin itself is a small protein, and its structure is very well known from X-ray crystallography[14]. Its native prosthetic group, haem, is easily removed and can be replaced by another porphyrin[15]. In deoxymyoglobin the haem Fe atom is 5-coordinate, the fifth ligand being histidine. Also, nonspecific porphyrin–apomyoglobin binding seems unlikely. We used Breslow's method[16] to prepare apomyoglobin from sperm whale myoglobin (Sigma), and subsequently purified the apoprotein by gel filtration chromatography using Sephadex G-75.

All the porphyrins so far substituted for haem in myoglobin or haemoglobin are, like haem and unlike Chl, water soluble. For simplicity, we chose to work with Mg chlorin e_6, a water-soluble derivative of Chl *a* commonly called chlorophyllin *a* (refs 17, 18) (Fig. 1). Chlorophyllin is Chl-like in that it has a central Mg atom and a reduced ring IV, but haem-like in having multiple carboxylates and no ring V. The structural differences between Chl *a* and Chln *a* cause only minor changes in the visible absorption spectra. We used an established procedure[17,19] to prepare Chln *a* from Chl *a* that was purified from spinach leaves by the method of Strain and Svec[20].

To prepare the complexes, Chln and apomyoglobin were mixed in 0.1 M borate buffer (*p*H 9.2)[16]. The freshly prepared mixture absorbs maximally at 644 nm, bathochromically shifted only very slightly compared with free Chln in the same buffer. (Figure 2 shows the various spectral forms of Chln–apomyoglobin mixtures.) When the mixture was irradiated with red light ($\lambda \geq 600$ nm), the 644-nm peak diminished, and a new peak appeared at 690 nm. (A similar peak has been observed for Chln bound to polyvinylpyrrolidone[17]. In this system, a peak at ~690 nm occurs slowly in the dark and more rapidly under red illumination. In our system the dark production rate is too small to be measured.) In the absence of O_2, the total absorbance in the red region of the spectrum is approximately conserved during the irradiation. The rate of photoconversion is temperature dependent; in the conditions used here it required ~2 h at 22 °C, while at 5 °C it was too slow to observe conveniently. In the dark at 22 °C, the 690-nm peak disappeared in a few tens of hours, and was replaced by a peak at 646 nm. Again, total red absorbance was approximately conserved, although this dark relaxation process is O_2 independent. At 5 °C, the dark relaxation proceeded ~10 times more slowly. In contrast to the original 644-nm absorbing form, the 646-nm form is insensitive to red light in the absence of O_2. The occurrence of the relaxed form apparently limits the photoconversion yield of the 690-nm form. However, we readily achieved an absorbance ratio, A_{690}/A_{645}, of 1.1.

Freshly prepared, irradiated and irradiated-and-relaxed samples (2 ml) of Chln–apomyoglobin mixtures were chromatographed separately on 1 × 50 cm Sephadex G-75 columns at 5 °C. In each case, protein and pigment co-eluted at a volume corresponding to the molecular weight of myoglobin

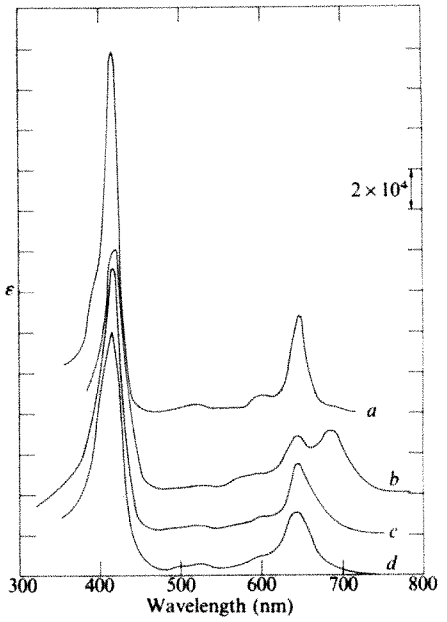


Fig. 2 Absorption spectra of: a, irradiated and relaxed; b, irradiated; c, unirradiated chlorophyllin–apomyoglobin complexes; and d, uncomplexed chlorophyllin *a*. The extinction coefficients for the protein complexes are expressed per mol apomyoglobin, and those for the uncomplexed chlorophyllin are expressed per mol pigment; the spectrum of the latter is scaled by Broyde's extinction coefficients[19]. All spectra have essentially zero absorbance at 800 nm. Irradiation is performed with a 75 W Xe lamp and a Corning 2-63 filter. Relaxed material remained in the dark at 22 °C for 7 days. All complexes are chromatographed on Sephadex G-75 and have 1:1 stoichiometry of pigment to protein.

(~18,000 MW), demonstrating that a complex was formed. Uncomplexed pigment eluted in much later fractions. Protein concentration was determined in the fractions by Lowry's procedure[21], using an equimolar mixture of Chln and apomyoglobin as a standard (pigment correction, 15%). Pigment was determined spectrophotometrically for the protein complex in aqueous buffer and also in 7:2 acetone/methanol solutions. The red absorbance maximum of the unirradiated complex (644-nm form) differs little in wavelength or extinction from that of Chln in buffer. The stoichiometry of this complex in ~18,000-MW fractions taken from the column is 1.0 ± 0.1 pigment molecule per protein when either 1.2:1 or 4:1 mixtures of pig-

ment/protein are loaded onto it. Because the red absorption of the complex is substantially changed on irradiation, the pigment content of both irradiated and irradiated-and-relaxed samples was estimated by assuming that the extinction coefficient at the maximum of the Soret band (~420 nm) is unchanged. On this basis, a stoichiometry of 1.1 ± 0.1 pigment per apomyoglobin in 18,000-MW fractions was found when either 1.2:1 or 3:1 mixtures of pigment/protein were irradiated and then chromatographed. The irradiated-and-relaxed complex has a stoichiometry of 1.2 ± 0.2 pigment per protein. The independence of the final stoichiometries from those in the initial mixtures is further substantiated by the constancy of the ratios of red band to Soret to protein tryptophan absorbance maxima in corresponding fractions of the two chromatographies. These results demonstrate the monomeric nature of the 690-nm absorbing Chln derivative that is formed as an apomyoglobin complex.

It is likely, though not proved, that other 690-nm absorbing forms of Chln, previously observed in protein-free systems and assumed to be aggregated forms of Chln[17,22] are also monomeric. In control experiments on irradiated Chln in protein-free borate buffer, a presumably monomeric 690-nm absorbing form is observed, together with a highly aggregated form of Chln that absorbs at 735 nm (Davis and Pearlstein, unpublished results). The latter form has also been observed in other protein-free Chln systems[17,22], but does not appear in solutions of apomyoglobin complexes.

The 690-nm monomer remains to be chemically identified. The close similarity of the absorbance spectra of the unirradiated and the irradiated-and-relaxed apomyoglobin complexes suggests that the chemical differences between these two are slight, and places a considerable constraint on the nature of the 690-nm form. The evidence suggests that the transformation to the 690-nm form is a property of the Chln itself. Although we do not know what this property is, one possibility is that it represents deformations of the Chln macrocycle that result from changes in axial ligation of the central Mg.

In conclusion, we have produced 1:1 complexes of apomyoglobin with the water-soluble chlorophyll derivative, chlorophyllin, that exhibit three spectral forms including one that, like chlorophyll *in vivo*, absorbs light at long wavelengths.

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An inhibitor protein of nuclear protein kinases

THE cyclical phosphorylation and dephosphorylation of proteins, catalysed by protein kinases and phosphoprotein phosphatases, respectively, are important ways in which cells regulate many of their metabolic activities. Cells seem to have at least two distinct phosphorylation systems, one in the cytoplasm, the other in the nucleus. The best studied function of the cytoplasmic protein kinases is their mediation of the effects of cyclic AMP in the modulation of existing metabolic pathways, whereas the nuclear protein kinases have been implicated in the regulation of gene activation[1]. This view is based on the rapid changes in nuclear protein phosphorylation that occur in response to stimuli of differentiation and cell growth[2–4], and because phosphorylated proteins are heavily concentrated in the actively transcribed regions of the genome[5]. For these reasons the nuclear protein kinases have been studied extensively, yet there is very little information concerning their regulation. It is thus of considerable interest to identify the proximal effectors of nuclear protein phosphorylation. We present here evidence for the presence in rat liver nuclei of a 150,000-molecular weight, heat-sensitive inhibitor protein that is specific for the nuclear protein kinases, being without effect on the cytoplasmic enzymes or their subunits. In addition to its potential importance as a negative regulator of nuclear protein kinases, the inhibitor may prove to be a valuable tool in assessing the role of nuclear protein phosphorylation in gene activation.

The nuclear protein kinases exist in multiple molecular forms that can be separated on ion-exchangers[8–11]. Attempts to demonstrate a specificity of individual isoenzymes towards some of their natural substrates has remained inconclusive because of the difficulty of obtaining substrate-free enzyme and enzyme-free substrate preparations. We have used affinity chromatography, which achieves the selective removal of the protein kinases from their endogenous phosphate acceptors[12]. Briefly, the procedure is to pass a crude nuclear extract, prepared as previously described[12], through a casein–Sepharose column. At 0.1 M NaCl the protein kinases are selectively retained on the column whereas the bulk of the proteins passes unretarded through the gel and is collected in the effluent (fraction I). The enzymes are then eluted from the column in a highly purified state with 0.6 M NaCl (fraction II). Enzyme activity in fraction II is demonstrable only on addition of casein or fraction I as phosphate acceptors. When incubated separately with [γ-^{32}P]ATP and magnesium neither fraction incorporated phosphate into trichloroacetic acid (TCA) precipitable material, but incubation of the combined fractions gave the same activity as the original extract[12].

The enzymes, essentially substrate free (fraction II), were resolved by chromatography on phosphocellulose into several peaks of activity demonstrable using casein as phosphate acceptor, as previously described[6]. The column fractions within three major peaks containing protein kinase activity were pooled and concentrated severalfold by pressure dialysis, then incubated with and without the endogenous substrates (fraction I) concentrated to contain 5 mg per ml of protein. The total reaction mixtures were analysed by electrophoresis in SDS-containing polyacrylamide gels. The results are shown in Fig. 1. Figure 1*a* depicts the proteins stained with Coomassie Brilliant Blue, Fig. 1*b* shows the autoradiograph of the same gel, depicting the proteins phosphorylated by each isoenzyme in the presence ('a' channels) and in the absence ('b' channels) of 100 μg protein of fraction I. It can be seen that the proteins contaminating the protein kinases purified by affinity chromatography and phosphocellulose are present in quantities too small to be stained by Coomassie Blue or to register as phosphate acceptors in the standard protein kinase filter assay that measures TCA-precipitable ^{32}P. Nevertheless, these contaminants sustain sufficient phosphate incorporation to become visible when analysed by

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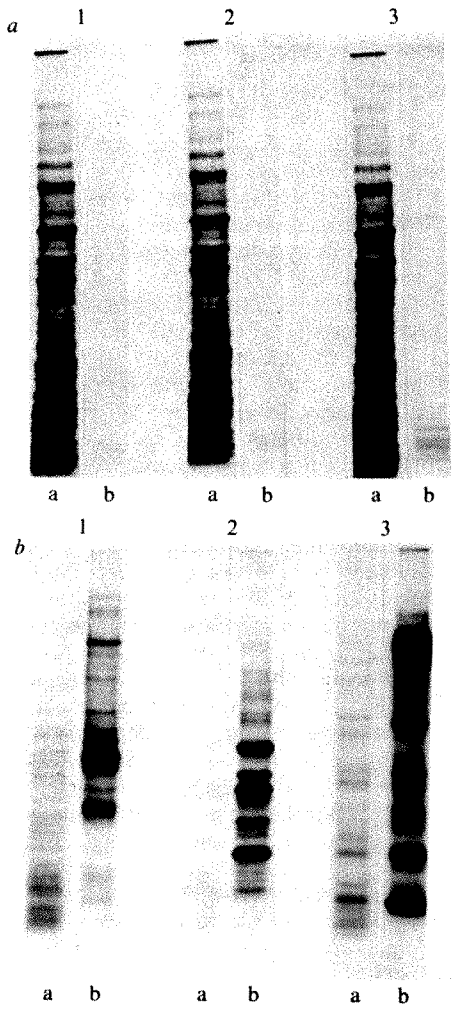


Fig. 1 Nuclear protein kinases were separated from their endogenous substrates by affinity chromatography[12] and separated into individual isoenzymes on phosphocellulose as previously described[6]. The active fractions within the three major peaks were pooled and concentrated by pressure dialysis. They were incubated for 20 min at 30 °C in 30 μl containing 3 μCi [γ-^{32}P]ATP and 15 nmol $MgCl_2$ in the presence ('a' channels) and in the absence ('b' channels) of 100 μg of enzyme-free concentrated fraction I. The reaction was stopped by addition of 3 μl 10% SDS and the entire reaction mixture was subjected to electrophoresis in SDS-containing polyacrylamide 7–15% gradient gels. The gels were stained with Coomassie Brilliant Blue to render the proteins visible (*a*), then dried and exposed for 16 h to NS 5T Kodak X-ray film (*b*). The twin-channels labelled 1, 2 and 3 refer to different isoenzymes.

autoradiography on gels after a 16-h exposure. It is clear that the latter detection method is considerably more sensitive than the filter assay. In the presence of 100 μg of protein in fraction I, however, phosphate incorporation is nearly abolished, whereas fraction I proteins supported significant phosphate incorporation when present at concentrations of 5–10 μg per assay. These results suggested that the fraction containing endogenous substrates also contained in inhibitor of protein kinase which, on concentration of crude, but enzyme-free, extracts severely inhibits phosphate incorporation into the natural substrates.

This preparation inhibited not only the phosphorylation of nuclear proteins but also that of casein. This permitted us to quantify the amounts of inhibitor present in nuclear extracts in the presence of saturating amounts of casein (1 mg per ml), thus eliminating the effects of varying amounts of endogenous substrates in the assay. Studies to characterise the inhibitor therefore measured the inhibition of casein phosphorylation. Figure 2 shows the inverse proportionality of remaining protein kinase activity with increasing amounts of inhibitor added to a constant amount (0.1 unit) of protein kinase purified by affinity chromatography[12]. One unit of inhibitor is defined as the amount giving 50% inhibition of 0.1–0.2 units of nuclear protein kinase in the standard assay[12].

Fraction I was adsorbed to a DEAE-cellulose column at 6 mg per ml of packed column volume in 50 mM Tris-HCl, *p*H 6.8, containing 0.1 M NaCl and 0.05% Nonidet P-40 (buffer I). Inhibitory activity eluted as a sharp peak between 0.3 and 0.35 M NaCl when the column was developed with a linear gradient in NaCl of 0.1–0.5 M in 50 mM Tris-HCl, *p*H 6.8, and 0.05% Nonidet P-40. The active fractions were pooled, concentrated by pressure dialysis and loaded onto a 0.6 × 50 cm column of Biogel P-150 (minus 400 wet mesh) equilibrated and developed in 50 mM Tris-HCl, *p*H 6.8, containing 0.5 M NaCl to minimise the possibility of molecular aggregation. The inhibitor emerged as a single, slightly skewed peak with an R_F of 0.9 relative to dextran blue, indicating an apparent molecular weight of 150,000. Inhibitory activity was destroyed on incubation at 60 °C for 10 min at *p*H 7 or at 37 °C for 30 min at *p*H 4. Incubation of the inhibitor with pancreatic RNase, DNase I or phospholipase A at 37 °C for 60 min had no effect, whereas incubation with trypsin or chymotrypsin rapidly destroyed its activity (data not shown).

Aliquots of an inhibitor preparation purified through the DEAE-cellulose step, containing 30–50 μg protein, induced 30–40% inhibition of each of the three major isoenzymes of nuclear protein kinase separated on phosphocellulose. However, they had no effect on the activity of a cytoplasmic protein kinase preparation (100,000*g* rat liver supernatant) either with casein or with histones as substrates, regardless of whether cyclic AMP was present in the assay. The results of a typical experiment are summarised in Table 1. The specificity of the inhibitor for the nuclear protein kinases ruled out the possibility that inhibition was due to some nonspecific interference with the assay, such as ATPase or protease activities in the extract, because such activities would also give an apparent inhibition of the phosphotransferase reaction catalysed by the cytoplasmic protein kinases. It did not eliminate the possibility that the inhibitor was a phosphoprotein phosphatase, as we have previously shown that the nuclear phosphoprotein phosphatases have a pronounced substrate preference for nuclear phosphoproteins over phosphoprotamine[13]. However, this possibility could also be discounted, as when nuclear phosphoproteins, prepared from livers of rats injected with ^{32}P 90 min before death, were incubated with the inhibitor for 30 min at 30 °C, there was no loss of phosphate from TCA-precipitable material.

We have previously shown that the nuclear protein kinases, in contrast to the cytoplasmic enzymes, are stimulated by polyamines, but not by cyclic AMP[6,7], and are not inhibited by the

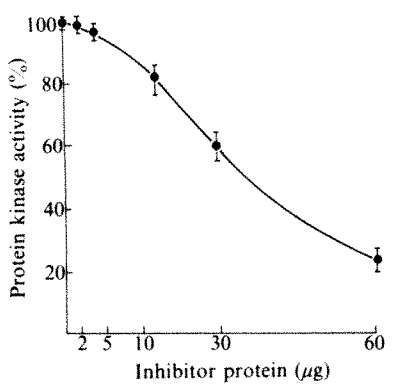


Fig. 2 Nuclear protein kinase (0.1 unit) was incubated with various amounts of inhibitor protein purified through the DEAE-cellulose step as described in the text. The inverse proportionality between remaining enzyme activity and amounts of inhibitor added is that predicted by the Michaelis–Menten relation for the reversible interaction between an enzyme and its inhibitor.

Table 1 Effect of the inhibitor protein on nuclear and cytoplasmic protein kinases

Protein kinase	Substrate	Other additions	nmol ^{32}P incorporated per min per mg	% Inhibition
Nuclear				
Phosphocellulose				
Peak 3	Casein	None	0.49	
	Casein	Inhibitor	0.34	31
Peak 4	Casein	None	1.32	
	Casein	Inhibitor	0.83	37
Peak 5	Casein	None	0.25	
	Casein	Inhibitor	0.15	40
Cytoplasmic				
	Casein	None	0.46	
	Casein	Inhibitor	0.46	0
	Casein	+cyclic AMP (5×10^{-6} M)	0.48	
	Casein	+cyclic AMP+ inhibitor	0.49	0
	Histone	None	0.76	
	Histone	Inhibitor	0.74	2.5
	Histone	+cyclic AMP (5×10^{-6} M)	3.05	
	Histone	+cyclic AMP+ inhibitor	2.97	2.5

Nuclear protein kinases were prepared and freed of endogenous substrate[12] and fractionated on phosphocellulose as previously described[6]. Cytoplasmic protein kinase was a 100,000g rat liver supernatant. Casein and histones were present at 200 μg in the 0.2-ml standard assay[12].

* Inhibitor protein (30 μg) was purified by the DEAE-cellulose step as described in the text.

cytoplasmic heat-stable inhibitor protein[6] that specifically inhibits cyclic AMP-dependent protein kinases by interacting with their catalytic subunit[14]. Here we report the presence in rat liver nuclei of a heat-sensitive inhibitor of 150,000 molecular weight that behaves as an acidic protein and is specific for the nuclear enzymes. Taken together, these findings suggest that the nuclear protein kinases are subject to controls that are specific and distinct from those regulating the cytoplasmic enzymes which primarily mediate the effects of cyclic AMP.

We believe this inhibitor to represent the only negative modulator of nuclear protein kinase activity described to date. It is suggested that the inhibitor may play an important part in the regulation of nuclear protein phosphorylation. Moreover, it can be expected to be a valuable tool in assessing the possible role of nuclear protein phosphorylation in nuclear RNA metabolism and gene activation.

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A chiasma–hormonal hypothesis relating Down's syndrome and maternal age

THE incidence of the human congenital abnormality known as Down's syndrome (or mongolism) has been observed to increase with maternal age[1]. The trend of sharply increased incidence beyond the age of 30 has been well documented[2], and various hypotheses have been formulated to explain it. These include cumulative ovum or uterine dysfunction (radiation[3], chemicals[4], disease[5]), acute effects of radiation and chemicals, hormonal effects[6], the 'production line' hypothesis[7,8] and changes in sexual behaviour[9]. We describe here our hypothesis that there is an interaction between the hormonally governed rate of meiosis and the timing of chiasma terminalisation. We propose that changing hormone levels during the menstrual cycle not only trigger resumption of meiosis in the ovum[10], but also control the rate of meiosis through the availability of a limiting substance[11]. As hormone levels and the length of the cycle change with advancing age of the mother, meiosis slows down, and chiasma frequencies decline[7,12]. Meiotic chromosome bivalents are held together by their chiasmata against strong mutual repulsion during late dictyotene and diakinesis[13], making smaller bivalents with fewer chiasmata, such as 21, the most vulnerable to premature separation during terminalisation[14,15]; premature separation can lead to trisomy 21, by far the most frequent cytological manifestation of Down's syndrome[16].

17β-Oestradiol may stimulate the production of luteinising hormone (LH) receptors in the follicle either directly through transcription[17,18] or indirectly by increasing the number of granulosa cells surrounding the ovum[19]. Thus, LH receptors could accumulate during the menstrual cycle as an integral function of oestradiol concentration. At corresponding points in the cycle, LH concentration varies little[20], but oestradiol decreases with age[20,21] (see Fig. 1 legend).

After meiosis resumes in the ovum at the LH surge, the rate of meiosis is assumed to be limited by a substance released in response to LH[19] (for example, cyclic AMP[10] or Ca^{2+} (ref. 22)). As LH concentration remains high during this part of the cycle, the intensity of the LH stimulus may be limited by the number of LH receptors rather than by LH concentration[19]. In this simplest case, the accumulation rate of the limiting substance is directly proportional to the number of receptors. By ignoring allosteric or other complexities of the rate-limiting reaction, the rate of meiosis can be expressed as the Michaelis–Menten function in equation (1) (Fig. 1 legend).

Estimating the minimal interval from LH peak to ovulation to be 12 h (ref. 23), we add 24 h for the duration of the LH surge[24] to obtain the interval from resumption of meiosis to ovulation (end of meiotic division I) in women at age 20; we can then use equation (1) to predict this interval for other ages. Bivalents become attached to spindle fibres roughly 18 h after the resumption of meiosis in women at age 20 (ref. 25), but equation (1) implies that this pre-alignment interval lengthens for older women, with an increasing chance of losing chiasmata before proper alignment. We assume that the starting times of terminalisation can be represented by a normal distribution independent of age. As a first approximation, let the frequency of premature univalents be given by the frequency of bivalents whose chiasmata terminalise before spindle attachment. Then the frequency of prematurely terminalised chiasmata is expressed by equation (2) (Fig. 1 legend). Note that if terminalisation of individual bivalents is rapid relative to the time scale of meiosis, as seems likely, the exact number and positions of chiasmata become unimportant.

Assuming that unaligned univalents 21 successfully attach to the spindle but segregate independently at anaphase I (ref. 8), allowing for a partial fetal viability (we use 0.235 (ref. 26)) and multiplying by 1,000, we obtain the incidence per 1,000 births of trisomy 21 as a function of maternal age in equation (3) (Fig. 1

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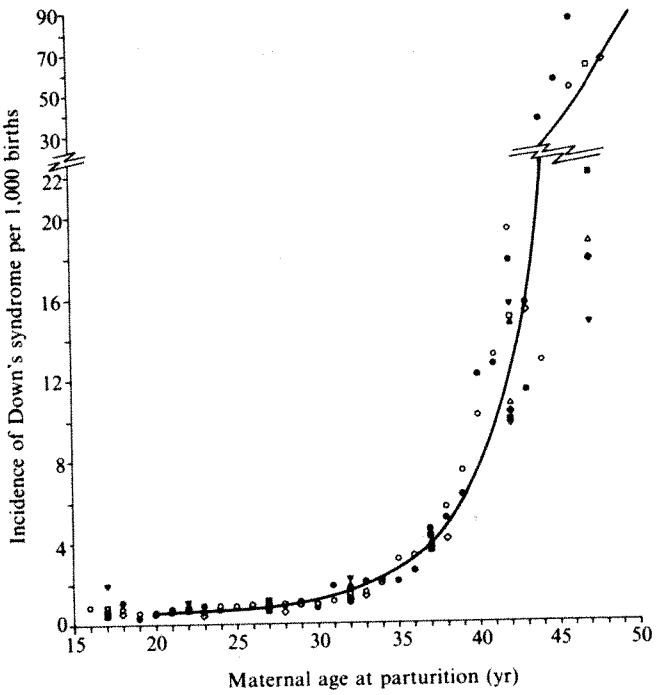


Fig. 1 Incidence of Down's syndrome per 1,000 births versus maternal age at parturition. Data from eight of the studies cited are for 5-yr age intervals; data from the last two studies are for 1-yr intervals. The curve illustrates one reasonable fit to these data of the model developed here, for which the two fitted parameters are $\sigma = 7.09$ and $K_m/k_1k_0 = 7{,}000\ h^2$. This result assumes a reverse sigmoid decline beyond age 20 in peak oestradiol concentration ($c(a) = 1{,}176/(a^2 - 40a + 1{,}576)$) and a linear peak shift ($p(a) = 603 - 8.53a$, where p is time in hours to the oestradiol peak from the beginning of the cycle) with age a in years. The general forms and parameters of these two functions are suggested by data in Sherman and Korenman[20]. Key equations from the derivation outlined in the text are:

$$\rho(a,t) = \frac{\rho_{max}M(a,t)}{K_m + M(a,t)} = \frac{\rho_{max}c(a)\int_0^t\int_{t_0}^s E(u)\,du\,ds}{K_m/k_1k_0 + c(a)\int_0^t\int_{t_0}^s E(u)\,du\,ds}, \quad (1)$$

where ρ is the rate of meiosis at time t during the cycle of a woman of age a, h^{-1}; ρ_{max} is the maximal rate of meiosis ('limiting' substrate present in excess), h^{-1}; M is the concentration of the rate-limiting metabolite, $ng\,l^{-1}$; a is maternal age, yr; t is time during the menstrual cycle, h; K_m is the Michaelis constant, $ng\,l^{-1}$; c expresses the decline in oestradiol concentration with age a, normalised such that $c(20) = 1$; t_0 is an initialisation time, taken here to be the beginning of the cycle; E is the oestradiol concentration during the cycle, $ng\,l^{-1}$; k_1 is the rate constant for metabolite accumulation, h^{-1}; and k_0 is the coefficient for the accumulation of LH receptors, $ng\,l^{-1}h^{-1}$.

$$F(fi) = \int_0^{fi} P(r)\phi(r)\,dr$$

where

$$P(r) = \begin{cases} 0 & \text{if } fi \leq r \\ \alpha(fi - r) & \text{if } fi > r \geq fi - 1/\alpha \\ 1 & \text{if } fi - 1/\alpha > r \end{cases} \quad (2)$$

and

$$\phi(r) = \exp[-(r-\tau)^2/2\sigma^2]/2\pi\sigma$$

and where F is the frequency of unaligned univalents; f is the pre-alignment fraction of the interval between resumption of meiosis and ovulation; i is the duration of this entire interval, h; $P(r)$ is the probability of terminalisation by time r(h) after the resumption of meiosis; α is the terminalisation rate, h^{-1}; ϕ is the frequency distribution of first terminalisation times, of which τ is the mean and σ is the standard deviation, h.

$$I(a) = 1{,}000vF(fi(a))/(2+v) \quad (3)$$

where I is the incidence per 1,000 births of trisomy 21, and v is the fraction of 21-trisomic zygotes surviving to birth divided by the fraction of all other zygotes surviving to birth. ◆, Ref. 33; △, ref. 34; ■, ref. 35; ▼, ref. 36; □, ref. 37; ▲, ref. 38; ▽, ref. 39; ◇, ref. 40; ○, ref. 41; ●, ref. 42 (corrected for sparcity of data).

legend). The curve in Fig. 1 illustrates a reasonable fit to the data of equation (3) found by appropriately adjusting the two parameters σ and K_m/k_1k_0.

The curve in Fig. 1 accelerates sharply in response to both the $i(a)$ function and the leading tail of the cumulative normal function $\int \phi(t)\,dt$. By the analysis presented here, neither the hormonal (i) nor the chiasma (ϕ) effects alone can bend the incidence curve sharply enough to fit the data, but taken together, as in equation (2), they can provide sufficient acceleration.

The chiasma–hormonal hypothesis proposed here yields some testable (but as yet untested) predictions: (1) The interval during the cycle between the LH peak and ovulation increases with maternal age (see refs 27, 28). (2) The incidence of trisomies will respond to experimental manipulation of intrafollicular hormone levels (see refs 29, 30). (3) Chiasma frequencies in the bivalents 21 of human females decrease with age during post-arrest dictyotene and diakinesis (see refs 7, 11 in mice).

Our hypothesis clearly emphasises the need for further experimental studies that link hormonal signals with cytological events, and for further information on the dynamics and significance of chiasmata before and during segregation. Thorough documentation of intrafollicular hormone levels during a cycle would be particularly useful, avoiding the necessity for extrapolating (as here) from systemic levels to follicular levels.

We have not formally considered the incidence trend below a maternal age of 20 yr in the model presented here. We suspect that the typically higher incidence of Down's syndrome in children of mothers younger than 20 (see Fig. 1) results from a higher variability of hormone levels among cycles as the complex neuro-endocrine feedback controls are still being established[31]; none of the other mechanisms assumed to operate in meiotic division I could so easily account for this higher incidence for very young mothers. The behavioural mechanism is not necessarily inconsistent with this pattern below age 20, but the finding that the majority of maternal meiotic segregation errors occur during division I (ref. 32) seems to preclude a significant behavioural effect.

Many other specific models, perhaps focusing on other hormones (follicle-stimulating hormone, progesterone) or other hormone interactions, could probably fit the data of Fig. 1 equally well. The parsimonious LH–oestradiol interpretation is primarily intended to illustrate the essential implications of the general chiasma–hormonal mechanism.

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Specific DNA methylation sites in the vicinity of the chicken β-globin genes

METHYLATION of cytosine is the only post-synthetic modification so far detected in the DNA of higher eukaryotes and thus has been made the basis of several proposed mechanisms of gene activity and cellular differentiation[1–3]. All evidence indicates, however, that the overall content of 5-methylcytosine in DNA does not vary significantly between different tissues of the same organism and does not change throughout at least some steps of differentiation[4–6]. As 5-methylcytosine occurs predominantly in the dinucleotide sequence CpG (ref. 7) and as a number of bacterial restriction endonucleases can distinguish whether this sequence is methylated or unmethylated, it is now possible to investigate DNA methylation patterns in the vicinity of single genes[8]. In this report, we use this technique to provide a correlation between DNA methylation and the activity of the chicken β-globin genes.

The restriction endonuclease *Hpa*II cleaves the DNA sequence CCGG (ref. 9) but does not cleave the methylated sequence C(MeC)GG (ref. 10). A second restriction endonuclease, *Msp*I, is able to cleave this same DNA sequence whether it is methylated or not[11]. Thus DNA from any cell or tissue type can be digested with either *Hpa*II or *Msp*I, the cleaved DNA electrophoresed on an agarose gel, transferred to a nitrocellulose filter[12] and hybridised to a gene-specific probe. Any bands seen in the autoradiogram which differ between the two enzyme digests can reasonably be ascribed to differences in methylation of CCGG sites in the vicinity of the specific gene. This kind of experiment, used here to examine the chicken β-globin genes, has recently been used to investigate a single methylation site in the large intervening sequence of the adult rabbit β-globin gene[13].

Figure 1 shows the autoradiogram from an experiment with chicken DNA, using the plasmid pHb1001 as a ^{32}P-labelled hybridisation probe (pHb1001 is a cDNA clone of the adult chicken β-globin gene[14], provided by Dr W. Salser). Figure 1*a* represents the *Hpa*II digest of adult chicken erythrocyte DNA and shows bands at 4.2 and 1.3 kilobase pairs. These fragment sizes should correspond to distances between unmethylated CCGG sites in the DNA regions containing β-globin genes. Figure 1*b* represents the *Msp*I digest of the same adult erythrocyte DNA and shows bands at 2.0 and 1.3 kilobases, as well as a faint but reproducible band at 0.4 kilobases. These fragment sizes should correspond to distances between both methylated and unmethylated CCGG sites in the β-globin gene DNA. The 1.3-kilobase fragment is of similar intensity in both digests.

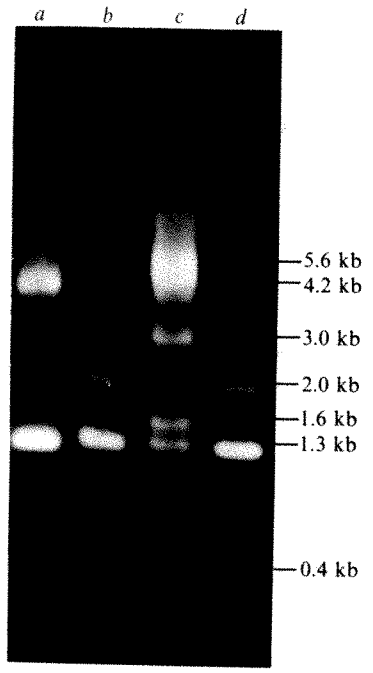


Fig. 1 DNA was isolated from chicken tissues by extensive digestion with proteinase K (Merck) in the presence of 0.2% SDS with or without previous isolation of nuclei. This was followed by at least three extractions with an equal volume of phenol–chloroform–isoamyl alcohol (25:24:1), three extractions with two volumes of chloroform–isoamyl–alcohol (24:1), and three ethanol precipitations. In a typical digestion protocol, 200 units of restriction enzyme were added to 100 μg DNA in a final buffer of 6 mM KCl, 10 mM Tris-HCl (*p*H 7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol and 100 μg ml^{-1} autoclaved gelatin. (The enzyme *Msp*I was obtained from New England Biolabs. The enzyme *Hpa*II was obtained from either New England Biolabs, Bethesda Research Laboratories, or Boehringer–Mannheim; all gave identical results. It was verified that, in the digestion conditions used, both *Hpa*II and *Msp*I gave identical cleavage patterns with the plasmid pBR322.) The reaction was incubated at 37 °C for 2 h, a further 200 units of enzyme were added and incubation was continued for an additional 2 h at 37 °C. Identical results were obtained if this second incubation was continued for 10–15 h. The completion of digestion was monitored by adding a small amount of bacteriophage λ DNA to an aliquot of either the first or the second incubation mixture and verifying by electrophoresis that the required cleavage products were present. After digestion, the DNA was repurified by repeating the proteinase K digestion and organic extraction steps described above, and 25–50 μg electrophoresed on each slot of a 1% agarose slab, 6 mm thick, for 2 h at 200 V. DNA was then transferred to Schleicher and Schuell nitrocellulose filters by the method of Southern[12], the filters hybridised to ^{32}P-labelled nick-translated pHb1001 for 24 h at 70 °C in 6 × SSC, washed in 3 × SSC, and finally washed for 30 min in 0.1 × SSC. Filters were exposed to Kodak XR5 X-ray film for 24–96 h at −80 °C, using two Dupont Cronex intensifying screens. A typical autoradiogram is shown above and described in more detail in the text. *a*, Adult erythrocyte DNA digested with *Hpa*II; *b*, adult erythrocyte DNA digested with *Msp*I; *c*, oviduct DNA digested with *Hpa*II; *d*, oviduct DNA digested with *Msp*I. Band sizes were calibrated relative to a *Hin*dIII digest of λ and a *Hae*III digest of ΦX174 DNA, run in an adjacent gel slot, and are probably accurate to 5%. Identical *Hpa*II digestion patterns were obtained with DNA from erythrocytes or reticulocytes isolated from several individual adult roosters and chickens, of both White Rock and Leghorn breeds. *Hpa*II digestion of a mixture of erythrocyte and oviduct DNA yielded a pattern which was the expected sum of the patterns of slots *a* and *c* above. A digest of the same DNA with a mixture of *Hpa*II and *Msp*I yielded only the *Msp*I digestion pattern. The *Msp*I digestion pattern of all DNA samples investigated was identical to that in slots *b* and *d* above. Digestion with several restriction endonucleases which do not have CG as part of their recognition site gave the same digestion patterns in different tissues (see also ref. 16).

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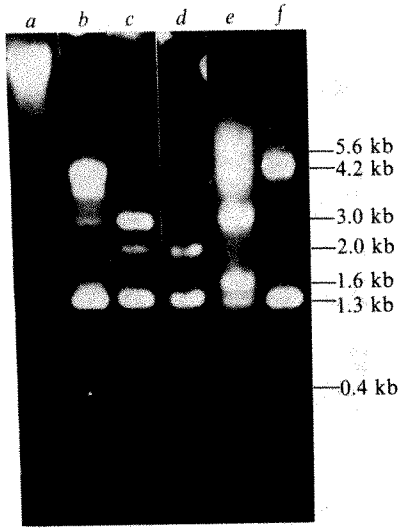


Fig. 2 *a–d*, Partial digestion of adult erythrocyte DNA with the restriction endonuclease *Msp*I; *a*, no enzyme control; *b*, 2 units; *c*, 12 units; *d*, 100 units of enzyme. All incubations were for 2 h at 37 °C with 50 μg DNA and using the buffer conditions described in Fig. 1. *e*, Complete *Hpa*II digest of erythrocyte DNA from 5-d-old embryos. For comparison, slot *f* is a complete *Hpa*II digest of adult erythrocyte DNA.

However, the *Hpa*II digest (*a*) lacks both the 0.4 and 2.0 kilobase fragments of the *Msp*I digest as well as any other bands which could correspond to a combination of the *Msp*I bands. This suggests that the CCGG sites at the ends of the 1.3-kilobase fragment are completely (>90%) unmethylated in adult chicken erythrocyte DNA and furthermore, that at least two other specific sites are completely methylated. DNA isolated from the circulating reticulocytes of phenylhydrazine-induced anaemic adult chickens gives digestion patterns which are essentially identical to those of adult erythrocyte DNA (data not shown). These results are in contrast to those for the rabbit β-globin gene[13], where in both marrow and spleen cells of anaemic rabbits, only partial methylation of a site in the large intervening sequence was observed; this difference could be due to the different positions of the cleavage sites within the gene sequence or, as the authors have pointed out[13], due to a heterogeneous cell population.

Figure 1*c* shows the *Hpa*II digest of oviduct DNA. The observed band pattern is not only different from the *Msp*I digest of the same DNA (Fig. 1*d*) but also different from the *Hpa*II digest of erythrocyte DNA (Fig. 1*a*). There are now prominent bands at 4.2, 3.0, 1.6 and 1.3 kilobases (as well as several weaker but poorly resolved bands at higher molecular weight). As Fig. 2*b* and *c* show, all these bands appear as partial digestion products of adult erythrocyte DNA by the enzyme *Msp*I. This suggests that, in the oviduct, CCGG sequences in the vicinity of the β-globin genes are only partially methylated. Nevertheless, the absence of the 2.0-kilobase band suggests that at least one such sequence is completely methylated. As shown in Fig. 2*e*, DNA isolated from the circulating red blood cells of 5-d-old embryos (in which the adult β-globin gene is not being expressed[14,15]) or from adult brain (data not shown) gives digestion patterns essentially identical to that of oviduct DNA.

The legend to Fig. 1 summarises control experiments which show that the above results are not due to partial digestions, DNA isolation methods, enzyme inhibitors, DNA sequence rearrangements or individual differences between chickens. Figure 2*a* also provides an important control, showing that undigested DNA is still transferred to the nitrocellulose filter for hybridisation; thus, any high molecular weight bands would have been detected.

One current difficulty in assigning the observed bands to their positions within the gene sequence arises from the fact that the adult chicken β-globin gene in pHb1001 shares considerable sequence homology with at least one of the embryonic β-globin genes[16], and both sequences can show up on the filter hybridisations. However, our preliminary results using a genomic clone (G.D.G., W. I. Wood and G. Felsenfeld, unpublished) indicate that the prominent 1.3-kilobase fragment shown in Figs 1 and 2 is derived from the adult β-globin gene sequence; moreover one end of this fragment lies within about 30 bases of the 5'-end of the coding sequence[14].

It is undoubtedly premature to relate DNA methylation patterns to a specific biological activity. It does seem clear, however, that the observed tissue differences do not solely reflect different rates of cell division, as both adult and embryonic red cells have ceased division and yet their DNA shows different methylation patterns. Moreover, brain, a non-proliferating tissue, shows the same pattern as the proliferating oviduct. On the other hand, at least some correlation can be made between these methylation patterns and globin gene activity. In cells which are expressing or have expressed the adult β-globin gene (adult reticulocytes and erythrocytes) the CCGG sites near the ends of the gene sequence (that is at the ends of the 1.3-kilobase fragment) seem to be completely unmethylated. In cells which are not expressing this gene (oviduct, brain and embryonic red blood cells) these sites can be at least partially methylated. The determination of how these specific methylation events occur during cellular differentiation must await a more detailed description of their exact positions in the gene sequence. Nevertheless, we believe these results to be the first correlation between site-specific DNA methylation and eukaryotic gene expression.

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Long regions of single-stranded DNA in human cells

WHEN DNA is isolated from actively growing animal cells by a variety of standard procedures, 1–2% of the total DNA is recovered in single-stranded form[1–5]. Pulse labelling experiments have established that the large majority of the single-stranded DNA is not newly synthesised[3,5]. However, a minor part of this DNA fraction consists of short fragments of newly replicated DNA released by branch migration during isolation[6,7]. Studies with synchronised human cells have shown that single-stranded DNA is mainly found during the period of DNA synthesis[3,4]. In agreement with this, a specific antiserum against denatured DNA used for immunofluorescence has revealed the presence of single-stranded DNA in nuclei of a human lymphoid

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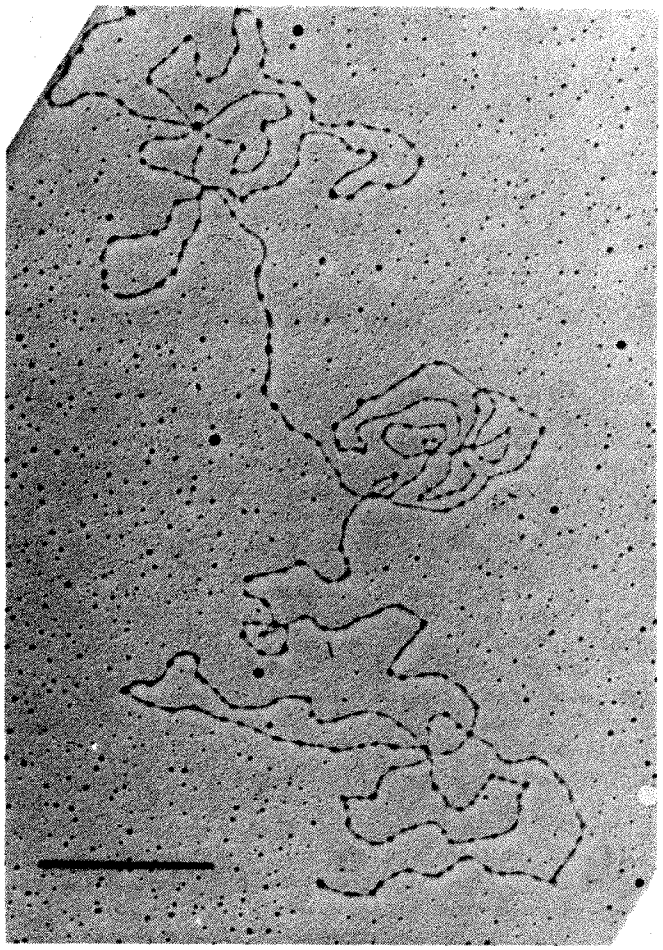


Fig. 1 Electron micrograph of a long single-stranded DNA molecule isolated from the human lymphoid cell line Molt-4. The DNA was spread in the presence of 30% formamide by the technique of Davis *et al.*[13]. In comparison with an internal reference, phage ΦX174 DNA, this molecule has a molecular weight of 40×10^6. Scale bar, 1 μm.

cell line during the S phase but not during the G1 phase[8]. DNA hybridisation measurements indicate that the single-stranded sequences belong to the non-repetitious fraction of the DNA and that at least part of this material contains sequences transcribed *in vivo*[2]. We have developed gentle isolation methods to purify large, covalently closed circular DNA molecules from human lymphocytes transformed by the Epstein–Barr virus (EBV)[9]. These methods do not cause the conversion of linear double-stranded DNA to single-stranded forms[5,9]. We have repeatedly observed by electron microscopy long single-stranded stretches of host DNA that initially co-purified with the guanine–cytosine-rich viral episomal DNA. This single-stranded DNA accounted for about 1% of the total cellular DNA in several different lymphoid cell lines derived from Burkitt lymphoma patients or from healthy donors, while the EBV DNA sequences in the same lines only constituted 0.02–0.1% of the total DNA. Clearly, the single-stranded sequences were mainly or exclusively of host origin. Long regions of single-stranded DNA, indistinguishable from those present in EBV-transformed cells, were also found in similar amounts in two EBV-negative human lymphoid cell lines of different origin, BJAB[10] and Molt-4 (ref. 11). This report looks at these single-stranded DNA molecules, which are considerably larger than any previously isolated from animal cells.

Molt-4 cells were grown in stationary suspension culture at 37 °C in RPMI-1640 medium (GIBCO) supplemented with 15% fetal bovine serum and antibiotics. High molecular weight DNA was prepared by treatment of washed cells with Sarkosyl and Pronase, followed by CsCl density gradient centrifugation[9]. Fractions of higher density (1.715–1.735 g cm^{-3}) than the main band DNA (1.700 g cm^{-3}) were recovered with the aid of a closed system collection device to minimise shearing forces[9]. This material, which contained about 5% of the total cellular DNA, was further fractionated by neutral glycerol gradient centrifugation in the presence of 1 M NaCl. The conditions were as described previously[9], except that the time of centrifugation was reduced to 2 h, polyallomer tubes were used, and the size marker (phage T4 ^{14}C-DNA) was run in a separate tube. The purpose of the latter fractionation step was to purify further large single-stranded DNA molecules, which sediment faster than double-stranded molecules of similar size[12]. Different fractions of a glycerol gradient were dialysed free from glycerol, and circular single-stranded DNA from bacteriophage ΦX174 was added as an internal reference. The fractions were then examined by electron microscopy, using the formamide modification of the Kleinschmidt technique[13].

The fastest sedimenting DNA detected had a sedimentation coefficient of 150–160S. This DNA seemed to be entirely single stranded, as judged by electron microscopy (Fig. 1). Using contour length measurements the DNA was estimated to have a molecular weight of 40×10^6. Note that a single-stranded DNA molecule of this size would be expected to have a sedimentation coefficient of 156S in neutral 1 M NaCl (ref. 12). Furthermore, these long DNA molecules as well as the ΦX174 DNA were no longer detected after treatment with pancreatic DNase or single-strand-specific S_1 nuclease, while they were resistant to pancreatic RNase.

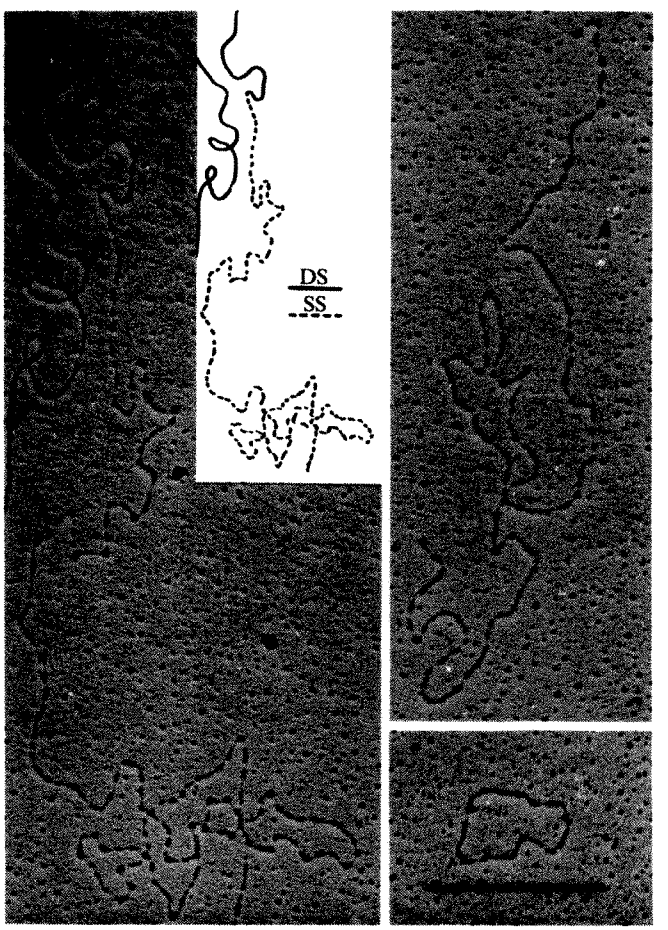


Fig. 2 Single-stranded DNA from Molt-4 cells. Left, a molecule comprising both double-stranded (DS) and single-stranded (SS) DNA. The length of the single-stranded region is 17×10^6 MW. Upper right, a well extended single-stranded DNA molecule of 14×10^6 MW. Lower right, single-stranded circular DNA from bacteriophage ΦX174 of $1{\cdot}8 \times 10^6$ MW. Scale bar, 1 μm for all pictures.

Table 1 Single-stranded DNA from cells in different growth phases

	Sample A (early S phase)	Sample B (mid S phase)	Sample C (G2 phase)
Number of double-stranded DNA molecules per field square	6.1	5.4	7.4
Number of double-stranded molecules measured	43	38	52
Average length of double-stranded DNA (μm)	38.5	43.2	32.1
Number of single-stranded DNA molecules per field square	1.2	0.9	0.6
Number of single-stranded molecules measured	29	23	16
Average length of single-stranded DNA (μm)	18.0	23.5	11.3
Proportion of DNA in single-stranded form (%)	9.2	9.1	2.9

DNA preparations were spread in the presence of 30% formamide[13]. Bacteriophage PM2 DNA in open circular form (MW 6.4×10^6) was used as size reference. A length correction (based on parallel measurements of double-stranded and single-stranded ΦX174 DNA) was performed to correct for differences in spreading between double-stranded and single-stranded DNA.

Most of the high molecular weight DNA investigated here sedimented as a broad peak around 70S on glycerol gradients. In the region of a gradient containing molecules sedimenting at >50S, which presumably would be free from any newly replicated DNA fragments of low molecular weight, about 20% of the total material was found in single-stranded form (Fig. 2). The size distribution of the single-stranded DNA recovered is shown in Fig. 3. It seems that the single-stranded DNA is not of unique size, and that a considerable part of this material is as much as $20–40\times10^6$ MW. However, the long DNA chains are very shear sensitive, and could be derived from even larger single-stranded or partly single-stranded molecules, fragmented during isolation.

To investigate the alkali stability of the material, a fast-sedimenting DNA fraction (100–120S) containing a relatively large amount of single-stranded DNA was brought to *p*H 12.7 by the addition of NaOH, left at 20 °C for 15 min, neutralised, and immediately spread for electron microscopy. A control experiment with bacteriophage λ DNA showed that double-stranded DNA was completely converted to intact single strands in these conditions. An aliquot of a 100–120S DNA fraction isolated from Molt-4 cells contained 26 DNA molecules, of which 22 were single-stranded molecules between 11 and 35 μm long, and 4 were double-stranded molecules. A similar aliquot from the alkali-denatured material contained 7–42-μm long single-stranded DNA molecules. There was no detectable decrease in the average length (about 20 μm) of the chains after exposure to alkali. These data show that the material shown in Figs 1–3 consists of long single-stranded DNA molecules rather than hydrogen-bonded aggregates of short chains.

As it has been reported that single-stranded DNA is primarily found in growing cells in S phase[3,4,8], it was of interest to see if the much longer single-stranded regions detected here were also mostly present during active DNA synthesis. Human lymphoid cells were therefore synchronised by the double thymidine blocking technique[14]. This procedure is of varying effectiveness with different lymphoid lines, but Raji cells respond well[14] and were used for this experiment. DNA was prepared from cells mainly in early S, middle S, and G2 phase (Fig. 4), and fractionated by CsCl gradient centrifugation. The high-density material was recovered and investigated by electron microscopy. The DNA preparations from S phase cells contained about three times larger amounts of single-stranded material than the preparation from cells mainly in G2 phase (Table 1). These data

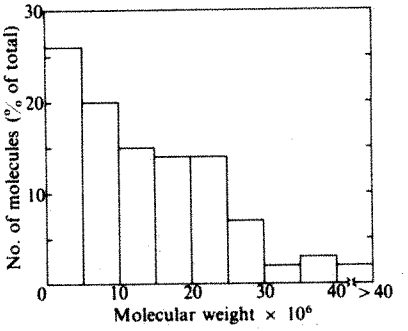


Fig. 3 Size distribution of long single-stranded DNA regions. Molt-4 DNA was fractionated by neutral CsCl and neutral glycerol density gradient centrifugations, and the material sedimenting at >50S was investigated. Both entire single-stranded molecules and single-stranded regions in partly double-stranded molecules were included, and 100 molecules were measured.

agree with previous findings[3,4,8] that single-stranded DNA is primarily (or exclusively) found in S phase cells.

The subcellular distribution of the single-stranded DNA was investigated by preparation of Raji cell nuclei by Nonidet 40 treatment as described previously[15]. High molecular weight DNA preparations were made in parallel from both whole cells and isolated cell nuclei, and after CsCl density gradient centrifugation, the DNA density fractions 1.720–1.740 g cm^{-3} was examined by electron microscopy. In both these preparations 10–15% of the high molecular weight material was single-stranded DNA. Thus, we conclude that most or all of the long regions of single-stranded DNA described here is of nuclear origin.

When Molt-4 cells were pulse-labelled with 10 μCi ^{3}H-thymidine per ml 30 min before collecting the cells for preparation of DNA, the total DNA had a specific radioactivity of 4,000 c.p.m. per μg. After enrichment of large single-stranded DNA molecules by CsCl and glycerol gradient centrifugation as above, a fast-sedimenting fraction representing about 0.3% of the total DNA and containing 50% single-stranded material was obtained. This DNA had a specific activity of about 3,000 c.p.m. per μg. Although the estimate of the DNA concentration is only semi-quantitative, these data strongly indicate that the large

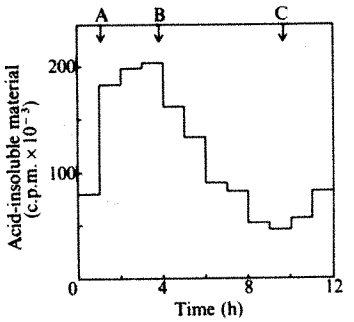


Fig. 4 DNA synthesis in synchronised lymphoid cells. Cells (60×10^6) of the Raji line were propagated in stationary suspension culture and synchronised by the double thymidine blocking technique as described by Hampar *et al.*[14]. After release from the block, DNA synthesis was monitored by 1-h pulse-labelling of aliquots of 5×10^5 cells with 5 μCi ^{3}H-thymidine. At the three time points indicated by arrows, 15×10^6 cells were collected and used for the preparation of high molecular weight DNA. Each DNA solution was fractionated by neutral CsCl density gradient centrifugation[9]. Fractions of density 1.720–1.740 g cm^{-3}, which represented about 2% of the DNA in the gradient, were pooled, dialysed and investigated by electron microscopy (see Table 1).

single-stranded DNA regions do not represent newly synthesised DNA, again confirming previous results[3,5].

Methods used previously[1-5] to isolate single-stranded DNA from mammalian cells yield larger amounts of material suitable for further characterisation. However, the approach used here reveals the unexpected length of much of this single-stranded DNA. It seems unlikely that such long regions could be due to DNA destabilised by transcription or DNA repair, or to DNA sequences serving as regulatory signals. Note that during replication of adenovirus DNA in cell nuclei, the replication process occurs in a completely asymmetric manner with the generation of single strands of entire parental virus DNA as replication intermediates[16,17]. Single-stranded DNA of the size range described here can thus exist in cell nuclei without being degraded. Also, mitochondrial DNA is synthesised in an asymmetric manner with the temporary generation of single-stranded regions of parental DNA[18].

Recently, it has been found that both adenovirus DNA and mitochondrial DNA are replicated by DNA polymerase γ (refs 19, 20), whereas most of the nuclear DNA is replicated by DNA polymerase α in a symmetrical, bi-directional way[21,22]. Both these polymerases are present in the nuclei of uninfected cells and are thought to act in DNA replication, as they are present in increased amounts in S phase cells[23]. It seems that two fundamentally different modes of DNA replication may occur in human cells, symmetric synthesis catalysed by DNA polymerase α and asymmetric synthesis catalysed by DNA polymerase γ (ref. 19). The long single-stranded DNA regions visualised here may then represent displaced strands of parental DNA in asymmetric DNA synthesis.

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Codon–anticodon interaction at the ribosomal peptidyl-site

VARIOUS models have been proposed to explain the interaction between an aminoacyl-tRNA molecule and the ribosomal A and P sites during one elongation cycle of protein biosynthesis[1-7]. In general all these models agree as to the existence of at least two tRNA binding sites on the 50S subunit, the A and P sites. These models, however, do not agree on the important question of whether mRNA–tRNA (codon–anticodon) recognition occurs exclusively in the A site or in both A and P sites. Previous experimental evidence adduced in favour of either hypothesis has usually been based on analysis of the effects of various antibiotics on the partial reactions of protein synthesis. However, molecular mechanisms of action of such antibiotics are still poorly understood, and definite conclusions as to which type of model is correct cannot be based solely on such data. Here we show that factor-mediated binding of aminoacyl–tRNA (aa–tRNA) to the ribosomal A site is dependent on the presence of both an mRNA codon in the P site and of a tRNA which recognises that codon. Thus binding of a ternary complex EF-Tu · aa-tRNA · GTP to the ribosomal A site occurs only if the P site is occupied by a tRNA for which codon–anticodon interaction is maintained (for a preliminary report see ref. 8).

Initially we studied the binding of Phe–tRNA to ribosomes in the presence of 8 mM Mg^{2+} using oligouridylate templates with a chain length of between 3 and 10: the chain length dependence was found to be different in the presence and absence of EF-Tu (Fig. 1). Considerable nonenzymatic Phe–tRNA binding to 70S ribosomes was observed (even at this low Mg^{2+} concentration) with each of the eight oligouridylates investigated. Even with the triplet UUU, 3 pmol of Phe–tRNA were bound to 100 pmol 70S ribosomes, and only a small increase was observed with the longer oligouridylates (Fig. 1). In striking contrast to nonenzymatic binding, no EF-Tu mediated binding of Phe–tRNA was observed unless the chain length of the oligouridylate was equal to or longer than 6 (Fig. 1).

The requirement for $(U)_6$ for enzymatic binding of Phe–tRNA suggests that two tRNA molecules form a codon-dependent complex with the ribosome. Aminoacylated Phe–$tRNA^{Phe}$, exclusively present as preformed ternary complex, bound into

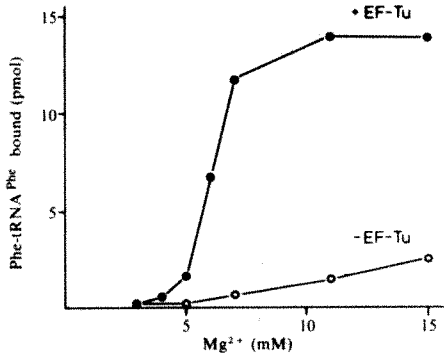


Fig. 1 Enzymatic (●) and nonenzymatic (○) binding of Phe-tRNA to 70S ribosomes in the presence of oligonucleotide $(U)_3$ to $(U)_{10}$. The binding of Phe-tRNA to 70S ribosomal tight couples[9] was assayed as follows: 75 μl of a mixture containing 20 mM Tris–HCl (*p*H 7.5), 50 mM NH_4Cl, 10 mM 2-mercaptoethanol, 8 mM magnesium acetate, 100 pmol 70S ribosomes and 25 nmol oligouridylate[10] was preincubated for 5 min at 37 °C and cooled on ice. For nonenzymatic Phe–tRNA binding the sample was then supplemented with 25 μl of a mixture containing the same salt concentrations as above, together with 100 pmol bulk [^{3}H]Phe–tRNA (2 Ci mmol^{-1}, charging degree 2%). When enzymatic Phe–tRNA binding was to be measured, 300 pmol EF-Tu[11] and 0.2 μmol GTP were included. The mixtures were incubated for 10 min at 37 °C. Ribosomal binding of Phe–tRNA was measured by the Millipore filter technique[12].

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Table 1 Binding of Phe-tRNA to 30S ribosomal subunits with oligonucleotide $(U)_3$ to $(U)_7$ in the presence and absence of EF-Tu

Oligonucleotide	Phe-tRNA^{Phe} bound (pmol)	
	EF-Tu absent	EF-Tu present
$(U)_3$	4.3	4.0
$(U)_4$	3.9	3.9
$(U)_5$	4.1	4.2
$(U)_6$	4.4	4.5
$(U)_7$	4.8	5.0

Experimental conditions as in Fig. 1 except that the binding was carried out with 100 pmol 30S ribosomal subunits at 16 mM Mg^{2+} with an incubation for 30 min at 0 °C.

the A site of 70S ribosomes, whereas the P site was exclusively occupied by deacylated tRNA^{Phe}. The evidence for this suggestion came from experiments which showed that no diphenylalanines were formed (as judged by peptide analysis) and none of the Phe-tRNA bound to the ribosome reacted with puromycin, that is, aminoacylated Phe-tRNA did not bind into the P site (data not shown).

In contrast, the degree of Phe-tRNA binding to 30S ribosomal subunits is independent of the oligouridylate chain length within the range of 3 to 7 (Table 1) and occurs to the same extent, regardless whether EF-Tu is present or not (Table 1). Thus each of the specific effects of EF-Tu described above requires 70S ribosomes.

The experiments with hexauridylates (Fig. 1) provided evidence that occupancy of the ribosomal P site by an uncharged tRNA was a prerequisite for subsequent enzymatic binding of Phe-tRNA^{Phe} to the 70S A site. To demonstrate that the occupation of the P site had to be codon-specific to permit subsequent enzymatic tRNA binding to the A site, the hexanucleotides AUGUUU, GUAUUU and UAUUUU were synthesised and used as templates. Four pure deacylated tRNAs ($tRNA_f^{Met}$, tRNA^{Val}, tRNA^{Tyr} and tRNA^{Lys}) were tested for this stimulatory activity. The results, summarised in Table 2, confirmed our predictions. EF-Tu-mediated Phe-tRNA^{Phe} binding was dependent on the presence of the tRNA cognate to the 5'-codon (Table 2). The combination of $tRNA_f^{Met}$ and AUGUUU gave the highest degree of Phe-tRNA^{Phe} binding, which was in each experiment above 40%. This effect was absolutely dependent on the presence of the 5'-AUG codon, since the stimulatory effect of $tRNA_f^{Met}$ was not observed with either GUAUUU or UAUUUU as templates (Table 2). This result is of special importance, since $tRNA_f^{Met}$ has a high intrinsic affinity for the P site[13]. It excludes the possibility that the observed enhancement of enzymatic Phe-tRNA^{Phe} binding to the A site with AUGUUU was only a consequence of the occupation of the P site by the initiator tRNA without concomitant codon–anticodon interaction. This was confirmed when use of tRNA^{Val} and tRNA^{Tyr} gave qualitatively similar codon-specific effects (Table 2). The effects of these two tRNAs on

Table 2 Enzymatic binding of Phe-tRNA^{Phe} to 70S ribosomes with AUGUUU, GUAUUU and UAUUUU is strictly dependent on the tRNA which is cognate to the first codon

Deacylated tRNA	Phe-tRNA^{Phe} bound (pmol)		
	AUGUUU	GUAUUU	UAUUUU
None	0.7	0.9	0.7
$tRNA_f^{Met}$	10.0	0.6	0.9
tRNA^{Val}	0.5	4.5	0.6
tRNA^{Tyr}	0.6	0.8	3.0
tRNA^{Lys}	0.5	0.6	0.5

Reaction conditions were as described in Fig. 1 except that the incubation mixtures contained 25 pmol 70S ribosomes, 25 pmol of pure [^{3}H]Phe-tRNA^{Phe}, 100 pmol EF-Tu, 5 nmol of hexanucleotide[10] and 50 pmol of pure deacylated tRNA as indicated.

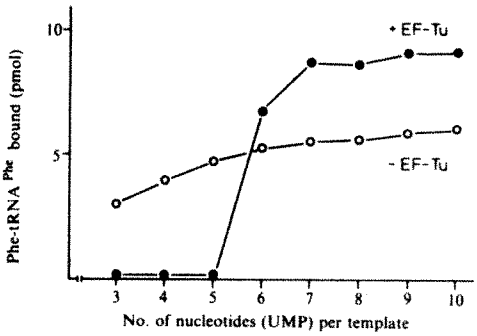


Fig. 2 Mg^{2+} dependence of the enzymatic (●) and nonenzymatic binding (○) of the Phe-tRNA^{Phe} to 70S ribosomes mediated by AUGUUU. Experimental conditions as in Table 2 except that the reaction mixtures contained 2 nmol AUGUUU and 50 pmol of pure deacylated $tRNA_f^{Met}$.

enzymatic Phe-tRNA^{Phe} binding were less marked than those seen with $tRNA_f^{Met}$, probably because of the lower affinity of deacylated non-initiator tRNA species for the ribosomal P site. tRNA^{Lys} had no effect (Table 2), which is as expected since the cognate codon for this tRNA (AAA) was not present in the templates.

Codon-specific occupation of the ribosomal P site with deacylated tRNA allows the subsequent binding of an aa-tRNA into the ribosomal A site to be studied in isolation, since peptidyl-transfer cannot occur in any of the systems described above. For AUGUUU/$tRNA_f^{Met}$ the active role of elongation factor EF-Tu in promoting aa-tRNA binding into the A site is not restricted to a unique Mg^{2+} concentration (Fig. 2). Negligible binding of Phe-tRNA^{Phe} to the ribosomes was observed from 5 to 15 mM Mg^{2+} in the absence of EF-Tu. However, in the presence of EF-Tu (and hence the ternary complex) maximal binding of Phe-tRNA^{Phe} was reached at and above 7 mM Mg^{2+} (Fig. 2).

Our findings are in accordance with the observations of others, who found a similar stimulation of enzymatic Phe-tRNA binding when the P site of poly (U)-programmed ribosomes was occupied either with *N*-acetyl-Phe-tRNA[14] or with peptidyl-tRNAs [15,16] but our data conflict with the conclusion[17] that the states of occupation of A and P sites are independent of one another. The latter experiments were, however, performed under nonphysiological conditions, namely at 20 mM Mg^{2+} and without elongation factor EF-Tu.

The experiments reported here prove that EF-Tu mediated Phe-tRNA^{Phe} binding to 70S ribosomes is possible if (1) the template $(N)_x$–UUU has a minimum chain length of 6 (Fig. 1) and (2) a tRNA is present which fits the 5'-neighbouring NNN codon. Non-cognate tRNAs are ineffective, even if they bind tightly to 70S ribosomes in the absence of their codon (for example, $tRNA_f^{Met}$; Table 2).

These observations can be explained by assuming that in physiological conditions (in the presence of EF-Tu and at low Mg^{2+} concentrations) the A site of the 70S ribosome can accommodate aa–tRNA only within the ternary complex EF-Tu · aa-tRNA · GTP and, even then, only when the P site is occupied by mRNA-cognate tRNA. Furthermore, the experiments strongly suggest that the A site only exists as such when the P site is so occupied. Further experiments will be required to show whether the codon-selected tRNA in the P site acts only as an effector molecule, which 'opens' the A site, or whether tRNA–tRNA interactions occurring within the ribosome are also involved.

Regardless of this uncertainty, our results show that codon–anticodon interaction is maintained by the P site-bound tRNA. Our results are consistent with conclusions drawn from recent experiments of Wurmbach and Nierhaus[18]. These authors analysed a series of purified deacylated tRNAs for their ability to block the P site binding of *N*-acetyl-Phe-tRNA to poly

(U)-programmed 70S ribosomes and to direct the same tRNA to theA site, and were able to show that only the cognate tRNA was effective.

The AUGUUU/$tRNA_f^{Met}$system may serve as an ideal and simple model system for investigations concerned solely with the binding of a ternary complex to the ribosomal A site, since the system is not dependent on the presence of initiation factors. Furthermore, the complexes of 70S ribosomes carrying Phe-$tRNA^{Phe}$ in the A site can be purified on a large scale by sucrose gradient centrifugation without loss of pre-bound Phe-tRNA (unpublished results). Thus the system may be useful for the study of the effects of some antibiotics and also for physicochemical investigations, such as fluorimetric studies, with tRNAs or templates specifically modified with flourescent probes.

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Atmospheric lead pollution of grass grown in a background area in Denmark

LEAD in the atmosphere of the Northern Hemisphere originates mostly from lead-alkyl petrol additives emitted from car exhausts[1]. A small proportion of this lead—estimated at about 10%[2,3]—is deposited within 50–100 m of the road while the rest is presumably transported long distances before deposition. There are reports of increasing concentrations of lead in roadside vegetation with decreasing distance from the edge of the road[1,4,5]. This apparent lead contamination ceases beyond 100–200 m from the road, and it is said therefore that airborne lead is not a significant source of lead in crops in general[5–7], although there is circumstantial evidence to the contrary[8,9]. We report here, however, that the lead taken up by grass in a remote rural area is predominantly from the atmosphere.

Table 1 summarises evidence of airborne lead in background areas far from roads and towns throughout the world[10,11]. If airborne lead were present everywhere, aerial deposition on vegetation could account for part of the lead content found in plants. Lead concentrations reported for protected plant organs are normally very much lower than for unprotected parts. The average rate of deposition of lead from the atmosphere in Denmark is about 1 mg m^{-2} per 30 d[12], which is much greater than the rate of accumulation of lead in an average crop, 0.1–0.2 mg m^{-2} per 30 d. To determine the uptake of lead by plant roots, specific activity (counts per 10^3 s per μg Pb) was measured in soil labelled with ^{210}Pb and compared with the specific activity in the plants. Any difference in specific activity in the plants had to be the result of uptake of nonradioactive lead by the plants. The site of our experiment was the experimental station at Højbakkegaard in Taastrup, west of Copenhagen. We know of no industrial sources of lead contamination within a radius of 5 km. The E66 highway runs east–west 1 km to the south (22,000 cars per 24 h), and the area is surrounded by agricultural farmland and forests.

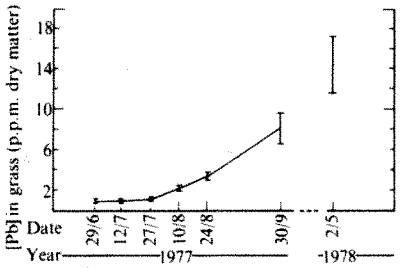


Fig. 1 Lead concentration in grass during growing season, p.p.m. dry matter. Vertical bars indicate $\pm\sigma$ on observations.

We carried out a pot experiment involving three different soils and a crop of Italian rye-grass (*Lolium multiflorum* Lam.). Pots were fertilised with normal amounts of N, P and K at the beginning of the season to give a normal growth rate. Six cuttings were taken during 1977 and one in 1978. Soils were labelled with ^{210}Pb (0.02 mCi per kg soil) 18 months before sowing. During this period the soils were mixed thoroughly several times and wetted to obtain isotopic equilibrium.

The surface area of the polyethylene pots was 250 cm^2, and the soil was 20 cm deep. Below the soil was a layer of 5 cm of acid-washed quartz on top of filter paper. Drainage was provided through a hole (1 cm diameter) at the bottom of the pot. The pots were sunk into a lawn, so that their edges were level with the soil surface, and placed on a layer of sand to secure drainage. After the experiment no roots could be seen emerging from the drainage hole in the pots. The height of the lawn was kept the same as that of the grass in the pots.

During the growing season the bulk precipitation of lead and the content of lead in the ambient air around the experimental station were measured. The rain was collected in a polyethylene funnel/bottle system and acidified with HNO_3 as preservant. Aerosols were sampled on an OECD standard sampler[15] modified with a 5-cm filter (Whatman no. 1), and sampling about 2 m^3 d^{-1}. The filters were extracted in 1M HNO_3 (boiling) before analysis.

Plant samples were dried in paper bags at 50 °C, and digested in HNO_3: $HClO_4$ (10:1). Soil samples were treated with three extractants to assess differences in isotopic labelling (1:1 HNO_3, 0.02 M EDTA or ammonium acetate (*p*H ~ 4, 8)). The determinations of lead in prepared sample solutions were made on atomic absorption spectroscopy with graphite atomiser[13]. Solvent extraction (DDDC in xylene) was used to eliminate interference[16]. The activity of ^{210}Pb in digests or extracts was measured by crystal scintillation counting.

A major objection to using radioactive tracers in a soil

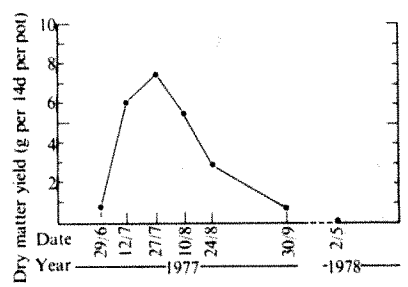


Fig. 2 Rate of dry matter yield, expressed as grams of dry matter (collected) per 14 days per pot.

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Table 1 Lead concentrations in air and lead deposition, and mean values for local background levels

Country and Locality	Period	Lead in air ($ng\ m^{-3}$)	Lead deposition ($mg\ m^3$ per 30 d)	Ref.
USA				
Intermediate non urban	1966–67	96		10
Proximate non urban	1966–67	210		10
Belgium				
Botrange	1972–75	212		11
Holland				
Background	1974–76		0.5	12
Rural area	1974–76		1.0	12
UK				
Plynlimon	1976	46	1.4	13
Denmark				
Jutland, Tange	1973	65		*
Rural areas, 12 stations	1976		0.8	14
Pot experimental site Taastrup	1977	132	0.9	This work

* The Danish Meteorological Institute, Air Pollution Section (1973).

experiment is the uncertainty of isotopic equilibration. However, as three methods of soil extraction gave the same specific activity, we believe that the added ^{210}Pb had attained true isotopic equilibrium with the indigenous lead. Further, if the added ^{210}Pb was less strongly bound than the indigenous lead, the observed specific activity in the grass would be too high, leading to an underestimation of the atmospheric lead supply to the plant.

The characteristics of the soils are given in Table 2. Average lead deposition and the average ambient air concentration are shown in Table 1. The values observed at the experimental site are higher than those found at remote sites, but they are within the range found in rural areas of western Europe and the US.

The total lead concentrations in the plant material that had been exposed to the atmosphere varied from ~0.6 to 8 p.p.m. dry matter for plants grown in the summer, while concentrations were considerably greater for those grown during the winter. The range of lead concentrations found is quite similar to the values summarised by Höll and Hampp[1] for grasses sampled in background locations. In research on the application of sludge to the land conducted in cooperation with three state research farms in Denmark we have found that the concentration of lead in grass from untreated fields is in the range 1–6 p.p.m. of dry matter, most values averaging around 2 p.p.m.

The observed increase in the lead concentration during the growing season is consistent with the findings of other authors[6,14]. This phenomenon may be explained as a decreasing dilution of the atmospherically supplied lead with newly grown grass, due to the decrease in growth rate after the midsummer peak.

Table 2 Characteristics of soils in the pot experiment

Origin and type of soil*	Rønhave (loam)	Askov (sandy loam)	Lundgaard (sandy)
Clay % < 0.002 mm	16	11	3
Silt % 0.002–0.02 mm	21	12	4
Fine sand % 0.02–0.2 mm	37	38	26
Coarse sand % 0.2–2 mm	24	37	65
Organic matter %	2	3	2
CEC mEqv per 100 g dry matter	13	12	5
pH_{CaCl_2}	6.6	6.3	4.6
Total lead p.p.m. in dry weight	17.3	14.4	11.1

* The soils are arable top soils (0–25 cm).

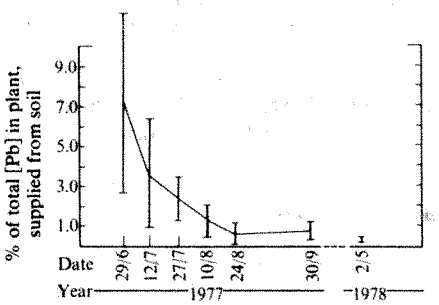


Fig. 3 Root uptake of lead expressed in percentages of total lead supply to plant. Vertical bars indicate $\pm\sigma$ on observations.

Figure 2 shows the average yield of dry matter per pot per 14 days. Yields are comparable to normal yields on grassland, being of the order of 6–10 ton of dry matter per hectare per season. The specific activity of lead in the soil was 1,500–3,100 counts per 10^3 s per μg Pb, and in the grass 4–200 counts per 10^3 s per μg Pb.

The total concentration of lead in the grass, and the calculated percentage of lead in the grass coming from the soil did not differ significantly between the three soils used in spite of differences in *p*H and texture. Figures 1–3 are thus the averages of results obtained for the three soils in six pots.

The percentage of lead in the grass crop originating from the soil was calculated as:

$$\%\ Pb_{soil} = \frac{\text{Specific activity of Pb in crop}}{\text{Specific activity of Pb in soil}} \times 100$$

Figure 3 shows that it was 0.2–10% of the total lead content of the green parts of the grass. This result will not be greatly affected by the earlier discussed possibility of isotopic inequilibrium in the soils, as an underestimation of the atmospheric contribution is hardly important when it is more than 90%. Thus deposition of atmospheric lead on the plant surface was responsible for between 90 and 99% of total lead content in the grass in this typical background area in Denmark.

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reviews

Defining sociobiology

Richard Dawkins

Sociobiology: Sense or Nonsense? By Michael Ruse. Pp. 231. (D. Reidel: Dordrecht, The Netherlands, and Boston, Massachusetts, 1979.)

WHAT on earth is sociobiology? If philosophers have a role to play in science it presumably includes clearing up semantic confusion. Michael Ruse is a distinguished philosopher of biology who would agree: "I am a philosopher, and for me, in John Locke's great words, 'it is ambition enough to be employed as an under-labourer in clearing the ground a little and removing some of the rubbish that lies in the way of knowledge . . . ' " (p164). Yet, after reading this sensible and pleasantly written book, I remain unable to answer the question with which I began. What is sociobiology and who are the sociobiologists?

Well, if anybody is a sociobiologist it is E. O. Wilson, so his definition must be taken seriously: ". . . the systematic study of the biological basis of all social behavior" (*Sociobiology: The New Synthesis;* Harvard University Press, 1975). A neutral discipline then, like history or geology. Geology or history as such cannot be true or false, yet Ruse debates the "truth" of sociobiology, and whether it is sense or nonsense. I sympathise. However neutral Wilson's definition may be, the widespread identification of "sociobiology" with a school of thought is an undeniable fact of current biological sociology, a linguistic oddity whose only recent biological parallel is the popular transmutation of "ecology" from a scientific discipline to a political opinion.

What, then, is this cluster of ideas, this bundle of concepts that have become linked in people's minds both with each other and with the name sociobiology? The answer is complicated because it depends on whether you read biologists or social scientists. Ruse has read both and absorbed both meanings, apparently without realising it. I will return to the social scientists' meaning later. The biological cluster is well represented by section headings in Ruse's chapter on "The Sociobiology of Animals": kin-selection, parental investment, reciprocal altruism, evolutionarily stable strategy. How the cluster came to be linked in our collective mind is not clear. Wilson's *Sociobiology* can hardly be the common denominator, for, although these concepts are mostly mentioned there, so is almost everything else. As befits his own definition, Wilson rightly devotes more space to the comparative study of social systems and their correlation with environmental variables, important topics which seem to be absent from the mysterious cluster and absent from Ruse's book.

My own slightly irreverent epitome ("sociobiology is the branch of ethology inspired by W. D. Hamilton") leaves the timing unexplained. A little research in *Science Citation Index* shows that Hamilton's now famous papers in the 1964 *Journal of Theoretical Biology* were seldom cited in the nine years up to 1973. Then, in 1974, the year before *Sociobiology,* the curve suddenly turns up sharply and has soared (almost exactly exponentially) ever since. When concepts that have been ignored a long time suddenly group themselves together and proliferate like fire through the imaginations of scientists, then philosophers and historians of science should have interesting things to say. Unless, that is, they are lured away from constructive analysis by a preoccupation with destructive criticism and the supposed need to reply to it. In this respect Ruse's book is a disappointment.

It is, however, not all disappointing. Ruse may not define his subject, but he does a good job of discussing it, whatever it is. He writes in an easy, unpretentious style, and his aim is clearly to communicate with his readers rather than to show off to them. "Of course, if I get my biology wrong I expect to be criticised by biologists; but that is nothing to what I expect from philosophers if I get my philosophy wrong . . .". This particular biologist has no complaints about the chapter on "The Sociobiology of Animals"; it might have been written by a biologist. Oddly enough, my most serious worry is over an issue that could be called philosophical, the one known by the cliché of nature and nurture.

The trouble begins to surface in the chapter on "The Positive Evidence" (for human sociobiology), where he makes the following strange remark: "I take it that the problem at issue is the extent to which human behaviour, specifically social behaviour, is essentially a function of the genes, where, by this, is meant that the behaviour will manifest itself under normally occuring environments without the need for any special learning input". We have now moved on to the social scientists' understanding of "sociobiology", which has nothing to do with Wilson's neutral definition (unless perhaps you think "biological" means "genetic"), nor with my "mysterious cluster". Sociobiologists are apparently supposed to believe that human behaviour is governed by genes as opposed to learning (I even recently heard Sir Cyril Burt quoted, not by Ruse of course, as an example of a sociobiologist!).

Let me briefly put my finger on this muddle, for it is a common one and I know it well. Whoever the sociobiologists may be, nobody would deny that they are interested in behaviour as Darwinian adaptation. In order to talk about the Darwinian evolution of anything we have to postulate the sometime existence of genetic *differences* between individuals — otherwise there would have been nothing for selection to choose between. This does not mean there has to be genetic variance any longer: any characteristic that has been strongly favoured by selection is likely now to have low heritability, the genetic variance having been used up. More importantly, a genetic contribution to differences is not the same as (indeed, it has nothing whatever to do with) behaviour manifesting itself "without the need for any special learning input". As Konrad Lorenz and others have emphasised for years, learned behaviour must itself be subject to Darwinian, therefore genetic, selection. Happily, Ruse does not fall for the even more extreme muddle, beloved of

extreme hereditarians and their unwitting allies among "scientific radicals", of equating genetic variance with irrevocable, unswerving genetic determinism.

Ruse is rather good at dealing with such people. In America there are some genuinely good scientists who have severely criticised sociobiology, and we have to listen to them. Ruse does so, understands them, agrees with them in places, but finally remains largely unimpressed. He also gives some reassuring pats to the ruffled feathers of social scientists who fear imminent takeover by a rapacious biological imperialism.

I have concentrated on places where the book could have been improved, but there is good in it and I hope it will be read by those who have managed to retain an interest in this rather overpublicised controversy. Ruse's sense of balance and agreeable style are well shown in his closing sentences: ". . . I am far from convinced that human sociobiologists have yet made their case. What I do plead is that their sins are not as grave as their critics argue. Human sociobiology should be given the chance to prove its worth. If it cannot deliver on its promises, it will collapse soon enough; but if it does prove viable, then its success could pay scientific dividends of the highest order". Who will disagree? □

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Interpreting visual motion

The Interpretation of Visual Motion. By Shimon Ullman. Pp. 229. (MIT Press: Cambridge, Massachusetts, and London, UK, 1979.) $17.50; £11.40.

THIS book is a slight revision and extension of Ullman's doctoral thesis researched at the Artificial Intelligence Laboratory of MIT. Various parts of it have also been published independently (in somewhat different form) in various journals (*Perception, Perception and Psychophysics, Proc. R. Soc. London).* Nevertheless, the book is worth prossessing in its own right if you are an experimental psychologist or an artificial intelligence researcher interested in vision, especially in motion perception, primarily because of its special methodological qualities.

Ullman investigates the phenomenon of apparent (or phi) motion, the illusion enabling us to watch television or movie pictures despite their discrete presentation. The phenomenon is conceptualised as a computational problem and is divided into two parts, each of which is studied independently. Solving a computational problem includes specifying the goal of the computation, and then investigating possible (and plausible) methods for attaining the goal. A theory of what is being computed is thus an integral part of the understanding of the information processing system being studied, in this case the human visual system. The phi phenomenon is seen to establish the correspondences between the elements in two successive "snap-shots" of a scene, and then interpreting the resulting displacements as motions of the objects.

Ullman shows that the correspondence computation amounts to computing the so-called minimal mapping, the match that minimises costs over the covers of the graph of possible (probability weighted) pairings. He shows that the initial candidates for a set of possible pairings for a given element in one snapshot can be restricted to elements (in the second snapshot) lying within some limited distance radius of this element. This means that the correspondence process can be effected by a simple network of locally competing processors of uniform structure. Furthermore, Ullman shows that the domain of the computation is a "token space" (D. Marr's "primal sketch": *Phil. Trans. R. Soc.* **B275**, 483-524: 1976), a symbolic representation consisting of blobs, line segments, edge fragments and the like, which is the result of pre-processing the raw image intensity distributions.

The next stage consists of interpreting the displacements obtained. Ullman develops the notion of a "rigidity test", operating locally on a nucleus of elements and leading to decisions about whether the displacements associated with the nucleus can possibly represent (and result from) a rigid object in motion. The analysis is based on his "structure-from-motion" theorem which states that the structure (relative position) of four non-planar points is uniquely recoverable from three orthographic projections. Such an interpretation does not assume any recognition of, or familiarity with the objects in question.

Finally, Ullman discusses "motion-from-structure" situations in which motion is established as a "filling-in" process linking two objects recognised as being an instance of the same object in the two successive static snapshots. He compares the two motion-analysing mechanisms and argues that various interpreting processes (analysis of static "snapshots", "motion-from-structure", "structure-from-motion", and so on) all contribute to one common representation and that they must compete or co-operate to produce the final result, the percept.

This monograph is clearly written. Within the limits he has set for himself, Ullman has achieved a great deal. This is an excellent and important piece of work.

K. Prazdny

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Protein synthesis inhibitors

Inhibitors of Protein Biosynthesis. By David Vazquez. Pp. 312. (Springer: Berlin, Heidelberg and New York, 1979.) $32.50.

NO matter how hard you try to keep up with the literature in this field, you usually find it slipping away from you. Well, here's a good chance to catch up with all of it. In this comprehensive survey David Vazquez cites 1017 references (I think), of which a mere 100 originated from his laboratory, and pulls so many rabbits out of the hat that one feels he could have directed *Watership Down.*

The book is bulging with information including the structures, empirical formulae, molecular weights (many thanks) and synonyms of antibiotics together with the names of the producing organisms. The subject of synonyms is particularly intractable and this reviewer is now glad to know that multhiomycin and nosiheptide are the same thing, to say nothing of anthelmycin and hikizimycin. All your old favourites are here and, when you turn to the appropriate Figure, you'll probably find they have a few cousins you didn't know about. It's inevitable (isn't it?) that something should have been omitted, but I'll have to check carefully before I complain that pulvomycin should have been included with the rest of the kirromycin group in Fig. 42. (You never know, it could be an alias for efrotomycin!)

All the alkaloids are in here too and when botanists have finished talking about them as being herbivore-repellents, or nitrogen excretion products, or whatever, they should note just how many of them are ribosome-inhibitors. The same is true for various other plant products known more generally as lectins and agglutinins.

Of course the main aim of this book is to deal with the action of inhibitors of protein synthesis. So, if you want to know what others think they know about the mode of action of any antibiotic or drug, David Vazquez will tell you. For this reason the book does not read like an essay; with so many facts and figures (61 of the latter) it is meant to be used as a reference book. As such it should find a place in research laboratories and in reference collections, although it cannot be recommended for students. However, no-one working in the fields of antibiotic action, protein synthesis or ribosome structure-function analysis should be without it. I warmly recommend it.

Eric Cundliffe

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Human information processing

Strategies of Information Processing. Edited by G. Underwood. Pp.455. (Academic: London and New York, 1978.) £20.

IN the 1950s and early 1960s scientists who discussed selective attention, perceptual discrimination, reading and the choice of responses called themselves Human Experimental Psychologists. They were earnest people prone to strange methodological obsessions. They used models derived from the theory of simple, passive communications systems and metrics derived from information theory and communications theory. Nowadays the same people are relaxed and expansive. Most have abandoned interest in methodology or metrics and many even in experiments. Nowadays we are Cognitive Psychologists. Among very young research workers it is a dogma that this euphoric transformation (including, incidentally, the titling of three prestigeous journals, at least two large departments of psychology, four honorific chairs and an Open University course) came about as a result of a single book, Ulrich Neisser's *Cognitive Psychology* published in 1967. Older workers are embarrassed to enjoy their new liberties without knowing what they must believe to earn them. They find it strange that a very slight book should be credited with a Cognitive Revolution and, by and large, those of us who still do research do the same things we have always done under new titles.

The middle-aged are not prone to rocking boats - even Ships of Fools - so it is right that complaints should come from a young research worker, Geoffrey Underwood, who has edited a collection of 12 essays under the title *Strategies of Information Processing*. Underwood takes his role as challenger of the new establishment as seriously as if he were writing in 1958. He insists that his book is a "statement of faith" (Preface, p1). Apparently he believes that classical models of human information processing may be revived within the context of models drawn from the theory of self-optimising control systems. He feels that these rehabilitated models will provide means for empirical investigations of intention, and purpose, and even perhaps sensible approaches to problems of consciousness. That is they will simultaneously teach Cognitive Psychologists to do proper experiments and disarm their critics (such as Gauld, Shotter and the anti-empiricists) who believe that the most important problems of human psychology are inaccessible to laboratory investigation.

Older research workers may be expected to brighten at this news. The dear familiar models of our youth are to be justified as the basis of the next conceptual advance. We have always lived beyond the current fashion without knowing it.

The publication of these papers will not quite achieve this. Critics who objected to "mechanistic" psychology in the guise of simple communications models are not likely to be placated by descriptions of purpose in terms of loops and branches of computer programs or conceptions of intentionality paraphrased as the search for the optimum among possible paths in a decision tree.

Control theory models have been common in ethnology since the late 1960s and excellent summaries of their applications by MacFarland, Oatley and Toates have formed the basis of undergraduate courses for some years. The omission of recent work on ethological systems is a sad ommission from this book. There is little about the contributions which suggests any striking novelty of approach — or even any daring retrogression. Neville Moray now provides a clear and useful summary of the lectures on optimisation of informational selectivity which he has given over the past six years. Max Coltheart usefully reviews his recent thinking about lexical access during reading. A review of hemispheric asymmetry effects by M.P. Bryden provides an updated bibliography of the unreliable literature in a field recently very popular among Cognitive Psychologists. The inclusion of the word *strategy* in the titles of contributions seems to reflect loyalty to the editor rather than any worked-out change in theoretical approach. For example, C.I. Howarth reviews his own, excellent and sadly neglected, work on motor skillls under the title "Strategies in the Control of Movement". There are useful digests of research on speech perception by Cole and Jakimek and on eye-movements during reading by Alan Kennedy. One paper certainly has a novel theme. John Fox provides an exegesis of hypothetical neurology of vision proposed by Dr. Marr. This work is fascinating, and original, but Marr's own papers are more lucid, much richer in their implications, more rigorous in argument and now generally available. Fox's paper is cheerful and entertaining, but why is it necessary for him to act as a sort of intellectual Basil Brush on behalf of a very talented colleague?

In sum, this is a very handy reference work, invaluable for teaching courses to third-year undergraduates. It does repeatedly stress the point that human beings must be considered as active rather than passive self-optimising systems and it illustrates this emphasis with data from a wide range of experimental situations. Librarians and teachers will find this a useful book to buy, but active research workers will be busy with the current issues of journals and have read it all long ago.

Patrick Rabbitt

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Biological transport

Mechanics and Energetics of Biological Transport. By E. Heinz. Pp. 159. (Springer: Berlin, Heidelberg and New York, 1978.) DM 49; $24.50.

THE swift development of biological transport as a discipline in its own right over the past two or three decades has proved something of a phenomenon, and has brought in its wake the publication of a near-avalanche of books. Predictably, the majority of these are edited proceedings of meetings or collections of review articles, and suffer from the lack of coherence both in style and content peculiar to this particular genre. Some notable exceptions are the well-known treatises of Stein, Schoffeniels, Kotyk and Janácek, and more recently Christensen, each of which is written from a highly individual standpoint and intended as an all-embracing treatment. What is missing at present is a comprehensive analysis devoted entirely to the energy transactions of the transport process, which would draw together material scattered in a forest of original reports and reviews.

In this regard, the monograph under consideration (offset printed from a typed manuscript) presents, first and foremost, a concise compilation of *kinetic* models and formulae. Still, a commendably broad range of topics is included, covering one-flow and two-flow systems, along with an approach to the description of coupled transport in terms of nonequilibrium thermodynamics. Among the topics touched on in summary fashion are diffusion, flux ratios, specificity, carriers and channels, coupling phenomena, isotope interaction, and primary and secondary active transport. Some examples indicating the range of specific coupled systems discussed are the ATPases (Ca^{2+}-activated, Na^+-K^+-activated, and proton translocating), the redox proton pump, and the phosphotransferase systems. For many readers the brevity of the treatment will be a great advantage, and the ready accessibility of a large number of formulae to suit every occasion (and taste) may well be, for some, the most attractive feature of the book.

However, this is by no means an eclectic account of transport bioenergetics. Quite the opposite is the case: it bears the unmistakable stamp of a single school. To readers familiar with kinetics this may not

Nature Vol. 280 2 August 1979

be uncongenial. The author places great emphasis on the so-called "Law of Mass Action" treatment, in which transport phenomena are formally described as if they were chemical reactions. The implication is that coupled processes such as active transport are intrinsically stoichiometric, that is, completely coupled, and that any slippage must be attributed to internal or external leakage, or to "leakage" of the driving reaction in the case of a pump. This view does not comprehend the wider possibility that incomplete coupling may be a fundamental property of the system and even confer on it some regulatory advantages. Nevertheless, a serious attempt has been made to combine and integrate the kinetic and thermodynamic analyses. It might have succeeded better if certain lapses in rigour had been eliminated — for example, the present reader was surprised to find the standard affinity being put forward as the effective driving force in active transport, and to be informed that completeness of coupling implies an efficiency of 100%.

Although the author has certainly provided a unified and consistent exposition of his theme, lacunae occur which are troublesome. Some of these are to be anticipated from the preface, in which he states: "The simplifying assumptions may often be unreal. Hence the equations should not be applied uncritically . . . ," and later ". . . no attempt has been made to quote the underlying literature completely and according to priority". Indeed, the sparing use of references will undoubtedly make critical evaluation of the equations a hard task for the tyro. Perhaps the most important omission is the absence of experimental data which might have been used to illustrate the usefulness and range of applicability of the theory.

The book is marred by the usual sprinkling of misprints, but is handsomely bound, as behoves a manual to be thumbed through at frequent intervals. With some reservations I recommend its purchase, suspecting that its utility will be greatest for confirmed *afficionados*.

S. R. Caplan

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Semiconductor systems

Problems of Linear Electron (Polaron) Transport Theory in Semiconductors. By M.I. Klinger. Pp. 931. (Pergamon: Oxford, 1979.) £62.50.

THIS is a very large and expensive addition to Pergamon's Natural Philosophy series, being some nine hundred pages in length. It covers the theory of linear transport and other properties of electrons in a range of semiconductor systems which become gradually more disordered as the book proceeds (reflecting the research trends of the past three decades). As one might expect in such a large book on a restricted topic, there is a vast amount of detail, with every major (and in some areas minor) theory described.

The first two chapters form an introduction to transport and solid-state theory. The interaction of an electron with the lattice vibrations to form polarons is then described in various mathematical and physical approximations. The dynamic and transport properties of the polaron in crystals are considered in the following three, very long, chapters. The final part of the book attempts to draw together the theories of electron conduction in disordered semiconductors.

The content of the book is, in one respect, excellent, in that almost everything one could wish for has been covered concerning the theoretical development of the subject up to the mid-1970s. It is not a textbook in the normal sense; however, there is a strong tendency to present the problems and the various solutions with little explanation. In some areas it was necessary to go back to the original papers to understand the author's over-concise version. Throughout, the emphasis is on the formalistic development of the subject where possible, experiment playing only a very minor role. Even with this omission, the treatment is very condensed and mathematical, especially in the first half of the book. In consequence, however, a large range of properties and physical models have been covered.

Very few concessions are made to the reader: a very high mathematical ability and a good background knowledge are assumed, making this a book for the theoretical specialist rather than the general research worker. In a field where experiment and simple physical models have played such a large part in aiding our understanding, especially in the disordered systems, this is unfortunate and gives a distorted view.

In conclusion this is a rather lopsided book; a more critical approach to the content could have reduced the size and still have allowed room for a more balanced treatment of the subject. What one has here is a very specialised reference book, undoubtedly very useful to a restricted number of people but not really fulfilling the need of the many experimental workers in this field.

J.C. Inkson

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Materials science

Science of Materials. By W. Brostow. Pp. 436. (Wiley-Interscience: Chichester, UK, and New York, 1979.) £20.

THIS book is intended as an undergraduate text in materials science, but differs significantly from existing books in its approach. This difference can best be summarized by saying that Professor Brostow has given us a physicist's view of materials science rather than a materials scientist's! Thus the solid state is not introduced until the seventh chapter, the first six being devoted to mathematical techniques, statistical mechanics, intermolecular forces, gases, liquids and solutions.

This early portion of the book is excellent, especially the treatment of statistical mechanics which is rarely introduced in any depth in materials texts. The treatment of intermolecular forces is also refreshing, escaping as it does from the simplistic classification of chemical bonds usually presented in books for undergraduates.

The amount of space devoted to the physics of matter means that some essential topics such as phase equilibria, crystal structure and defects are treated in a very basic fashion, appropriate only to a first-year level. This tendency to touch on subjects without dealing with them adequately increases as the book proceeds. Thus, polymeric materials are covered completely in eight pages (although rubberlike elasticity provides a further six pages in a later chapter.) Composite materials have a separate chapter, but more than half of its twelve pages are devoted to wood, while fibre reinforcement is dismissed in less than a single page. The mechanical properties of all forms of solids and liquids occupy a single chapter and strengthening mechanisms in crystalline solids receive only a three-page coverage. Electrical and dielectric properties are treated at what seems to be a sub-degree level, and a final chapter on the testing of materials contains only four pages of text and no diagrams.

In a book of manageable size, of course, it is quite impossible to achieve both depth and breadth. Professor Brostow has settled for depth in the physics of matter and breadth in materials science, and this balance will no doubt appeal to teachers of physics. By the same token, however, it will not satisfy the needs of materials and engineering undergraduates. The high cost of the present hardcover edition will also discourage its use as an undergraduate text.

E.H. Andrews

E. H. Andrews is Professor and Head of the Department of Materials Science at Queen Mary College, University of London, UK.

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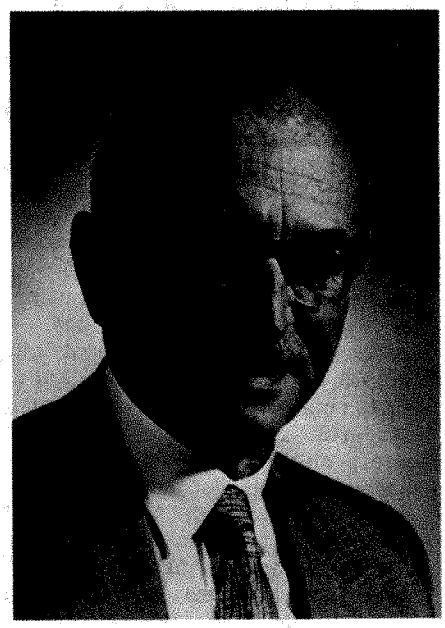
obituary

Dennis Gabor, 1900-1979

DENNIS GABOR, who received the Nobel Prize for Physics in 1972, for the invention of holography, died on 9 February 1979. One can surmise that he would have approved of such a one-sentence summary — holography was the crowning achievement, contemplated with great pleasure. Yet, it gives a distorting emphasis on a man who contributed radically new ideas to a number of quite distinct areas of applied physics, and who moved with effortless facility across the cultural divide.

Gabor was born in 1900 in Budapest, where he spent all his school days and completed three years at the Technical University. At the age of 21, he moved to the Technische Hochschule in Berlin, where, after receiving his Diploma, he commenced his doctorate studies under Professor Mathias. These centered on the use of cathode ray tubes for the observations of transient waveforms — thereby initiating a development, which has continued to this day, always in pursuit of the faster transients that have marked the path of progress in electronics. A by-product of this research was the development of the first electron lens; it had been known for some time that electrons could be focused by means of a 'concentrating coil', but it was Gabor's use of a magnetic circuit to concentrate the magnetic field over a short region which led to the first practical lens. Although he did not, at the time, fully understand its mode of action (it was just before the celebrated paper by Busch which marks the beginnings of modern electron optics), the invention was to play an important role in the realisation of the first electron microscope. In fact, the first demonstration of such a microscope by Borries and Ruska used the actual lens created by Gabor.

Gabor had himself speculated on the possibility of realising an electron microscope, as he has recounted in a fascinating article on the History of the Invention of the Electronmicroscope (*Elektrotech. Z. Ausg. A.* **78**, no. 15, 522-30, 1957) — but enthusiasm for undertaking the development was dampened by the expectation that any objects of interest would be rapidly carbonized by the electron beam. By the time Borries and Ruska embarked on the venture, Gabor had moved to the Siemens



Research Laboratory.

It was a narrow escape from greatness at an early age. Perhaps that early disappointment served to sharpen his subsequent enthusiasm for the inventive process; Shockley has attributed his invention of the junction transistor to his chagrin at having missed the earlier point-contact device.

The work at Siemens was largely devoted to a new type of gas discharge lamp. Whilst this research failed to lead to a commercially exploitable product, it marked the beginning of a life-long interest in the plasma state and its applications. It also led to a move to the Central Research Laboratories of B.T.H., Rugby, in 1933 — Gabor's response to the election of Adolf Hitler in Germany. The move to Rugby was facilitated by inspired talent-spotting on the part of Professor T. E. Allibone.

While continuing his work on gas discharge lamps, Gabor never entirely abandoned his interest in electron optics. He invented what we would now call a mechanical analogue computer for electron trajectory tracing; coincidentally with Irving Langmuir, he discovered the basic limitations on electron density in electron optical systems.

During the war, by reason of his status as an alien, Gabor was not able to contribute to the main stream of research — it was a time when the damning fact of foreign origin was more than a match for rational assessment. He was, nonetheless, able to make pioneering contributions to infra-red imaging and to develop a deeper understanding of the interaction between electrons and electromagnetic fields, the latter leading to a series of classic papers published in the last two years of the war. It also enabled him to continue work on electron optics and, above all, to do battle with what he perceived as the key problem — how to improve the quality of electron optical lenses. The constraints imposed by Laplace's equation deprive the electron optical designer of most of the weapons which are available in classical optics. The aberrations of electron optical lenses seemed to bar the path to the achievement of the ultra high resolution of which electron microscopes should have been capable — a resolution which could image single atoms.

Gabor tried to find room for manoeuvre by suggesting the introduction of space charge into lenses, to correct the aberrations — a technique which, as it eventually turned out, could not be effective, on account of the blurring occasioned by direct electron-electron interaction. Then, in 1947, quite suddenly, he perceived that the way to deal with bad lenses was not to strive to make them a little better, but, rather, to get rid of them altogether. The strategy envisaged by Gabor was made up of a number of quite distinct and quite brilliant insights — the ability to record the phase of a wavefront using materials sensitive only to intensity, by including a 'reference' wave; the possibility of recovering the information contained in the original wave by a reconstruction process with another, complementary wave; the recognition that the recording and reconstruction waves need not have the same wavelength — that an electron 'hologram' might be reconstructed at optical wavelengths. Gabor was able to demonstrate the principle at optical wavelengths, using the most coherent source then available — a mercury arc illuminating a pin hole. He published the results in a celebrated communication to *Nature* in 1948. It was a long way from the bold scheme envisaged for improving electron micrographs;

indeed, to this day, such improvements have not been realised — though the hope of eventual success is not dead.

Gabor then developed a comprehensive theory of holography, but few more experiments were conducted for a dozen years. It was not until the invention of the laser provided experimenters with highly coherent sources that holography had its second birth, in a series of inspired researches undertaken by Leith and Upatnieks.

The subsequent story of holography is one of explosive growth. It is now the basis of a large and growing industry; it has inspired new forms of art. It is still far too early to assess its ultimate impact on science, technology and the conduct of human affairs.

How should such a discovery be weighed and judged? Gabor, himself, wondered how one should assess the value of a discovery in applied science, in the aforementioned paper on the history of the electron microscope. The following is a translation from the German of one of the opening paragraphs in that paper:

'It is hardly to be debated that those whose work is most highly regarded are those who have pioneered a new field which has subsequently been shown to be fertile. In this there is an element of luck, possibly even of injustice; honours are not bestowed — as they are, for example in mathematics — in proportion to the mental effort required to reach the result. In fact, I would go further and suggest that if a scientific achievement is to receive the highest accolade, it should not be wholly rational; there must be some element of boldness and risk. We regard the discovery of electromagnetic waves by Hertz as a greater achievement than Hamilton's theory of conical refraction not only because Hamilton's work put the finishing touch to the theory of crystal optics whilst in contrast, Hertz opened up a wide and new field, but also because, when Hertz began his classic investigations, Maxwell's theory was by no means established; moreover, there was not the slightest ground for the expectation that the conductivity of the electric spark could decay in 10^{-9} seconds. This was a matter of boldness and risk — not one of logical expectation.'

The passage was written with reference to electron microscopy. It was 1957, and Gabor certainly had not the slightest expectation that holography could ever be viewed in such terms. Certainly, the research students he acquired when he moved to Imperial College as a Reader in 1948 — and these remarks are written from the vantage point of one of these — regarded holography as a laboratory curiosity. Yet, with hindsight, the passage is, surely, an entirely apt commentary on the invention of holography.

Often one finds that an inventive step follows very hard on the heels of another discovery, or some advance in technology which made it possible. Holography, however, was ready for birth at least half a century before the idea occurred to Gabor. Moreover, although the demonstrations in optics were difficult, there was no inherent reason why the principle should not have been demonstrated in acoustics — where, indeed, it has found application in recent years. By this criterion also, the invention of holography must be seen as an extraordinary stroke of brilliance.

In this period, Gabor also produced his pioneering contributions to what we now call communications theory — beginning with a remarkable paper published in the *Journal of the Institution of Electrical Engineers* in 1946. This work was inspired by the desire to gain a deeper understanding of the structure of information transmission; yet, included in the motivation there was, as always with his work, a thrust towards the applied, towards the inventive step. It led him to investigate means for transmission of information using 'band-width compression', a theme which remains an exciting area of research to this day.

When Gabor moved to Imperial College in 1948, he presented a remarkable and, at first, unfathomable phenomenon to his research students. He had little comprehension of the difficulty encountered by ordinary mortals in attempting to gain an appreciation of his aims in a particular research endeavour, or to follow a leaping thought line without the help of stepping stones. His lecture courses on electromagnetic theory, statistical physics, the plasma state, electron optics, seemed memorable but hardly capable of assimilation — at least, until the realisation dawned that they were not so much lectures as master classes. It was necessary to 'know' the subject before attending these occasions; but then the experience was enormously worthwhile — providing both the long historical view as insight in depth and generality. There must be many who still treasure the copious lecture notes typed at high speed by Gabor himself using an amazing two finger technique on a battered, ancient portable. There must be many who regret that these notes never found a more permanent expression in what would have amounted to a unique set of books; but research, invention and original thought was always the greater attraction and the more compelling master of his time.

There was no doubt amongst his students that Gabor could and should receive the Nobel Prize for something — we debated just what it might be. Nor was this a symptom of hero worship, of which we were largely innocent. We could assess the magnitude of the intellect but found it harder to discern its direction. We were also aware of a personality of great warmth yet of inherent shyness. Communication was not always easy. And the attempts to capture that personality, the highly charged, penetrating comments, the humour, the perfection and elegance of expression, though clothed in a Hungarian accent of some substantial depth, was the supreme task of those endowed with any ability of mimicry — though one doubts whether their skill will ever again be displayed.

Recognition outside his immediate group was slow to come. Gabor was a physicist who saw himself as a practical engineer — a reversal of the more usual aspiration for role changing. Not all physicists recognised this situation. I remember a seminar, perhaps around 1953, to an audience largely of physicists, where Gabor discussed the interpretation of discrete energy losses suffered by electrons when transmitted through thin metal films, in terms of plasmon excitation — an explanation which we now know to be essentially correct. The criticisms which he met at the time were fierce, and seemed to transcend the requirements of scientific debate — there seemed to be an element of a demarcation dispute.

By the early fifties, Gabor had completed his work on holography, made pioneering contributions to information theory, to electron optics, to electron-electromagnetic interactions and to plasma theory. Yet, he was still a Reader in the University and repeatedly failed to achieve election to the Royal Society. The omission was resented by his students and others who knew him well; it was deeply felt by Gabor himself. It was hard to comprehend at the time; it is no easier to do so in retrospect. Throughout this period, however, Gabor's creative work never faltered. Flat television tube research involving the most ingenious electron optical conceptions lived side by side with learning machines, with holographic microscopy and, a little later, with schemes for direct generation of electricity from heat using a plasma — a technique which echoed some of his work on gas discharge lamps of three decades earlier. Many of these schemes remain as fascinating concepts, devoid of practical implementation. But it would be unwise to write them off; holography had a gestation period of 15 years.

In 1956, Gabor was, at long last, elected a Fellow of the Royal Society and two years later became the Professor of Applied Electron Physics at Imperial College. His inaugural address marked a totally new direction in his publications and his thought, — to the socio-political problems faced by mankind as the direct consequence of advances in technology. The lecture, later published in the form of a most compelling contribution to *Encounter*, eventually formed the basis of a celebrated book, *Inventing the Future*. The trilemma facing mankind: war, excess population and leisure, is now revealed wisdom — part of the backcloth of our existence. It was not so when the book was first published in 1963. The approach taken in this book — an appraisal of the

enormous dangers, coupled with a guarded optimism that, with their recognition, the worst could be avoided — Gabor pursued and developed in a series of further publications. He became a founder member of the Club of Rome, and thereby became embroiled in the fierce debates which followed the publication of *Limits to Growth* by Forrester and Meadows. His stance was one of cheerful and total support for these authors — though a careful examination of his expressed opinions reveals that this support did not, in fact, rest on his assessment of the computer model calculations which caused such widespread alarm.

Just before *Inventing the Future*, it became clear that holography, transformed by the discovery of the laser, was turning into one of the most rapid growth topics in applied science. The range of applications appeared to grow month by month; holography was recognised as a principle of such generality tht it could touch many branches of physics, biology and engineering. Gabor, himself, contributed several inventions to this growing field. He lectured widely on the subject. Many will never forget a brilliant discourse at the Royal Institution which he gave in 1969. The recognition, so long denied, or belatedly bestowed, came in full flood. Honours of all kinds were showered upon him and, in 1972, the Nobel Prize.

There remained some good years. They were devoted to scientific work at the CBS Laboratories in the USA, to Imperial College and London, and to a villa in Italy. Gabor continued to contribute to what became his dominant and passionate concern — the adjustment of the human condition to the new world, which the explosive development in science and technology had wrought within his own lifetime and experience. Prophecy is the hardest of arts; scientists as a body are not notably more successful than others in its practice. Yet, if any doubt that there are some, a very few, who can penetrate that ultimate unknown at least some little distance, they should re-read *Inventing the Future* of 1963, or, perhaps, the Fawley Lecture of 1972, *The proper priorities of Science and Technology*.

Dennis Gabor married Marjorie, née Butler, in 1936, a marriage which brought great happiness and continuing support, in a life which knew both bitter disappointment as well as triumph and total fulfilment.

Eric A. Ash

Airey Neave

A GENERAL ELECTION is a deep parliamentary gulf dividing sharply one time from another. I have returned to the House of Commons not only to a new parliament and a new government but to unfamiliar faces that crowd the benches as solidly and confidently as did those whose places they have taken. But there is outstandingly one face absent from the chamber, one expected figure not again to be met in the long corridors. I refer to my friend Airey Neave, the victim of a cowardly political murder within the precincts of that same Palace of Westminster where we meet.

Leading figures in public life, the newspapers, radio and television have all said so much deservedly in tribute to Mr Neave, his life and work, that it might seem presumption on my part to add more. Nevertheless these columns give me an opportunity to enlarge a little on his considerable services to the Select Committee on Science and Technology of the House of Commons, for Airey Neave was a founder member of the committee from its establishment in 1967 until 1975 when he became opposition spokesman on Northern Ireland.

I was the first chairman of the select committee but in 1970 when the Conservative government of Mr Heath came to power, in accord with parliamentary practice, Airey Neave succeeded me for the parliamentary sessions of 1970-1974. Every chairman, I suppose, has his own style and Airey's was very much his own. In dealing with witnesses, especially those (and there are a few) who think that politicians cannot possibly have knowledge of anything except the suspect business of getting elected, he could be severe to near inquisitorial. Yet never for an instant did he go beyond the needs of the inquiry being conducted. As a good select committee chairman should, he saw his primary duty to parliament, not to governments or parties. I remember well how he gave a rising Conservative ministerial star caught out in a mistake a wigging that led to withdrawal and public apology. But to the general run of helpful and co-operative witnesses, enduring the unfamiliar ordeal of questioning in public, Airey was the soul of courtesy.

In the chair Airey Neave was vastly impressive to witnesses as he was to colleagues on the committee because of his detailed knowledge of the subject under consideration. His capacity for work was truly prodigious. Among the inquiries he handled either as chairman of the main committee or as chairman of a sub-committee, were population control, the Rothschild proposals and the computer industry.

But I suppose the inquiry that brings me closest to Airey Neave in memory was the one that the select committee carried out, through a sub-committee, into the choice to be made by Britain for the 'third generation' of nuclear reactors, the Magnox reactor being counted first generation and the advanced gas-cooled reactor second generation. This was during the time of the Heath government, when Mr Peter Walker was, as Secretary of State for Technology, the responsible minister.

The assumption had been that no firm decision would be taken on the choice to be made without a ministerial statement and the normal opportunity for parliamentary questioning. Then a long special article appeared in a leading Sunday newspaper by a science correspondent, to the effect that the Central Electricity Generating Board had made up its mind already and that American style pressurised water reactors were to be bought on a large scale for installation in the British electricity supply system, with the co-operation of our manufacturers and the apparent approval of the government.

Simple parliamentary questioning took us little further forward and Airey and I both agreed that this was just the kind of situation for which the select committee had been established.. After all, the British taxpayer had either spent or had guaranteed large sums of money in the past on the assumption that Britain had the most efficient and safest nuclear power arrangements in the world with the gas-cooled systems and now for parliament to be told that we had been wrong all the time took more than a little swallowing.

Because of his declared interest as a director of a plant manufacturer engaged in the nuclear field, Airey Neave felt he should not take the chairmanship of the inquiry and I occupied his place, though he served on the sub-committee. To a great extent it was the safety of the PWR in an emergency situation that took the attention of the inquiry and on this point critical evidence was received from Sir Alan Cottrell, then Chief Scientist to the government — evidence which had not been published before.

The conclusion of the select committee, reported to the House of Commons, was that the case for abandoning British reactor systems and adopting the American system could not be sustained by the evidence submitted and we advised caution over the PWR. Needless to say, some but not all parts of the nuclear technological establishment attacked the select committee for political meddling and worse. Nevertheless, a full day's debate in the Commons found members of parliament on both sides impressed by the committee's findings and Airey and I followed up the debate with a joint appearance on the national television network which engendered a large correspondence. Many of our correspondents were obviously impressed by the way in which a select committee of the Commons could, while supporting nuclear development in general, run up a danger flag about one particular proposed development. Who in the light of Harrisburg could to-day say we were wrong?

I spoke to Airey Neave last on the Thursday night before his death on the following day. We met by chance in the Commons lobby and discussed the future of the select committee on Science and Technology in the light of suggested procedural reforms for select committees in a new parliament. I did not know then I was never to see him again.

Arthur Palmer

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CARTER, Melvin W., MOGHISSI, A. Alan, and KAHNZ, Bernd. (ed.) Management of Low-Level Radioactive Waste. Vol. 1: Pp.1 — 633. Vol. 2: Pp.637 — 1214. ISBN-0-08-023907-2. (Oxford and New York: Pergamon Press, 1979.) £62.50.

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ELECTRICAL LOAD-CURVE COVERAGE. (Proceedings of the Symposium on Load-Cruve Coverage in Future Electric Power Generating Systems, organized by the Committee on Electric Power, United Nations Economic Commision for Europe, Rome, Italy, 24-28 October 1977.) Pp.xix + 546. ISBN-0-08-022422-9. (Oxford and New York: Pergamon Press, 1979.) £35.

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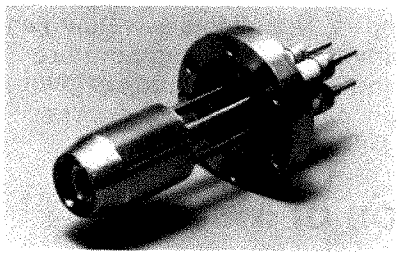
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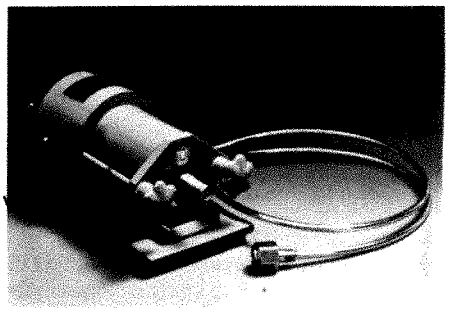
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The Technilab Frigimat

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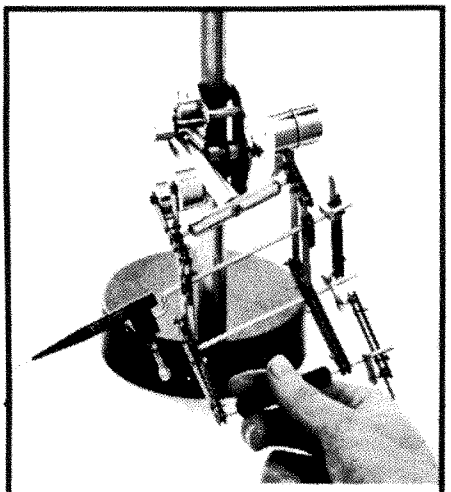
Chemistry Kit. Wheaton Scientific announces a new chemistry kit featuring Wheaton Connections — unique 1-piece seals that let you screw components together in assemblies that cannot fall apart. Components in the Wheaton semi-micro kit feature screw threads for the Wheaton Connections. No clamps, grease or springs and hooks are needed to join the glassware. The kit is ideal for schools and universities. A 12-page brochure is available with complete information on the Wheaton semi-micro kit and an array of components and accessories using the Wheaton Connections.
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Refrigerated centrifuge. Burkard Scientific announce a new version of Koolspin, their table-top refrigerated centrifuge. Now the Koolspin range includes a large bowl version, taking up to 8 × 15 ml tubes in a swing-out rotor. This increase of bowl size has been achieved with little change in case size and overall weight. Bowl air temperature control remains at +10° to -10°C, and the speed range of this new version will be 0 - 10,000 r.p.m. Acceptable tube size range is 200 μl - 25 ml. These Koolspins all have a fully automatic lid-lock system that prevents the lid from being opened when the rotor is moving.
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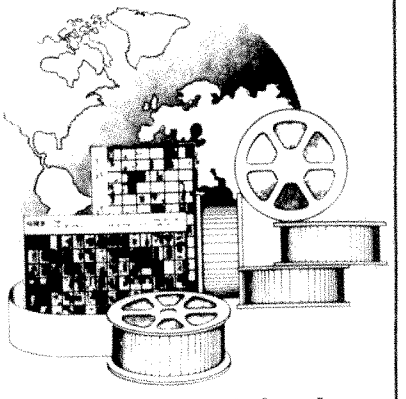
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W272(A)

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Applications can be obtained from the Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS. Telephone 031-556-2930. 2791(A)

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DEPARTMENT OF IMMUNOLOGY
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Applicants should have a postgraduate degree and experience in tissue culture and the cloning of cells.

Persons interested in the post should contact Professor I.C.M. MacLennan's secretary, Department of Immunology, the Medical School, Vincent Drive, Birmingham B15 2TJ.

Applications, three copies, including curriculum vitae and the names of two referees to the Assistant Registrar, Faculty of Medicine, University of Birmingham, Birmingham B15 2TJ by 30th August 1979. Please quote ref: RA/IMM/49. 2739(A)

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2724(A)

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2757(A)

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Applications are invited for the above post in the College's Department of Marine Biology, to commence on 1st October, 1979, or as soon as possible thereafter. Applicants should be graduates of chemistry, biochemistry or microbiology. Previous experience in aquatic chemistry or microbiology would be an advantage but is not essential.

The appointment will be within the range £3,689 to £4,232 per annum, and will be for one year in the first instance, with the possibility of extension for a further two years.

Applications (two copies), giving details of qualifications and experience, together with the names and addresses of three referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG. Informal enquiries can be made to Dr C P Spencer (Bangor 712641, Extension 6642). Closing date for applications is 13th August, 1979.

2755(A)

UNIVERSITY OF EDINBURGH

Mitochondrial Genome Expression

POSTDOCTORAL RESEARCH FELLOW

required to join a group led by Dr C J Leaver working on mitochondrial genome expression during plant development. Previous experience in the field of mitochondrial biogenesis or function together with an interest in membrane protein isolation and purification would be an advantage but not a necessity. The successful candidate will also be required to use various techniques involved in nucleic acid isolation and *in vitro* translation already in use in the laboratory.

This post is supported by the SRC and is available from 1st October 1979 for three years but a suitable applicant may start at a later date. Salary on the 1A Scale for research staff, plus USS benefits.

Applications with curriculum vitae and naming two referees should be sent to Dr C J Leaver, Department of Botany, The King's Buildings, Mayfield Road, Edinburgh EH9 3JH, Scotland, as soon as possible. Please quote reference 5037.

2734(A)

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Applications are invited from suitably qualified persons for the above positions.

Qualifications:
Ph.D. with 5 or more years postdoctoral research experience in the field of Insect Ecology. Previous acquaintance with social insects, particularly Termites will be of advantage, but high quality research for Ph.D and evidence of excellence since postdoctoral experience will be a critical criterion.

Responsibilities:
The successful candidate will carry out research in Termite Ecology, particularly the study of termite foraging activity in competition with other animals, termite population density and their role in the semi-arid savannah environment.

He will be expected to work in collaboration with the large research staff in other aspects of termite ecology and in termite physiology.

Salary:
Negotiable and includes attractive benefits.

Closing Date:
Applications giving detailed information on general education, professional qualifications, experience, marital status, age, present terms of employment (including salary), names and addresses of 4 referees (including one personal), and photocopies of relevant certificates and diplomas should be addressed to:

Administrative Manager,
ICIPE Research Centre,
P.O. Box 30772, NAIROBI.

to reach him as soon as possible.

W262(A)

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY
Nairobi, Kenya

Applications are invited from suitably qualified candidates for the following position:

RESEARCH SCIENTIST (SENSORY PHYSIOLOGY RESEARCH UNIT)
REF: SCI/79/7

Qualifications:

At least a Ph. D., degree in Zoology, Entomology or Physiology. Preference will be given to candidates with a broad background in Chemistry, Physics and General Physiology.

Suitable candidates should have considerable practical experience in sensory electrophysiological techniques, preferably at single-cell level; quantitative and qualitative behaviour studies, including the bioassay of natural products. Experience in histology, transmission and scanning electron microscopy would be an asset.

Responsibilities:

The successful candidate will be required to work in the field of sensory physiology and behaviour of insects and ticks restricted to one or several of the ICIPE target organisms: tsetse flies, termites, ticks, armyworm moths, mosquitoes, stem-borers and shoot-flies. The project work will be defined in greater detail with target-oriented research programme teams. Willingness to collaborate with colleagues in other disciplines is of first order importance. In spite of the relative adequate availability of research equipment at the Centre, the situation calls for innovative ability for improvisation.

Salary:

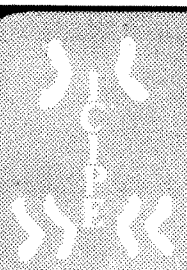
Negotiable and include attractive benefits.

Closing date:
Applications giving detailed information on general education, professional qualifications, experience, marital status, age, present terms of employement (including salary) names and addresses of four referees (including one personal), and photostat copies of relevant certificates and diplomas should be addressed to:

Administrative Manager
I.C.I.P.E.
P.O. BOX 30772,
NAIROBI, KENYA.

to reach him as soon as possible.

W262(A)



THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY

Nairobi, Kenya

Applications are invited from suitably qualified candidates for the following positions:

RESEARCH SCIENTIST (MEDICAL VECTOR RESEARCH PROGRAMME)

REF: SCI/79/1

Qualifications:

Ph. D., with five or more years of postdoctoral experience in biological and physiological sciences and demonstrable experience in research on mosquito ecology. Evidence for excellence since postdoctoral training will be a distinct advantage.

Responsibilities:

The successful candidate will work on malaria epidemiology in Tropical Africa with special interest on immunology and culture parasites. This new project will be linked with one on bancroftien filariosis epidemiology.

SENIOR RESEARCH SCIENTIST (BASES OF PLANT RESISTANCE TO INSECT ATTACK)

REF: SCI/79/4

Qualifications:

Ph. D., with five or more years postdoctoral research experience. High quality research for Ph. D., and envidence for excellence since doctorate degree will be a critical criterion. If an internationally recognised first-class senior scientist with previous experience in tropical environment applies, he may be considered for the leadership of the research programme.

Responsibilities:

The successful candidate will carry out research in planthost/insect relations, in some instances, including mass-rearing techniques and screening for resistance. The long-term objective of the research programme is to investigate thoroughly and establish the bases of plant resistance to insect attack. The research effort will specifically cover cereal stem-borers, legume pod-borers, and maize streak virus disease, at least, initially.

SENIOR RESEARCH SCIENTIST (CROP BORERS PROGRAMME)

REF: SCI/79/5

Qualifications:

Ph. D., with five or more years of practical experiance in the field of borers research. High quality research for Ph. D., and evidence for excellence since doctorate degree will be a critical criterion. If a first-class senior scientist of international status, with relevant technical expertise and a wide experience in tropical environment applies, he may be considered for the leadership of the research programme.

Responsibilities:

The successful candidate will carry out research in the field of crop borers, whether basic or applied. If appointed a leader, he will formulate the programme in collaboration with the ICIPE Director, supervise the research activities of the staff in the programme and handle some administrative and accounting problems.

RESEARCH SCIENTIST OR SENIOR RESEARCH SCIENTIST (FINE STRUCTURE RESEARCH UNIT)

REF: SCI/79/6

Qualifications:

Ph. D., in a biological science with considerable training and technical expertise in electron microscopical and associated techniques.

Responsibilities:

The successful candidate will be responsible for the organisation of the work of the Fine Structure Research Unit.He will also be able to pursue his own research in insect fine structure, including cytochemistry and autoradiography, and to assist other scientists with their work involving electron microscope techniques. The position is a very responsible one and requires a senior mature and experienced electron microscopist with wide organisational ability and enormous technical expertise.

Salary:

Negotiable in respect of all posts and includes attractive benefits.

Closing date:

Applications giving detailed information on general education, professional qualifications, experience, marital status, age, present terms of employment (including salary), names and addresses of four referees (including one personal referee) and photocopies of relevant certificates and diplomas should be sent to:

Administrative Manager,
ICIPE Research Centre,
P. O. BOX 30772,
NAIROBI.
Kenya.

to reach him as soon as possible.

Chemists

Two vacancies have arisen at our Welwyn Garden City site for Chemists, male or female. We offer excellent working conditions in modern well-equipped laboratories. The salary paid will be commensurate with experience and additional benefits include a Christmas Award, contributory pension scheme, free BUPA membership and a subsidised restaurant.

Organic Chemist -Research

Applications are invited from those with GRIC or BSc in Chemistry, this post is not suitable for someone with a PhD qualification. Research experience in the pharmaceutical industry would be an advantage but not essential. The chemist would assist a senior chemist in our Chemistry Department in the design and investigation of new procedures used in the synthesis of a natural product of chemotherapeutic importance.
Reference RC55 - Mrs. Clewley.

Quality Control Chemist

Applications are invited from those with an HNC or BSc in Chemistry for work on a range of analytical instrumental techniques, particularly the GIC analysis of pharmaceutical raw materials and products. This post would be suitable for a newly qualified graduate or someone who may also have had some experience of instrumental techniques.

Reference QC/ACJ - Mrs. Judge.

For further information and an application form, please telephone or write to the Personnel Department quoting the appropriate reference, Roche Products Limited, PO Box No 8, Welwyn Garden City, Hertfordshire, AL7 3AY telephone Welwyn Garden 28128.

ROCHE

2762(A)

FORSCHUNGSINSTITUT BORSTEL — FEDERAL REPUBLIC OF GERMANY

APPOINTMENT OF DIRECTORS

After retirement of the former director, there is a vacancy for the management of the Forschungsinstitut Borstel, (Institute for Experimental Biology and Medicine (district Bad Segeberg, situated at abt. 50 km distance from Lübeck, Kiel and Hamburg) which in future shall be made up of a board of directors consisting of 3 scientists.

The scientific orientation of the institute shall be concentrated on the research of diseases the clinical phenomena of which are accessible to medical-theoretical, experimental and scientific analysis. Priority will be given to infectious and related diseases the course of which is largely influenced by immunologic factors. This implies close collaboration of medical and scientific disciplines. The members of the board of directors shall represent the following scientific fields:

1. Clinical Medicine
2. Theoretical Medicine
3. Biosciences

The directors will be appointed professors for a lifetime (C 4), vizt. the medical fields at the Christian-Albrechts-University of kiel, and the bioscientific field at the Medical School (Medizinische Hochschule) of Lübeck.

The Forschungsinstitut Borstel includes a 180-bed hospital as well as medical-theoretical and scientific laboratories in which 38 scientists and 67 technical assistants are working at present. A total of abt. 400 people are employed at the institute. An additional laboratory with an effective area of 3.800 sqm will be built in 1980.

Applications, including curriculum vitae, list of publications and references should be sent by September 30, 1979 to the Vorsitzenden des Verwaltungsausschusses des Forschungsinstitutes Borstel, Ltd. Ministerilarat, Dr von Scheliha, 2300 Kiel, Düsternbrooker Weg 64-68
Der Kurator, Dr Helmut Lemke, Landtagspräsident.

W269(A)

UNIVERSITY OF NATAL
DEPARTMENT OF PHYSICS
Durban
PLASMA PHYSICIST

Postdoctoral Research Fellowship available October 1979 for at least one year, for experimental research in plasma waves and beam-plasma interactions. Salary range R8,100 to R11,200 p.a., plus travel allowance each way. Recent postdoc or sabbatical visitor suitable.

Send application, resumé and three referees' reports by September 1st quoting reference ADVD38/79 to Prof. M. A. Hellberg, Plasma Physics Research Institute, University of Natal, Durban, South Africa. W265(A)

UNIVERSITY OF BRISTOL

CHAIR IN PHYSIOLOGY

The University invites applications for appointment to the Chair in Physiology which becomes vacant on the appointment of Professor T.J. Biscoe to the Jodrell Chair of Physiology at University College, London. The appointment will carry with it Headship of the Department for a five year period in the first instance.

A clinical qualification is desirable but not essential. Candidates are invited to submit applications, including the names of three referees, not later than 12th October, 1979, to the Registrar and Secretary of the University, Senate House, Bristol, BS8 1TH, from whom further particulars may be obtained. 2782(A)

ST THOMAS'S HOSPITAL
MEDICAL SCHOOL
(University of London)
LONDON SE1 7EH
RESEARCH POST LEADING TO PhD

Physiologist or Biochemist with good first degree to study placental transfer and fetal metabolism.

Applications to Professor M Young, Department of Gynaecology. 2730(A)

KING'S COLLEGE LONDON
DEPARTMENT OF BIOPHYSICS
RESEARCH TECHNICIAN

Applications are invited for the post of Technician to work on Wellcome Trust supported research on the mechanism and control of chromatin transcription. Experience in sub-cellular fractionation techniques, protein and nucleic acid biochemistry and restriction endonuclease mapping would provide useful background.

The appointment would be for one year in the first instance starting 1 September (or sooner). Salary on scale £3,455 pa to £3,860 pa or £3,998 pa to £4,580 pa (inclusive) according to qualifications and experience.

Applications with curriculum vitae and the names of two referees should be sent to Dr J Allen (Ref: 217825/N), Department of Biophysics, King's College London, 26-29 Drury Lane, London WC2B 5RL. 2666(A)

With the escalating price of crude oil, its time to focus on the coal-to-oil technology of tomorrow

73' 75' 77' 79'

Stimulating opportunities for Chemical, Mechanical and Electrical Engineers on a permanent or contract basis in South Africa

Sasol Limited has the only commercial proven oil from coal plants in the world. In addition to petrol, diesel and industrial gas, a variety of petrochemical products and raw materials are produced and with the exporting of waxes, kogasins and alcohols to all parts of the world Sasol are earning valuable foreign currency for the country.

The Company was founded in the early fifties with the first plant situated in the northern part of the Orange Free State – some 50 miles south of Johannesburg – where a vast coalfield exists close to the Vaal River and the concentrated marketing areas. The second plant which is now being constructed on a site approximately 80 miles east of Johannesburg, will be considerably larger than the first plant in production capacity and will be commissioned early in 1980. A start has already been made with a third plant in the vicinity of the nearly completed second plant, at a cost of approximately £2 000 million. This brings the total cost of Sasol II and III to more than £3 300 million.

The successful applicants will be based in Sasolburg and Secunda.

Chemical Engineers

Applicants are required at all levels of seniority and should have a B.Sc. (Chemical Engineering) or equivalent qualification, preferably with experience in the petrochemical or refining industry. Successful candidates will have the opportunity of combining their engineering skills with creative ability to contribute to the effective functioning of the production process. Responsibilities will include the development of new processes, process design and project evaluation, the improvement of existing processes and the solving of process problems. Production orientated men will be responsible for the optimisation of the operation of chemical plants under their control.

Mechanical Engineers

Applicants are required at all levels of seniority with a B.Sc. (Mechanical Engineering) or equivalent qualification for the evaluation and optimisation of design studies for process equipment, high and low temperature systems as well as for static and rotary equipment. They will also be closely involved in an extensive range of projected and construction work. Those seeking the challenge and demand of maintenance or production work take full responsibility and authority for the specific function in an area of the Sasol plants.

Electrical Engineers

Applicants with a B.Sc. (Electrical Engineering) or equivalent qualification will be appointed either as area engineers (maintenance) or as project engineers. The area engineer will be responsible for all maintenance and switching in an area of the factory. He must ensure continuity of electrical supply, control budgets and a personnel of about 100 men as well as handling technical and administrative work.

Electrical project engineers, with at least 5–10 years experience of project and construction work are also needed.

These positions are based at Sasol II in Secunda.

Working at Sasolburg or Secunda offers . . .

* Residential locations where the most modern planning methods were employed in the development of the towns.
* Established and active multilingual communities with many schools, churches, clubs and cultural societies.
* Excellent recreational facilities which cater for virtually every sporting activity including nearby golf and sailing.
* The benefits of South Africa's pleasant climate and reasonable costs of living.

. . . with these benefits

* Assistance with travelling expenses to South Africa.
* A settlement allowance of up to £750 plus £55 per child.
* Financial assistance with the transportation of personal effects.
* Pension and medical fund and Group Life Assurance.
* Leave bonus of 8,3% of annual salary.
* 30 days annual leave and long leave after six years' service.
* Company housing plus 100% home purchase schemes.

If you have the talents we are looking for and the ability to use them there will be room for you to become a member of our permanent staff or you could join the Company on a contract basis. Interviews will be conducted in the U.K.

Please send a detailed curriculum vitae to Mr D. D. Rogers, 14a Cross Street, Reading, Berks. *quoting Ref. SS/6*

SASOL

2738(A)

BIOGEN S.A.

a young company engaged in basic and applied research in the field of molecular biology and microbiology, and with the objective of utilizing the potential of genetic engineering for the production of substances of commercial and medical interest in such areas as health care, agriculture and energy, is inviting applications from

SENIOR AND JUNIOR SCIENTISTS

with experience in molecular biology, nucleic acid biochemistry and microbiology,

for its **Research Laboratory in Geneva,**

Switzerland.

Excellent working conditions, in close contact with leading scientists from Europe and the US.

Salary scale: Group Leaders = SFr 60-85,000, Research Associates = SFr 35-55,000.

Applicants with real interest in both basic research and its applications should send their curriculum vitae (with a description of previous experience and a list of publications) to BIOGEN Laboratory, 3 Route de Troinex, 1227 Geneva, Switzerland. W248(A)

ASSISTANT SCIENTIFIC SECRETARY

Cancer Research Campaign

(British Empire Cancer Campaign for Research)

Due to the growth in both the extent and scope of its scientific activities the Cancer Research Campaign, one of the largest medical charities with an annual turnover in excess of £7 million, is expanding its Scientific Department and requires an Assistant Scientific Secretary.

The Scientific Department is responsible for the administration of the Campaign's scientific (as distinct from fund-raising) endeavours and the Assistant Scientific Secretary will help the Scientific Secretary and his staff in most aspects of their work, particularly in the processing of grant applications and the subsequent administration of those that are successful: at present the Campaign is supporting over 450 projects in 80 centres. A capable and enthusiastic individual will eventually have the opportunity to undertake most facets of the administration of scientific research.

The successful candidate is likely to be aged between 28-38 with some experience of biomedical research at a postdoctoral level and with a knowledge of science and medicine in the broadest terms, as well as an interest in a career in scientific administration. The appointment will be at the level of Senior Scientific Officer with a salary scale (currently under review) of £5,678 to £7,422 (with superannuation provision), and subsequent to satisfactory completion of a two-year probationary period can be permanent.

Applications by letter, accompanied by a curriculum vitae and the names of three referees, should be sent *marked "Personal"* and not later than 14 September 1979 to:-

The Administrative Officer
Cancer Research Campaign
2 Carlton House Terrace,
London SW1Y 5AR Tel 01-930 8972

2779(A)

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF ZOOLOGY

Applications are invited from men and women for an

SRC RESEARCH ASSISTANT (Range IB)

to work with Dr D.E. Kime on androgen biosynthesis and reproductive development in teleost fish. Tenable three years from 1 October 1979.

Initial salary £3,775 per annum (under review). Applications, with names of two referees to the Registrar and Secretary, the University, Sheffield S10 2TN by 17 August 1979.

Quote ref: R 348/G. 2753(A)

SCIENTIFIC OFFICER

BLOOD GROUP REFERENCE LABORATORY

A graduate is sought for research and development of new techniques relating to the standardisation of reagents (including albumin and enzymes) used in blood group serology. Some experience in protein chemistry or immunochemistry desirable.

Applications in writing with details of experience and the names of two referees to Miss K. Lomas, Blood Group Reference Laboratory, Gatliff Road, off Ebury Bridge Road, London SW1W 8QJ

2714(A)

UNIVERSITY OF SURREY

DEPARTMENT OF METALLURGY & MATERIALS TECHNOLOGY

RESEARCH STAFF

We have several vacancies for research staff to work on projects concerned with the mechanical properties of advanced fibre-composite materials. This is an opportunity to join an active and successful team working in well equipped laboratories and supported by Government and Industry. In appropriate cases consideration would be given for registration for a higher degree on a part time basis.

The posts vacant are:

RESEARCH OFFICER

Qualifications: BSc or equivalent in appropriate science or engineering discipline and good experimental aptitude. Salary from £3,689 according to qualifications and experience.

Note: A postdoctoral candidate might be considered for the post.

JUNIOR RESEARCH OFFICERS

Qualifications: Science A levels or equivalent. Salary from £1,722. These posts might suit a candidate who wishes to work for a year between school and university. In both cases appointments would be for 1 year with a possibility of extension to 2 or 3 years.

RESEARCH STUDENTSHIPS

We also have available an SRC CASE studentship in association with ICI Plastics Division for work on Toughness and Strength of Fibre-Filled Thermoplastics.

Applications which should include details of academic and industrial experience and the names of two referees should be submitted as soon as possible to: Mr M G Bader, University of Surrey, Department of Metallurgy & Materials Technology, Guildford, Surrey GU2 5XH. Tel: (0483) 71281 Ext. 895/623.

2756(A)

SUNDERLAND POLYTECHNIC

Faculty of Science

DEPARTMENT OF BIOLOGY

LECTURER II IN ECOLOGY

Salary Scale: Lecturer II: £4,470 to £6,597 Bar — £7,149.

Applicants should have considerable field experience and a strong background in computing and quantitative techniques possibly related to the analysis of plant communities.

An application form and further particulars may be obtained from the Personnel Officer, Sunderland Polytechnic, Chester Road, Sunderland, SR1 3SD, and should be returned as soon as possible. 2775(A)

CALIFORNIA INSTITUTE OF TECHNOLOGY

seeks candidates for a position in

PLANETARY SCIENCE

with emphasis on planetary surfaces or planetary atmospheres, and or observational programs using space-craft Appointment preferable at the assistant professor level.

Please contact Dr B. Kamb, Chairman Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, California 91125 giving résumé, bibliography, and research interests. An equal opportunity employer

W268(A)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

DEPARTMENT OF PHYSICS

LECTURER

The successful applicant is expected to participate in the auxiliary teaching of the department and in its research which is done both experimentally and theoretically in the main areas of nuclear and solid state physics (some preference will be given to an experimental solid state physicist). Duties are to be assumed on a date to be negotiated.

Salary will be in the range R8 100 — R13 200 per annum. (£1 = R1.89 approx).

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion or colour. Further particulars relating to this practice and to the above post are included in an information sheet obtainable from the London Representative, University of the Witwatersrand, Chichester House, 278 High Holborn, London WC1V 7HE, or from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, with whom applications should be lodged by 20th August 1979. 2725(A)

OXFORD UNIVERSITY

PHYSICAL CHEMICAL LABORATORY

APPOINTMENT OF POST DOCTORAL RESEARCH ASSISTANT

Applications are invited from chemists or physicists to work on the laser diode, infrared spectroscopy of weakly bound van der Waals complexes. Applicants should have some experience either of high resolution spectroscopy or of working with lasers.

The appointment is for 2 years in Research Support grade 1A, with membership of U.S.S. Starting salary is up to £4,776.

Applications with curriculum vitae and the names of two referees should be sent as soon as possible to Dr B.J. Howard, Physical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ. 2777(A)

UNIVERSITY OF OXFORD

Departments of Pharmacology and Physical Chemistry

POSTDOCTORAL RESEARCH ASSOCIATE IN HIGH PRESSURE PHYSIOLOGY AND PHARMACOLOGY

A postdoctoral position is available for research related to diving and the effects of high pressure on man and animals, in an interdisciplinary research team conducted by Prof. Sir William Paton and Dr E B Smith. A number of lines of research are being actively pursued, ranging from physical chemistry to human physiology. The successful candidate could work in any of these areas, although preference may be given to those interested in neuropharmacological approaches.

Applications, including statements of research experience and publications, with the names of two referees, should be sent as soon as possible to Prof Sir William Paton, Department of Pharmacology, South Parks Road, Oxford OX1 3QT. It is hoped to make an early appointment to enable the successful candidate to take up the post by 1 October 1979, although this need not rule out applicants wishing to start later. 2672(A)

Microbial Geneticist

Shell Research Limited have a vacancy in the Fermentation and Microbiology Division of their Sittingbourne Research Centre for a Microbial Geneticist.

We are looking for a young graduate, preferably with some postgraduate experience who will be interested in the challenge of contributing to our substantial research programmes in biotechnology – for example biocatalysis, fermentation processes and biopolymers. Appropriate technical assistance will be available.

We offer a competitive salary and a range of excellent benefits including pension, flexible working hour system, staff restaurant and a recreational club. Please write or telephone for an application form to:

Shell Research Limited, Recruitment Division (N1), PNEL/11, Shell Centre, London SE1 7NA. Telephone: 01-934 2948.

Shell

2790(A)

UNIVERSITY OF NATAL

DEPARTMENT OF BIOCHEMISTRY

PIETERMARITZBURG

Applications are invited from suitably qualified persons regardless of sex, religion, race, colour or national origin, for appointment to the posts of

LECTURER (Ref. PMB 44/79) and SENIOR LECTURER (Ref. PMB 45/79)

Incumbents will be required to take up their duties on 1 January 1980, or as soon as possible thereafter.

The salaries will be in the range: Lecturer R8,100 to R13,200 pa. Senior Lecturer R11,400 to R15,600 pa.

The commencing salary will be dependent on the qualifications and/or experience of the successful applicant. In addition, an annual vacation savings bonus is payable, subject to Treasury regulations.

Application forms, further particulars of the posts and information on pension, medical aid, group insurance, staff bursary, housing loan and subsidy schemes, long leave conditions and travelling expenses on first appointment are obtainable from the Registrar, University of Natal, PO Box 375, Pietermaritzburg, 3200, South Africa, with whom applications, on the prescribed form, must be lodged not later than 30 September 1979, quoting the relevant reference number. W266(A)

UNIVERSITY COLLEGE CORK

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH IN BIOCHEMISTRY

Applications are invited for two postdoctoral positions supported by grants from the National Board for Science and Technology. Applicants should have recently obtained, or expect to obtain in the near future, the PhD degree in biochemistry or chemistry. The projects are as follows:

1. Studies on the structures and activities of lipases from milk. The research programme will be under the joint direction of Professor S. Doonan, Department of Biochemistry and Professor P.F. Fox, Department of Dairy and Food Chemistry. The successful applicant will be based in the Department of Biochemistry which has extensive facilities for structural studies of proteins.
2. Studies in purine metabolism in muscle with particular reference to meat production. Some experience in the areas of purine metabolism and/or high performance liquid chromatography would be advantageous, but not essential. Good facilities are available for the isolation and quantitation of purines in tissues.

The appointments are for one year in the first instance with the possibility of extension for one or two further years. The initial salary is £3,837 (under review).

Applications, including a curriculum vitae and the names of two referees should be sent to arrive on or before *27th August, 1979* to Professor Shawn Doonan, Department of Biochemistry, University College, Lee Maltings, Prospect Row, Cork. 2778(A)

Wellcome

The Wellcome Research Laboratories Beckenham

Department of Parasitology

From £5,900

An opportunity has arisen in the research group studying malaria immunity and chemotherapy. The work involves department of methods of cultivation of malaria parasites.

Candidates should have post-doctoral experience in cell culture techniques and previous experience in malaria or other protozoan cell culture would be an advantage.

This appointment will be for a period of three years in the first instance.

The Wellcome Research Laboratories are situated in pleasant parkland surroundings, within easy reach of Central London. Excellent conditions of employment include subsidised canteen, pension scheme, 4 weeks' holiday and sports and social facilities.

For an application form, please write to the Personnel Manager, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, giving brief details of background and experience. 2795(A)

1979

Southampton THE UNIVERSITY

DEPARTMENT OF BIOLOGY

A RESEARCH ASSISTANT

is required from 1 October to work with Dr C E Bennett on a SRC project to study the secretory products of the common liver fluke (*Fasciola hepatica*) and its avoidance of the host immune response.

Applicants should have a good honours degree in a biological science and have some background of parasitology or immunology. A knowledge of iso-electric focussing and of *in vitro* culture would be an advantage. Where appropriate the assistant may be permitted to register for a higher degree.

The appointment will be for three years at a starting salary of £3,775 per annum with annual increments and USS arrangements.

Applications with details of education, experience and the names of two referees should be sent to Mrs E C P Sears, Staffing Section, The University, Southampton SO9 5NH, quoting reference 1503/R/N. 2774(A)

ISRAEL

SENIOR PHYSIOLOGIST

Faculty of Health Sciences in Beer-Sheva requires a Senior Scientist to be responsible for the teaching and research in physiology in a recently established Medical School and a School of Allied Health Professions. Challenging position offering progressive Faculty with innovative curriculum.

Write enclosing CV and references to Dean, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel. W273(A)

POSTDOCTORAL POSITION to study luminescence of DNA and related compounds. Excitation is produced by bombardment with ultraviolet photons, X-rays, and high energy heavy-ions. Experience in luminescence spectroscopy of biological materials and with lasers and microprocessors is desirable. Deadline for applications is September 20, 1979. Send curriculum vitae and names of three references to: Dr Malcolm Daniels, Chemistry Department, Oregon State University, Corvallis, OR 97331. Oregon State University is an Equal Opportunity/ Affirmative Action employer and complies with Section 504 of the Rehabilitation Act of 1973. W263(A)

UNIVERSITY OF OXFORD

Sir William Dunn School of Pathology

RESEARCH ASSISTANT

Grade 1A

Salary £4,232 to £7,145

Applications are invited for a Research Assistant supported by the Cancer Research Campaign, to assist with research into the biochemical mechanisms of virus growth. Experience with mammalian tissue culture would be an advantage. Applicants should possess a degree or equivalent qualification.

Applications, in writing, together with the names and addresses of two referees, to the Administrator, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE. 2643(A)

UNIVERSITY OF BRISTOL

DEPARTMENT OF BIOCHEMISTRY

Applications are invited from biochemists or microbiologists for the post of

S.R.C. POSTDOCTORAL RESEARCH ASSISTANT

to study the electron transport chains of bacteria which obtain energy for growth from the oxidation of ammonia (e.g. Nitrosomonas). Previous experience in microbiology is desirable. The appointment is for three years with a salary up to the rate of £4,776 per annum. (Scale subject to revision with effect from 1st October, 1979).

Applications, including a brief curriculum vitae and the names of two referees should be sent by 17th August, 1979. to Dr P.M. Wood, Department of Biochemistry, Medical School, University of Bristol, University Walk, Bristol. BS8 1TD. 2747(A)

LIVERPOOL POLYTECHNIC
Department of Biology

RESEARCH ASSISTANT

To investigate the characterisation of Tipula Polyhedrosis Virus.

Applications are invited for a Local Authority Research Assistantship in the Department of Biology. The appointment is for two years in the first instance, the candidate being required to register for a research degree of the Council for National Academic Awards (MPhil/PhD). Subject to successful registration for PhD or transfer from MPhil by CNAA the appointment may be renewed for a third year.

Applicants should hold or expect to obtain a first or upper second class honours degree in an appropriate biological subject. Letters of application, together with a curriculum vitae and the names and addresses of two referees, should be sent to:

Dr J B Carter, Department of Biology, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF, from whom further details on the research topic and conditions of service may be obtained. 2764(A)

GUY'S HOSPITAL MEDICAL AND DENTAL SCHOOLS

RESEARCH ASSISTANT
(Biochemist/Immunologist)

Pre- or post-doctoral biochemist having experience in immunochemistry or biochemistry of bacteria required for three years for a research project investigating the immune responses to specific components of Streptococci.

Salary according to qualifications and experience, with £502 London Allowance and superannuation.

Apply in writing, with full curriculum vitae, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. I.M.2. 2735(A)

CHURCHILL HOSPITAL RESEARCH INSTITUTE
(University of Oxford)

Vacancies exist for two RADIOBIOLOGISTS

in an expanding research group that is examining the biological effects if ionising radiations on a number of normal tissues and organs. These investigations are primarily aimed at improving the treatment of malignant disease by radiotherapy with both conventional photon therapy and fast neutrons. Post suitable for persons with higher degree or equivalent research experience. Responsibilities will depend on the interests and experience of the successful applicants although preference will be given in the case of one vacancy to someone with a qualification in Physics or experience in radiation dosimetry.

Further details can be obtained from Dr J W Hopewell, Churchill Hospital, Research Institute, Headington, Oxford, to whom application should also be sent with the names of two referees by 20th August, 1979. 2670(A)

BRUNEL UNIVERSITY

SCHOOL OF CHEMISTRY

POSTDOCTORAL RESEARCH

CATALYST PREPARATION AND CHARACTERISATION

Applications are invited from suitably qualified candidates for a postdoctoral research appointment on a 2 year SRC-supported programme which will be available from 1 October 1979 or as soon as possible thereafter. The programme, which is supervised by Dr Paul A. Sermon, concerns novel methods of preparing and characterising supported catalysts. A wide variety of techniques is available for catalyst characterisation and testing, including SEM, XPS, XRD and adsorptive and catalytic probes and spectroscopic techniques. Starting salary would be £4,952 per annum but this is under review. Candidates should send a detailed curriculum vitae to Establishment Secretary, Brunel University, Uxbridge, Middx UB8 3PH by 17 August, 1979. 2750(A)

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(University of London)
Keppel Street, WC1E 7HT

PREVENTIVE TERATOLOGY UNIT

Applications are invited for a

MEDICAL LABORATORY SCIENTIFIC OFFICER IN BIOCHEMICAL TERATOLOGY

to work on congenital malformations associated with environmental agents.

The appointment is for up to 6 months at a salary, depending on age, experience and qualifications, on a scale which rises to £3,930 (under review) + £354 London Weighting.

Applications, stating qualifications and experience and the names of two referees, should be sent to the Secretary (A1) at the School. 2758(A)

Synthetic Organic Chemistry

DELALANDE, an international pharmaceutical group actively involved in research, is expanding the drug discovery programme at its Research Centre and is seeking an additional organic chemist.

Candidates should have a real interest in all aspects of synthetic chemistry and have a PhD with up to one year postdoctoral experience in synthetic organic chemistry.

Knowledge of French is not essential.

The Research Centre is located in the vicinity of Paris.

Applications should be sent to:

Dr M. LANGLOIS
Head, Organic Chemistry
CENTRE DE RECHERCHE DELALANDE
10, rue des Carrières
92500 RUEIL MALMAISON — FRANCE

DELALANDE

W271(A)

HARVARD UNIVERSITY (CDB)

ASSISTANT PROFESSORS OF BIOLOGY

The Cellular and Developmental Biology subdivision of the Department of Biology expects to make one to two appointments at the Assistant Professor level during the next academic year in the fields of Cell Biology and Plant Biology. Applicants should have the PhD; preference will be given to candidates with post-doctoral training, records of outstanding research accomplishment, and teaching experience. Please have three professional persons well acquainted with your work send letters of recommendation directly to the search committee.

Applications should include resumé, copies of most significant publications, and summary of research goals. Send to: Chairman, CDB Search Committee, Department of Biology, Harvard University, 16 Divinity Avenue, Cambridge, Massachusetts 02138.

Women and members of minority groups are specifically encouraged to apply. Harvard University is an Equal Opportunity/Affirmative Action Employer. W270(A)

UNIVERSITY OF BRISTOL

DEPARTMENT OF PATHOLOGY

RESEARCH ASSISTANT

A Postdoctoral Research Assistant is required immediately to work in the Department of Pathology on a project funded by the Nuffield Foundation, to investigate the pericellular environment of macrophages during connective tissue breakdown.

The work will involve biophysical and biochemical techniques including Ion Specific Micro-electrodes; experience with micro-electrodes and/or cyto-chemistry is desirable. The initial salary will be within the range £4,232 to £5,048 p.a. (Salary scale subject to revision from 1st October, 1979).

For further details, apply to Professor I.A. Silver, Department of Pathology, The Medical School, University Walk, Bristol. BS8 1TD. Completed applications, together with a curriculum vitae and the names of two referees should be lodged by 14th September, 1979. 2783(A)

THE UNIVERSITY OF HULL

UNIT OF GENETICS

GRADUATE RESEARCH ASSISTANT

A Biologist interested in the interactions between animals and plants is required to join a team studying the effects of seed and flower parasites on the reproductive capability of different forms of *Lotus corniculatus* (birdsfoot trefoil). The project is both field and laboratory based and is funded by the SRC. Some experience of electrophoretic techniques and/or of genecology would be advantageous. The appointment is tenable from 1st October 1979 for a period of three years. It may be possible for the successful applicant to register for a higher degree.

Salary will be at the appropriate point on the scale 1B for Research Staff: £3,775 to £5,488.

Further information may be obtained from Professor D A Jones, Unit of Genetics, Department of Plant Biology, Hull, HU6 7RX, to whom applications (two copies) giving details of age, qualifications and experience, together with the names of two referees, should be sent by 27th August, 1979. 2787(A)

HOUGHTON POULTRY RESEARCH STATION

Higher Scientific Officer (Band II)

LIAISON/INFORMATION OFFICER

Applications are invited for the post of Liaison/Information Officer, the duties of which include overall responsibility for a scientific library, liaison with, and an information service for, the poultry industry, veterinary profession, Agricultural Research Council and Ministry, and Press, journals and educational organisations, and the preparation of publications and exhibits.

Applicants should possess a degree, HNC or equivalent in a science subject, with at least 5 years relevant post qualifying experience. Experience in public relations and editorial work would be an advantage. Salary in Higher Scientific Officer grade (£4,727 × 8 to £6,211) salary scale currently under review.

Further particulars from the Secretary, Houghton Poultry Research Station, Houghton, Huntingdon, Cambs PE17 2DA. Closing date for applications Friday, 14th September 1979. 2727(A)

Australian Institute of Marine Science

Senior Research Appointment

The Australian Institute of Marine Science was established as a Statutory Authority of the Commonwealth Government in 1974 and, in 1977, took occupation of a modern and comprehensively equipped laboratory complex at Cape Ferguson, near Townsville, well situated for studies in tropical marine science including phenomena at the land-sea interface, on the complex environment of the Great Barrier Reef and in the adjacent ocean. The ongoing programs of the Institute are augmented by active collaboration and interaction with visiting investigators. Staffing of the Institute is expanding to match developing commitments in comprehensive studies of the marine environment, notably of the tropics. Enquiries are invited from professional researchers interested in a senior appointment in any one of the following fields: Physical Oceanography, Marine Chemistry and Community Ecology. At least one and possibly more are being considered.

Duties: The appointee(s) being sought will be expected to accept a substantial responsibility for guiding and participating in studies aimed at developing a fundamental understanding of the environment of the Great Barrier Reef and its living communities, for encouraging collaborative activity in such studies and, where necessary, to manage contract investigations relevant to assessing responses to real or potential human impact within the region.

Qualifications: The appointee(s) will have received a PhD degree in a relevant field of study, will have had at least 10 years postdoctoral experience and a substantial research reputation. In addition, the appointment will call for experience in and a strong capacity for leadership, the stimulation of scientific interaction and a willingness to accept and execute managerial responsibilities while maintaining a vigorous and effective involvement in research.

Applications should be accompanied by a complete cv, the names of at least 3 but no more than 5 referees and should be forwarded to the address given below no later than August 31, 1979.

The Secretary, Australian Institute of Marine Science, P.M.B. No. 3, Townsville M.S.O. 4810, Queensland, Australia. 2759(A)

UNIVERSITY OF MANCHESTER
DEPARTMENT OF PATHOLOGY
A RESEARCH ASSISTANT

is required to participate in a study of ageing in the human placenta. The work will consist mainly of Electron microscopy. Initial salary £3,775 p.a. Applications to Professor H. Fox, Department of Pathology, Stopford Building, Oxford Road, Manchester M13 9PT. 2740(A)

To place your advertisement in these pages Phone: 01-831-6901

ANALYTICAL CHEMIST FOR NFT NURSERY IN SUSSEX

Nutrient Film Technology Ltd is a new and expanding company engaged in both primary production and associated research and development programmes connected with the nutrient-film technique. We require an analytical chemist to operate an atomic absorption spectrophotometer, a visible spectrophotometer and a specific ion meter, as an aid to developing programmes for commercial crop production in the UK and overseas.

This is an excellent opportunity for a young and enthusiastic person to become involved in an exciting new venture.

Apply to: Dr. A. J. Cooper, Managing Director, Nutrient Film Technology Ltd, Hook Lane, Aldingbourne, Chichester, W. Sussex, PO20 6TE. 2760(A)

UNIVERSITY OF SINGAPORE
PROFESSORSHIP IN ZOOLOGY

Applications are invited for the post of Professor in the Department of Zoology. Applicants should possess outstanding academic qualifications, have considerable University teaching and research experience, supported by authoritative publications. The successful candidate will be expected to provide leadership to the teaching and research programmes of the department. The major areas of research are ecology (with an emphasis on aquatic aspects) and comparative physiology, but developing lines of research include taxonomy, biometrics and genetics. Gross monthly emoluments range from S$4044/- to S$6236/-, the initial amount depending on the candidate's qualifications and experience. In addition, the University pays a 13th month annual allowance of one month's salary in December of each year.

Leave, medical and provident fund benefits are available. The University at present contributes to the staff member's provident fund at the rate of 16½% of his monthly salary. Subsidised housing will also be provided at current rentals ranging from S$100/- to S$350/- p.m.

Candidates should write to: *The Registrar, University of Singapore, Kent Ridge, Singapore 0511,* giving curriculum vitae (bio-data), with full personal particulars and also the names and addresses of three referees. W274(A)

ROTHAMSTED EXPERIMENTAL STATION
Harpenden, Herts AL5 2JQ

ENTOMOLOGIST

required to work on the overwintering mortality and migratory behaviour of aphids infesting potato crops in spring. Three-year post funded by the Potato Marketing Board.

Qualifications: 1st or 2(1) hons. degree in Zoology, Applied Entomology or Agricultural Zoology.

Appointment in the grade of Scientific Officer £3,289 to £5,085 (£3,591 to £5,486 from 1.1.80). Starting salary according to qualifications and experience. Non-contributory superannuation.

Apply in writing to the Secretary, giving names and addresses of two referees and quoting Ref. 403 by 9th August, 1979. Further details on request. 2793(A)

UNIVERSITY OF MANCHESTER

RESEARCH ASSOCIATE IN MEDICAL BIOCHEMISTRY

Applications invited from post-doctoral candidates for this post (available for three years) to work on "The Synthesis and Secretion of Elastin". Some experience in the field of nucleic acid isolation, protein synthesis or connective tissue biochemistry would be an advantage.

Starting salary in the range £4,333 to £4,910 p.a.

Applications with full curriculum vitae and the names of two referees should be sent as soon as possible to: Dr M.E. Grant, Department of Medical Biochemistry, University of Manchester, Medical School, Manchester M13 9PT. 2771(A)

THE UNIVERSITY OF HULL
DEPARTMENT OF BIOCHEMISTRY
RESEARCH TECHNICIAN
Grade 3

is required for an established post for varied and interesting work in the Departmental Chemostat and Fermenter Suite. Minimum qualifications: ONC in Sciences (or equivalent) plus 3-4 years background experience in Biochemistry. Additional experience in Microbiology would be advantageous. Salary on the scale £2,931 to £3,336 per annum.

Applications giving details of age, qualifications, experience and the names of two referees should reach the Technical Staff Officer, University of Hull, HU6 7RX by Friday, 17 August 1979 quoting Ref. No. TBCH/4. 2786(A)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF GENETICS

Applications are invited from graduates in a suitable discipline for the post of

RESEARCH ASSISTANT

to work with Dr P. Strike on error-prone DNA repair processes enhanced by bacterial plasmids. The project, which is supported by the S.R.C., if financed for a period of three years from 1st October, 1979, and will involve both conventional genetic techniques and *in vitro* DNA manipulation. Initial salary will be £3,689 p.a. The possibility exists for a suitable candidate to register for the degree of PhD.

Applications, together with the names of three referees, should be received as soon as possible, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/717/N 2745(A)

UNIVERSITY OF BRISTOL
SCHOOL OF CHEMISTRY
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Postdoctoral Research Assistant to join a group investigating the metabolism of gibberellins in higher plants. Some experience in the synthesis of isotopically-labelled compounds would be advantageous.

The appointment, funded by the SRC, is for a maximum of two years. Salary in the range £4,232 to £4,776 p.a. (scale subject to revision from 1st October, 1979).

Applications, enclosing a curriculum vitae and the names of two referees should be sent to Professor J. MacMillan by 31st August, 1979. 2650(A)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF BIOCHEMISTRY
TECHNICIAN GRADE 5 (ELECTRON MICROSCOPY)

For Electron Microscope unit to carry out work in association with several research groups. Applicants should posses HNC or equivalent as minimum qualification and graduates would be welcome. Candidates should possess experience in Electron Microscopy.

Salary within a range of £3,474 to £4,056 p.a.

Application forms available from The Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref: RV/723/N 2746(A)

FLOUR MILLING AND BAKING RESEARCH ASSOCIATION

MICROBIOLOGIST

The Flour Milling and Baking Research Association carries out a wide range of research and service work for the Milling and Baking Industries and holds a number of research contracts from the Ministry of Agriculture, Fisheries and Food. The laboratories are situated in an attractive semi-rural area on the edge of the Chilterns with ready access to London.

A GRADUATE MICROBIOLOGIST

is required to work on applied research topics and on microbiological problems of the milling and baking industries. Candidates should have experience of food microbiology and preferably have spent some time in the food industry. Preferred age, under 30; salary on the Scientific Officer (£3,194 to £4,767) or Higher Scientific Officer (£4,530 to £5,903) scales, entry position depending on experience.

Applications, including the names of two referees, should be sent to Professor Brian Spencer, Flour Milling and Baking Research Association, Chorleywood, Rickmansworth, Herts. WD3 5SH, from whom further details of the work of the Research Association may be obtained. 2732(A)

QUEEN ELIZABETH COLLEGE, KENSINGTON (Universiy of London)

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Quadrupole double resonance

Applications are invited for a post-doctoral research post, tenable for two years, in double resonance spectroscopy with particular reference to the detection of ^{17}O and ^{33}S quadrupole resonance in natural abundance in solids. Candidates should be graduates in Chemistry, Physics, or Chemical Physics and should have recently obtained, or expect shortly to obtain, a PhD degree. An interest in instrumentation and/or computing would be an additional qualification.

This post is funded by S.R.C. and has a salary (under review) in range £4,763 to £5,307 (including London allowance). Applications with a curiculum vitae and the names of two referees should be sent to: Professor J.A.S. Smith, Department of Chemistry, Queen Elizabeth College, Campden Hill Road, London, W8 7AH. 2723(A)

MLSO/

DEPARTMENT OF MEDICINE

Applications are invited for a research technician to assist in initiating a programme studying protein metabolism and in lung disease. Applicants should hold relevant HNC or equivalent and must be experienced in biochemical techniques with experience in chromatography and surgical techniques an advantage.

Applications in writing, stating date of birth, qualifications, career details and the names of two refeerees should be sent to The Secretary, Cardiothoracic Institute, Fulham Road, London, SW3 6HP. Quote Ref BC-1-79. 2731(A)

LONDON UNIVERSITY

CARDIOTHORACIC INSTITUTE, DEPARTMENT OF MEDICINE.

JUNIOR or MEDICAL LABORATORY SCIENTIFIC OFFICER

equired. Experience in immunology lesirable. Work will mainly involve the aboratory investigations of mmunological responses in patients with ung cancer.

Further information and applications, iving details of age and experience to: Mr. R.A. Perkins, Secretary, Cardiothoracic Institute, Fulham Road, London, SW3 6HP, quoting Ref 01/79. 2780(A)

HERIOT-WATT UNIVERSITY

MARINE TECHNOLOGY CENTRE

RESEARCH ASSOCIATES

Applications are invited for Research Associates in the SRC supported Heriot-Watt Marine Technology Centre.

The Centre is seeking to recruit graduates for work in Marine Technology and related fields; subjects include marine instrumentation, underwater plasma operations, aquaculture engineering, physical modelling of single-point moorings, offshore supply vessels, theoretical modelling of residual currents, dynamics of offshore cranes, concrete coating pipelines.

Applicants should have an Engineering or Science based degree. These posts offer an excellent opportunity for both new graduates and those already experienced in Marine Technology to develop specialised expertise in areas related to the offshore industry.

The work content involves theoretical studies, experimental work and also offshore trials.

Salaries: £3,689 to £6,108 (expected to be £3,775 to £6,355 p.a. with effect from 1 October 1979) depending on qualifications and experience.

Application forms from the Staff Office, Chambers Street, Edinburgh EH1 1AX. Ref. No. 107/79. 2733(A)

AGRICULTURAL RESEARCH COUNCIL

INSTITUTE OF PHYSIOLOGY

Babraham, Cambridge, CB2 4AT.

SCIENTIFIC OFFICER

required to assist with research in neurochemistry. H.N.C., pass degree in appropriate subject, or equivalent. Chemical knowledge essential. The position is financed by the Medical Research Council, at present until the 30th September 1980.

Salary in scale £3,289 to £5,085 p.a. according to qualifications and experience. Non-contributory superannuation scheme. 4 weeks paid annual holiday.

Applications with full details should be sent to the Secretary of the Institute quoting Ref. M.R.C.6. by the 13th August 1979. 2794(A)

Public Health Laboratory Service

Centre for Applied Microbiology & Research. Porton Down

Immunologist or Biochemist

The Vaccine Research & Production Laboratory has a vacancy for an **Immunologist or Biochemist** with immunological experience to take a leading role in developing a programme concerned with methods for the assessment of potency and safety of vaccines. Preference will be given to candidates with an interest in developing animal model systems.

Appointment may be at either Senior Grade (£5,451 to £6,837) or Basic Grade Microbiologist (£2,991 to £4,899) depending upon qualifications and experience. A good honours degree or equivalent is essential and for a Senior Grade position a record proven research ability. Salaries are under review.

Further details of the posts can be obtained from Dr J. Melling, Director, Vaccine Research & Production. Tel 0980-610391.

Applications stating date of birth, qualifications, experience and publications and naming three referees should be sent to Mrs M.Bushby, CAMR, Porton Down, Salisbury, Wilts SP4 0JG by 1 September 1979. 2799(A)

PHLS

UNIVERSITY OF GLASGOW

DEPARTMENT OF VETERINARY PHARMACOLOGY

RESEARCH ASSISTANT

Applications are invited for appointment as a Research Assistant in the Department of Veterinary Pharmacology.

The project is to study adrenergic mechanisms in the horse, using both *in vivo* and *in vitro* techniques. Experience in radiological methods and catecholamine assays would be helpful.

This project is funded by the Horserace Betting Levy Board and the position is available in the first instance for two years.

Applications can be made to Dr D Snow, Department of Veterinary Pharmacology, Veterinary School, Bearsden Road, Bearsden, Glasgow G61 1QH. 2761(A)

UNIVERSITY OF BRISTOL

School of Chemistry

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant to continue studies on the identification of reaction products from the ozonisation of organic compounds in potable waters. Instrumentation includes HPLC, capillary GLC and computerised GC-MS. Technical assistance is available. The project is financed by the Water Research Centre and relates to the purification of drinking water by ozone.

The appointment is for one year, with the possibility of renewal for a second year. Starting salary within the range £4,232 to £5,048 per annum. (Scale subject to revision with effect from 1st October, 1979).

Please send applications, by 20th August, with details and the names and addresses of two referees, to Dr J R Maxwell, Organic Geochemistry Unit, School of Chemistry, University of Bristol, Bristol BS8 1TS. 2749(A)

GUY'S HOSPITAL

DEPARTMENT OF HAEMATOLOGY

QUALIFIED LABORATORY SCIENTIFIC OFFICER

(H.N.C. or equivalent) required until 31st March 1981 for M.R.C. research project to study nutritional and biochemical aspects of vitamin K in the human. Previous experience in analytical techniques used in lipid biochemistry desirable but not essential.

Salary for candidate qualified as above not less than £3,261 (under review) plus £354 London Weighting.

Apply in writing, stating age and giving details of qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. H.M.1. 2736(A)

NUFFIELD INSTITUTE FOR MEDICAL RESEARCH

University of Oxford (on John Radcliffe Hospital site)

MEDICAL LABORATORY SCIENTIFIC OFFICER (WHITLEY COUNCIL)

The post involves assisting with research related to fetal development and its regulation. Experience in radio-chemical techniques is desirable.

Applicants should be state registered MLSO or possess an appropriate science degree or equivalent qualification together with at least one year's relevant laboratory experience. The post is available immediately. Salary scale £3,261 to £4,680 pa (as at 1 April 1978).

Applications (please quote ref MRC/R) with curriculum vitae and citing the names and addresses of two referees should be sent to The Administrator, Nuffield Institute for Medical Research, Headley Way, Headington, Oxford, OX3 9DS, by 15 August 1979. 2767(A)

COLAISTE NA HOLLSCOILE CORCAIGH
UNIVERSITY COLLEGE CORK
RESEARCH FELLOWSHIP

BIOCHEMICAL GENETIC/BACTERIAL PHYSIOLOGIST

required to work on regulatory aspects of nitrogen fixation in Rhizobium.

Minimum qualifications: First or upper second class honours degree in appropriate subject and at least two years relevant post-qualifying experience. Preference will be given to applicants with a PhD, degree. This post, funded by the National Board for Science and Technology, is tenable for up to 3 years and available from 1 October 1979. The salary range is £3,365 to £4,476 (post-doctoral £3,837 to £4,476) and starting salary will depend on experience (salary under review).

Applications, giving full particulars of qualifications and experience and names of two referees should be sent to:
Dr F. O'Gara, Department of Microbiology, University College, Cork, (Ireland).
2801(A)

COLAISTE NA HOLLSCOILE CORCAIGH
UNIVERSITY COLLEGE CORK
RESEARCH ASSISTANT

A Research Assistant is required for 1 October 1979 with postgraduate experience in enzyme purification and characterisation to work on oxygen and hydrogen peroxide metabolism in latic acid bacteria.

Candidates should preferably have a PhD in microbiology/biochemistry. The appointment is funded by the National Board for Science and Technology of Ireland. Starting salary is £3,837 for a post-doctoral worker (salary under review). The post is renewable for a second year.

Curriculum vitae with names of two referees should be sent to:
Dr S. Condon, Department of Microbiology, University College, Cork, (Ireland).
Closing date for receipt of applications: Friday, 31st August 1979. 2082(A)

UNIVERSITY OF WARWICK

POSTGRADUATE RESEARCH ASSOCIATE

in the

DEPARTMENT OF CHEMISTRY AND MOLECULAR SCIENCES

Applications are invited to the SRC funded post of Postgraduate Research Associate to work with Dr O.W. Howarth on a ^{13}C nuclear magnetic resonance study of the physical dynamics of biomolecules and macromolecules. Candidates should hold a good honours degree in chemistry or biochemistry.

A suitably qualified candidate will be permitted to register for a higher degree on appointment. The post is tenable for three years with a starting salary up to £4,333 p.a. on the Research Range 1B scale £3,775 to £5,488 p.a. depending on age and experience.

Applications, together with the names of two referees, should be sent as soon as possible to the Academic Registrar, University of Warwick, Coventry, CV4 7AL. Please quote Ref.No: 52/2A/79.
2785(A)

CENTRAL PUBLIC HEALTH LABORATORY

DIVISION OF ENTERIC PATHOGENS

MEDICAL LABORATORY SCIENTIFIC OFFICER

Suitable qualifications would be A.I.M.L.S., a relevant science degree or H.N.C. with experience in Microbiology.

The post is in the phage-typing and drug resistance unit. The successful applicant is to assist in the routine work of the unit but will be expected to participate in the research program. The officer would be encouraged to work for higher qualifications such as F.I.M.L.S. by thesis or higher degrees.

The Division of the National Reference Centre for Salmonella, Shigella and Escherichia coli. The World Health Organisation Collaborative Centre for Phage-typing and Drug Resistance of Enterobacteria is located in the Division.

Applications with full details of age, experience and qualifications to the Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London N.W.9. 5 H.T. Telephone 01 205 7041.
2737(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL
(University of London)
Denmark Hill,
London SE5 8RX

DEPARTMENT OF OBSTETRICS & GYNAECOLOGY

Applications are invited from graduates or suitably qualified people for the post of

RESEARCH TECHNICIAN

on a project involving tissue culture and cytogenetics and some histological and histochemi techniques in the area of pre-natal diagnosis of inherited diseases. Starting salary £3,669 inclusive (under review).

For informal enquiries please contact Dr J Singer on 01 274 6222 extension 2349. Applications in writing giving full details of qualifications and experience should be sent to the Secretary of the Medical School at the above address by 19th August, 1979. (Previous applicants need not re-apply.)
2770(A)

UNIVERSITY OF BRISTOL

LECTURER IN MEDICAL MICROBIOLOGY (VIROLOGY)

Applications are invited from honours science graduates, with experience of microbiology, for the post of Lecturer in Medical Microbiology (Virology). Salary scale £4,232 to £8,452 per annum, (under review), together with superannuation.

The lecturer will work mainly in the laboratories of Bristol Royal Infirmary, and responsibilities will include clinical laboratory work, teaching of medical students and research. Training will be provided in clinical virology, and in other aspects of medical microbiology. Applications, giving full details of age and experience, together with the names and addresses of three referees, should be sent by 25th August, 1979, to the Registrar and Secretary, University of Bristol, Senate House, Bristol, BS8 1TH, from whom further particulars may be obtained. Please quote reference TLJ. 2765(A)

UNIVERSITY OF DUNDEE

DEPARTMENT OF CHEMISTRY

There is a vacancy for a

POSTDOCTORAL RESEARCH ASSISTANT

to work with Dr J.A. Miller in the above Department on the synthesis of organic molecules designed to interact favourably with DNA.

The appointment will be available for one year from 1 October 1979 (or same later date agreed). Salary within the range £4,333 to £4,910. Superannuation under USS.

Applications containing details of age, qualifications and research experience and the names of two referees should be sent by 20 August 1979 to The Secretary, The University, Dundee, DD1 4HN. Please quote Ref: EST/76/79 J 2743(A)

UNIVERSITY OF DURHAM

DEPARTMENT OF ZOOLOGY

POST-DOCTORAL RESEARCH ASSISTANT

required from 1st October 1979, for a three-year project on control of lipogenesis and membrane biogenesis during hormone-induced cell differentiation in avian liver. Research experience in biochemistry or a related field is essential and experience of cell culture/immunochemistry would be an advantage.

Initial salary in the range £4,333 to £4,910 per annum within range 1A, plus superannuation.

Applications (3 copies) including curriculum vitae and naming three referees should be sent by 10 August 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham, DH1 3LE, from whom further particulars may be obtained. 2655(A)

RESEARCH ASSISTANT required for three years to work on a CRC financed project on the invasiveness of malignant cells in culture. Applicants should have a good degree in the Biological Sciences with some training in Cell/Developmental Biology or related subjects and post-graduate experience in one or more of the following fields will be an advantage: Tissue culture techniques and light microscopy of cultures. Quantitative analysis of motile behaviour in cells. Tumour cell biology. Biochemistry/biophysics of contractile systems. The successful applicant will be expected to show some resourcefulness in the design and interpretation of experiments. Starting date October 1979, starting salary up to £4,300 pa (depending on age and qualifications). Please apply in writing, enclosing a full curriculum vitae and the names of two referees to: Dr G A Dunn, Strangeways Research Laboratory, Wort's Causeway, Cambridge, CB1 4RN.
2729(A)

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

TECHNICIAN 5

(IMMUNOLOGY)

required to assist in a project unde Dr B. Anderton involving the isolation o brain proteins and the production o monoclonal antibodies to those proteins HNC/degree and experience in protei chemistry, or immunochemistry or tissu culture essential.

The appointment is for 3 years and i sponsored by the Wellcome Trust Emoluments £3,474 to £3,699 (unde review) plus £275 p.a. London allowance Application forms and further particular from the Sector Administrator, Sector 2 St. George's Hospital Medical School Cranmer Terrace, Tooting, SW17 0RE Closing date 3rd September 1979.
2798(A)

To place your advertisement in these pages

Phone: 01-831-6901

THE ROYAL SOCIETY

ROYAL SOCIETY METEOROLOGICAL OFFICE RESEARCH FELLOWSHIP IN DYNAMICAL METEOROLOGY

Applications are invited by the Council of the Royal Society for a

ROYAL SOCIETY METEOROLOGICAL OFFICE RESEARCH FELLOWSHIP IN DYNAMICAL METEOROLOGY

tenable in the department of atmospheric physics, University of Oxford. Candidates should supply the usual personal details and the names of two referees, together with a full description of the research proposed.

The appointment, which will be subject to the Society's general regulations governing research appointments, will be tenable for five years from a date to be mutually agreed in the academic year starting on 1 October 1979. The commencing salary which will depend on age and experience, will be within the scale £4,863 to £8,019 per annum, with appropriate superannuation benefits.

Applications should be made on forms to be obtained from The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG, and should be received not later than 30 September 1979. 2772(E)

THE QUEEN'S UNIVERSITY OF BELFAST

RESEARCH FELLOWSHIP

DEPARTMENT OF MICROBIOLOGY & IMMUNOBIOLOGY

This post involves work on macromolecular aspects of neurological disease. Preference will be given to applicants with experience in virology, immunology, particularly in immunassay methods and/or mucleic acid hybridization techniques. The fellowship is tenable for two years but may be renewable for a third year. Salary in the range of £6,000 to £7,000.

Application forms may be obtained from the Personnel Officer, the Queen's University of Belfast, BT7 1NN, Northern Ireland. Closing date 14th September, 1979. 2752(E)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF GEOLOGICAL SCIENCES

STUDENTSHIP IN URANIUM-THORIUM HYDROCHEMISTRY

Applications are invited for the above studentship. The study will include the measurement of uranium and thorium isotope concentrations in groundwater and the interpretation of their hydrogeological significance and the studentship holder will be permitted to work for the degree of PhD. The work is supported by a research grant from the Harwell Research Establishment. Applicants should have a good honours degree or an MSc in Chemistry or Geology or Physics. The Studentship is tenable for three years.

Applications should be sent as soon as possible to the Assistant Registrar, Science and Engineering, University of Birmingham, P.O. Box 363, Birmingham B15 2TT. 2781(F)

UNIVERSITY COLLEGE CARDIFF

Applications are invited for an

SRC POST DOCTORAL FELLOWSHIP

available for suitably qualified candidates to work with Dr J W T Wimpenny in the

DEPARTMENT OF MICROBIOLOGY

on quantitative aspects of the growth of micro-organisms in solute gradients. The successful applicant will join a group investigating the biology of three different diffusion systems, and will be responsible for generating mathematical models capable of defining and predicting the growth and interactions of organisms in solute gradients. Applicants are sought from relevant subjects disciplines including for example chemical or biochemical engineering, physical chemistry, operational research, computing science, and pure or allied mathematics.

The appointment is for 2 years from October 1st, 1979 at a starting salary of £4,333 (under review).

Applications (2 copies) including curriculum vitae and the names and addresses of two referees should be forwarded to the Vice-Principal (Administration) and Registrar, University College, PO Box 78, Cardiff CF1 1XL, as soon as possible. Reference 1870. 2748(E)

POST DOCTORAL FELLOWSHIP available for work on the causation of lung cancer by environmental and industrial metal particulates and the possible interaction of these with systemic agents. The project is based in Cambridge and involves collaboration with the Industrial Toxicology Unit of the Dept of Safety and Hygiene, University of Aston. Funding is provided by a 3 year grant from the Health and Safety Executive starting 1st October 1979. MRC conditions of service, starting salary in the range £5,048 to £5,850 depending on age and qualifications. Applications (2 copies) from persons with good previous experience of the techniques of experimental pathology should be forwarded to Administrative Secretary, Strangeways Research Laboratory, Worts Causeway, Cambridge CB1 4RN, together with full c.v. and the names of two referees. 2728(E)

UNIVERSITY OF LONDON KING'S COLLEGE

DEPARTMENT OF PHYSIOLOGY

Applications are invited for a three year MRC-financed

POST-DOCTORAL FELLOWSHIP,

initial salary on scale 1A, £4,333 p.a. plus London Allowance £502 p.a. effective from 1st October 1979.

The project involves electron-probe, physiological and electron microscopical studies of solute and water transport in rabbit ileum. The work will be at two centres, the physiological part at King's College London under Dr R.J. Naftalin and the electron microprobe and electron microscopy in the Zoology department, Cambridge, under Dr B. Gupta. Provision for travel and short stay expenses in Cambridge. Applications and names of two referees to Dr R.J. Naftalin, Department of Physiology, King's College, Strand, London WC2, telephone 01-836 5454. 2796(E)

SENIOR RESEARCH FELLOW IN ATOMIC/ MOLECULAR PHYSICS

The University of Maryland and the National Bureau of Standards in Washington, D.C. are announcing a one year fellowship for a senior research scientist to pursue research at the NBS Synchrotron Ultraviolet Research Facility (SURF). The SURF fellow will hold an appointment in the Institute of Physical Science and Technology at the College Park Campus of the University of Maryland. In addition to his research activities at SURF, the appointee will interact with the faculty and students at the University, principally through lectures and seminars.

Applications are now being accepted for the academic year 80-81, with detailed schedule to be negotiated. Salary and faculty rank will be commensurate with current position. We are initially seeking an individual interested in pursuing atomic/ molecular physics at SURF. Experience in the use of synchrotron radiation and/or photoelectron spectroscopy of gases will be considered particularly valuable for the appointment.

Send resume, desired starting date, and research plans to:
Prof. Joseph Silverman, Institute for Physical Science and Technology, University of Maryland, College Park, Maryland 20742. W267(E)

UNIVERSITY OF NOTTINGHAM

SRC. RESEARCH FELLOWSHIP IN PHARMACEUTICAL CHEMISTRY

Applications are invited for this post which is tenable for two years. This is a joint project between Professor B.W. Bycroft (Pharmacy) and Dr M.A.F. Dove (Chemistry) and concerns the application of fluorinated amino acids as reporter groups for the study of peptide antibiotic and protease inhibitors.

Salary in the range £4,333 to £4,910 per annum depending on age and qualifications. Applications in writing including full curriculum vitae and the names of two referees to the Staff Appointments Officer, University of Nottingham, Nottingham, NG7 2RD. 2773(E)

UNIVERSITY OF KENT AT CANTERBURY

RESEARCH FELLOWSHIPS IN CHEMISTRY

Applications are invited for three Post-Doctoral Research Fellowships in connection with the following research projects:

(i) Kinetics of fast reactions in solution by laser flash and temperature-jump apparatus and high-pressure cell (with Professor E.F. Caldin and Dr B.H. Robinson).

(ii) Tunnelling in proton-transfer and hydrogen-atom-transfer reactions in solution (with Professor E.F. Caldin).

(iii) Kinetics of very fast reactions in solution by microwave temperture-jump method (with Professor E.F. Caldin).

The projects are supported by the Science Research Council and the appointments will be for a minimum period of two years. Salary range — £4,232 to £5,321 (under review). Application forms and particulars may be obtained from the Senior Assistant Registrar, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH to whom applications should be returned by 31 August 1979. Please quote ref: A42/79. 2800(E)

FELLOWSHIPS

UNIVERSITY OF NOTTINGHAM

POST-DOCTORAL RESEARCH IN ENVIRONMENTAL SCIENCES

Fog interception by vegetation — a pathway for pollutant deposition

A post-doctoral research fellowship is available for study of the quantity and quality of fog water captured by shrubs, trees and extensive forests exposed to wind-blown fog and low cloud. In areas where fogs contain dissolved air pollutants it is believed that fog capture may be an important and previously unmeasured pathway for pollutant deposition. The project is in collaboration with the Institute of Terrestrial Ecology, Penicuik, Edinburgh, where some of the field studies will be made.

Applicants with backgrounds in environmental sciences, or in physical, chemical or biological science will be considered. The post is funded by the Natural Environment Research Council for three years, starting as soon as possible. Salary in the range £4,333 to £4,910 p.a.

Applications, including curriculum vitae and names of two referees should be sent to Dr M.H. Unsworth, Department of physiology and Environmental Studies, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leics. U.K., from whom further details are available. 2742(E)

PRESTON POLYTECHNIC

SRC POSTGRADUATE RESEARCH STUDENTSHIP

Applications are invited for an SRC Postgraduate Studentship on one of the following topics: 1) Climatic and biotic factors governing the colonisation of timber joinery by micro-fungi; 2) Photo-synthesis in algae and bacteria; 3)Energy metabolism: effects of diet and exercise.

For further information, interested persons should telephone The School Clerk (Chemistry and Biology), on Preston 51831 ext. 250. 2789(F)

UNIVERSITY OF WARWICK

RESEARCH FELLOWSHIP IN VIROLOGY

Applications are invited for a Postdoctoral Fellowship in the Tumour Virus Research Group in the Department of Biological Sciences. The successful applicant will work in a small group using molecular biology (including recombinant DNA) techniques to study mouse RNA tumour viruses, with particular reference to their ability to transform cells.

Starting salary will be between points 1 and 6 on the Research 1A scale: £4,333 (£5,777) £7,145 p.a. The Fellowship (funded by the Medical Research Council) is available from 1st October, 1979 and is renewable on an annual basis until September 1981.

Further details can be obtained from Dr R.J. Avery in the Department of Biological Sciences. Application forms from the Academic Registrar, University of Warwick, Coventry, CV4 7AL quoting Ref.No. 52/A/79. Closing date for receipt of applications is 24th August 1979. 2784(E)

FELLOWSHIPS continued

UNIVERSITY OF SUSSEX

SCHOOL OF BIOLOGICAL SCIENCES

Biochemistry Laboratory

RESEARCH FELLOWSHIP IN CELL BIOLOGY

A postdoctoral fellowship is available for the study of the involvement of cyclic nucleotides in the regulation of the differentation of muscle. The project, which is financed by the MRC, is specifically concerned with the mechanism of action of cyclic AMP and will involve an examination of protein kinase activities and protein phosphorylation in chick myoblasts as they prepare to differentiate *in vitro*. Experience with tissue culture techniques and two dimensional gel electrophoresis, would be an advantage. Available from 1 September 1979 for two years in the first instant. Initial salary will be in the range £4,232 to £4,776 per annum (scale under review from 1st October 1979).

Applicants should send curriculum vitae and names of two referees to Dr Rosalind Zalin, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, Sussex. 2751(E)

FELLOWSHIP AT THE FRIEDRICH MIESCHER-INSTITUT, Basel, Switzerland

In the Friedrich Miescher-Institut, a fellowship (2-3 years) is available for a PhD or MD in a research group examining the role of endogenous C-type viruses in leukemogenesis and lymphocyte function. Salary in accordance with the Swiss National Foundation. Travel costs will be covered.

Candidates, preferably with experience in immunology, virology or nucleic acid chemistry, please write to:
Dr Christoph Moroni, Friedrich Miescher-Institut, P.O. Box 273, CH-4002 Basel, Switzerland. W253(E)

QUEEN MARY COLLEGE
University of London

CHEMISTRY DEPARTMENT

Applications are invited for a Postdoctoral

RESEARCH FELLOWSHIP

funded by Hoffmann-La Roche to investigate with Dr G P Moss the structure of caroteno-proteins by the synthesis and recombination of modified carotenoids and a study of the physical properties of the products. Initial salary in range (under review) £4,835 to £5,412 pa (including London Allowance).

Applications, with curriculum vitae and the names and addresses of two referees, should be sent to The Registrar, (N) Queen Mary College, Mile End Road, London E1 4NS. 2776(E)

COURSES

COMBUSTION FUNDAMENTALS

A one week course of post experience lectures will be held at Imperial College from 22 to 26 October 1979. Details from Professor F.J. Weinberg, Department of Chemical Engineering and Chemical Technology, Imperial College, London SW7 2BY. 2722(D)

STUDENTSHIPS

THE ROWETT RESEARCH INSTITUTE

Greenburn Road, Bucksburn, Aberdeen, AB2 9SB

A.R.C. RESEARCH STUDENTSHIP

Applications are invited for the award of a Post-graduate Research Studentship of the Agricultural Research Council tenable for three years, from October 1979, in the Lipid Biochemistry Department for work on metabolic inter-relationships between lipid and protein metabolism.

Candidates should have a first or upper second class Honours degree (preferably in biochemistry) and will be expected to register for a higher degree. The Studentship comprises a tax-free maintenace allowance of £1,820 p.a., plus compulsory fees and travelling expenses.

Further particulars may be obtained from the Secretary, Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB, to whom applications (giving details of career to date) should be sent. 2792(F)

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AWARDS

ASSISTANTSHIPS

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF CHEMISTRY AND DEPARTMENT OF MATERIALS SCIENCE

Applications are invited for a

POSTDOCTORAL RESEARCH ASSISTANTSHIP

tenable from October 1st 1979 for 3 years to work with Professor W.J. Albery and Dr B.C.H. Steele on the development on novel oxide electrodes for fuel cells and solid state oxygen sensors. The research assistant will be responsible for applying advanced electrochemical techniques to the elucidation of the kinetics and mechanism of the oxygen reaction and for the design and development of new materials for these electrodes. The project is part of the Oxford Imperial Energy Group.

Initial salary of up to £4,776 plus £29 supplement plus £502 London allowance (under review from1-10-79).

Applications with curriculum vitae should be sent to Professor J. Albery, Department of Chemistry, Imperial College, London SW7 2AY. 2797(P)

UNIVERSITY OF OXFORD

DYSON PERRINS (ORGANIC CHEMISTRY) LABORATORY

POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a postdoctoral Research Assistantship supported by the S.R.C. to investigate the mechanism of action of enzyme catalysed phosphoryl and nucleotidyl transfer reactions. Experience in phosphate chemistry and enzymology is desirable.

The appointment is for one year from 1st October or as soon as possible thereafter. The salary will be on the Research Support Grade Scale 1A, initially not higher than £4,776 per annum plus U.S.S. benefits.

Applications, with curriculum vitae and the names and addresses of two referees should be sent as soon as possible to Dr G. Lowe, Dyson Perrins Laboratory, South Parks Road, Oxford. OX1 3QY. 2768(P)

UNIVERSITY OF DURHAM

DEPARTMENT OF PHYSICS

Applications are invited for an SRC funded

POSTDOCTORAL SENIOR RESEARCH ASSISTANTSHIP

tenable for 2 years from 1st October, 1979 to work in the field of Gamma Ray Astronomy with Professor A W Wolfendale FRS, and Dr A W Strong.

The work involves analysis of gamma ray data accumulated by satellite borne detectors and has as a major objective a study of the distribution of cosmic ray particles in the Galaxy (and beyond) whose interactions with the interstellar gas generate some of the quanta. Preference will be given to applicants with Research experience in Galactic structure, the interstellar medium or related fields. Computer experience would also be an advantage.

Initial salary in the range £4,333 to £4,910 on Range 1A plus superannuation.

Applications (3 copies) naming three referees should be sent by 31st August 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE from whom further particulars may be obtained.

Further particulars: Applications are invited for a Science Research Council supported Postdoctoral Senior Research Assistantship to work in the field of Gamma Ray Astronomy with Professor A W Wolfendale FRS, and Dr A W Strong.

The work is part of a programme relating to elucidating the origin of cosmic rays and the way in which they are propagated through the Galaxy and through space in general. Some of the gamma rays originate in interactions with gas in the ISM and a detailed analysis of gamma ray arrival directions coupled with a knowledge of the distribution of gas in the Galaxy and beyond allows cosmic ray intensity distributions to be examined.

Data from satellite-borne detectors (principally COS-B) are the main source of gamma ray information and this is analysed along with a variety of radio data on the distribution of H, CO.. in space.

The Research Assistant will work primarily in Durham (where good computing facilities exist) but opportunities exist for visits to other laboratories, largely in Europe.

The appointment will be for 2 years from 1st October, 1979, or as soon as possible thereafter. 2754(P)

AWARDS

UNIVERSITY OF GLASGOW

SRC CASE AWARDS

Applications are invited for two CASE awards in the Department of Chemistry for work on the following projects:

1. "A molecular interpretation of transport properties of dense fluids and fluid mixtures."
2. "Thermal conductivities of dense fluid mixtures."

Each project involves some experimental work at elevated pressures which will be carried out at the National Engineering Laboratory, East Kilbride.

The co-operating body will supplement the SRC Studentship rate by £500 p.a.

Applicants should possess a good honours degree. Further details may be obtained from Dr. J. H. Dymond, Department of Chemistry, University of Glasgow, Glasgow, G12 8QQ. In reply please quote Ref. No. 4491M. 2788(N)

BACK PAIN ASSOCIATION

offers a Medal and honorarium of £200 for outstanding new work in the field of back pain. To qualify, the work must have been presented to a scientific meeting or published in a scientific journal in the English language during the year ending 30th September 1979.

In the event of two candidates being adjudged winners, the honorarium will be divided equally and each candidate will receive a medal.

There are no restrictions as to nationality or academic qualifications but particular consideration will be given to the work of young researchers.

The Association shall have the right to publish a synopsis of the work or to reprint the complete paper providing that, if it has already been published elsewhere, copyright permission shall have been obtained.

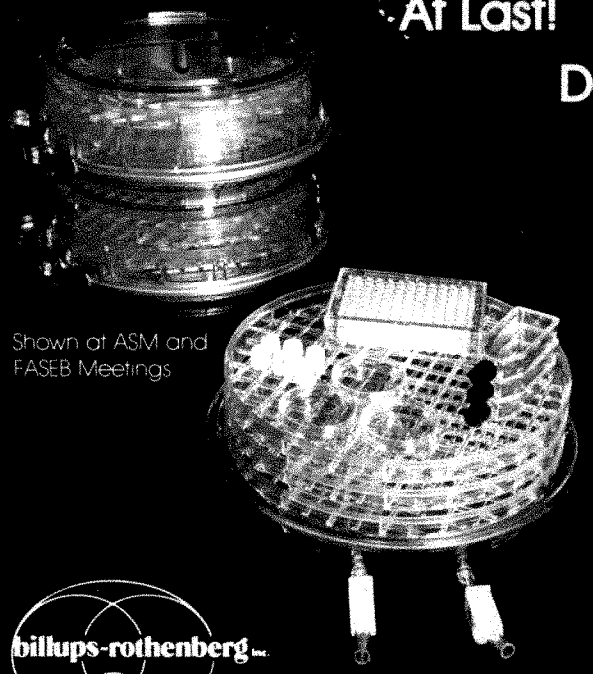
The Awards Committee reserve the right to withhold the award.

All submissions, which should be fully documented, will be considered by the Awards Committee and must reach The Back Pain Association, Grundy House, Somerset Road, Teddington, TW11 7TD not later than 30th September 1979. 2769(N)

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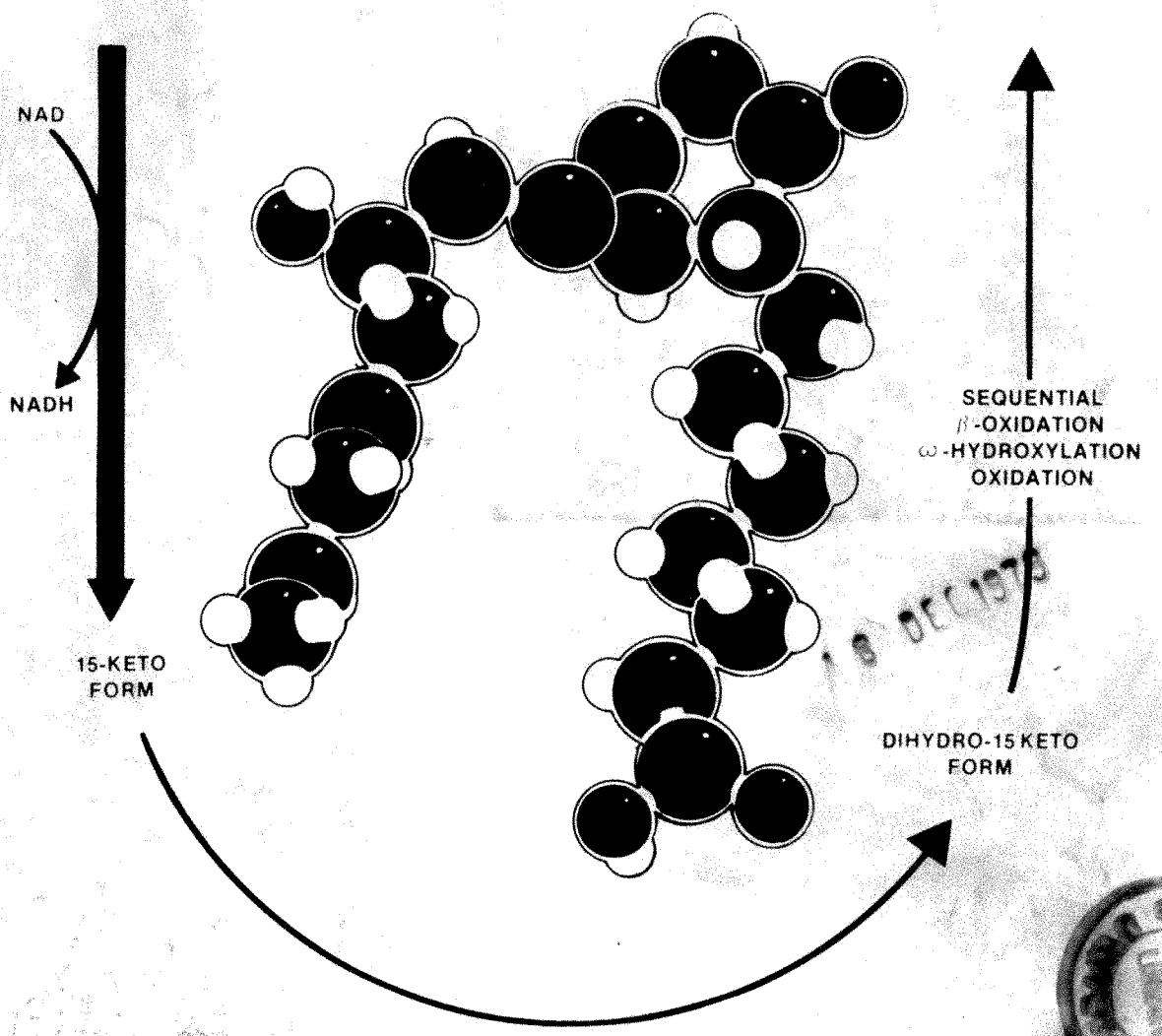
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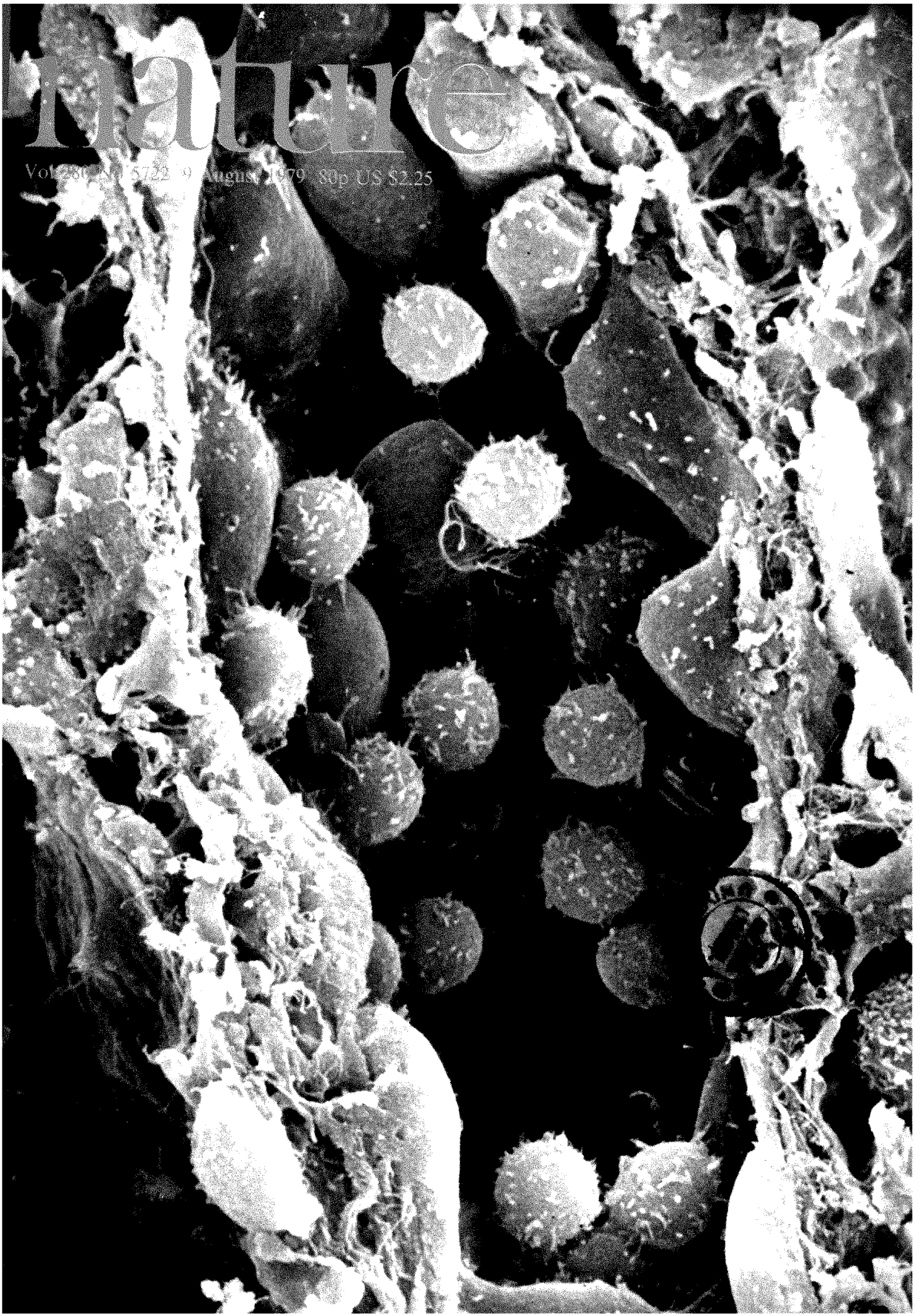
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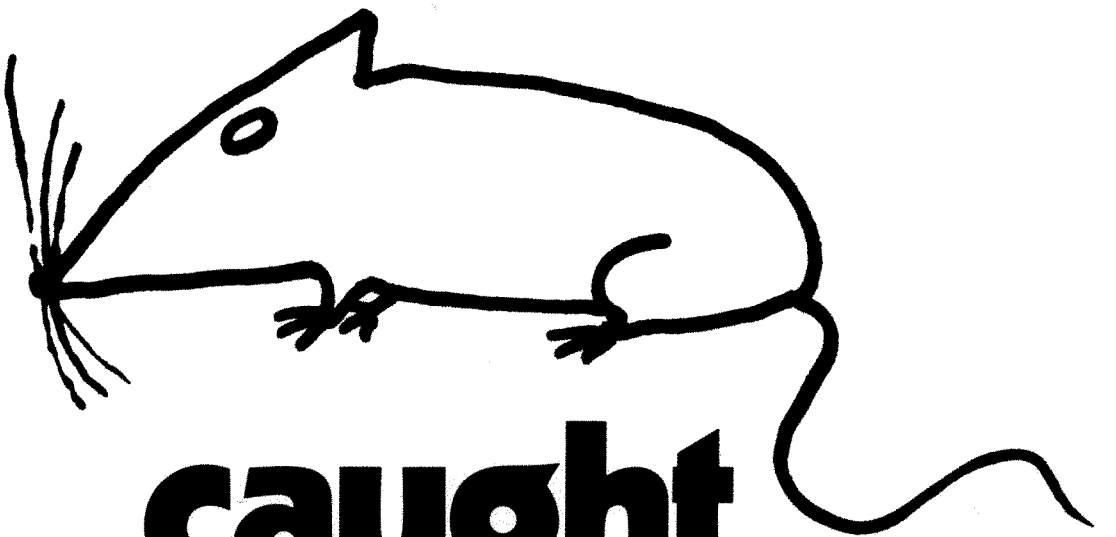
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Cover picture

Scanning electron micrograph showing lymphocytes binding to endothelical cells in specialised lymph node venules in the mouse (× 5,585). Evidence that cell-surface structures mediating lymphocyte adherence have been ramdonly altered during evolution is presented on page 496.

Vol.280 No.5722 9 August 1979

nature

Volume 280 9 August 1979

Who participates . . . and how? 435
Third World institute approved/HSE reviews safety at Windscale/ Uranium enrichment in Pakistan 436
Comecon tackles the energy crisis 437
Crisis for Spanish science 438
In brief 439
'Always on tap, never on top' — a scientists' view of the IPCS strike 440
Ireland, land of priests . . . and engineers 441
How industry lost an enthusiastic engineer 442
Sadly missed: a key contributor to science policy 443
Correspondence 444

NEWS AND VIEWS
Hypercycles and the origin of life/More twists and turns to recombination/ Theoretical difficulties with the binary pulsar/Structure and control of phosphorylase/Minding the psi's and q's/Naming names 445

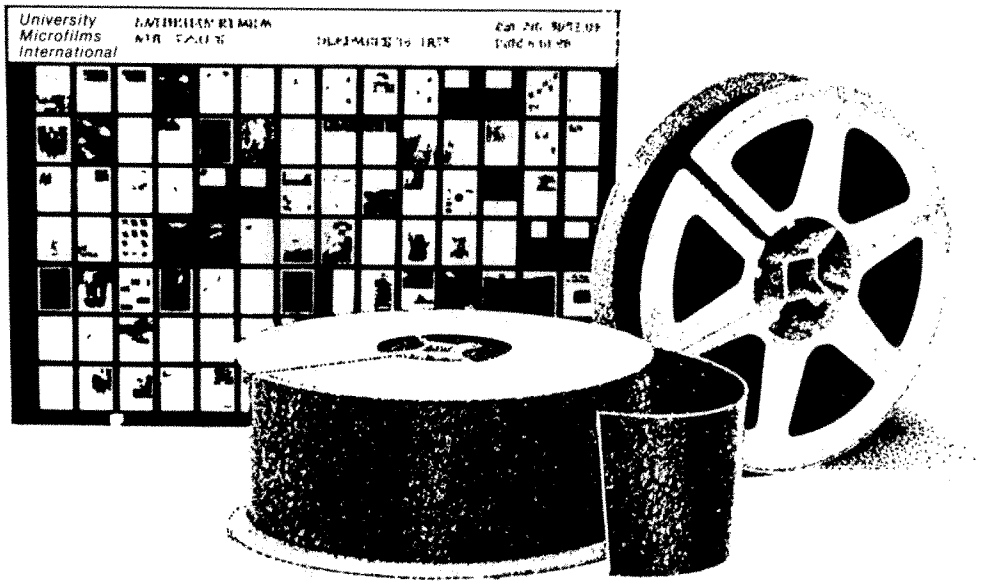
REVIEW ARTICLES
Evidence for the quantum nature of light — *D.F. Walls* 451
Population biology of infectious diseases: Part II — *R.M. May and R.M. Anderson* 455

ARTICLES
Radio studies of the double QSO, 0957 + 561A, B — *G.G. Pooley, I.W.A. Browne, E.J. Daintree, P.K. Moore, R.G. Noble and D. Walsh* 461
The amino acid sequence of the α-chain of human fibrinogen — *R.F. Doolittle, K.W.K. Watt, B.A. Cottrell, D.D. Strong and M. Riley* 464
The membrane attachment protein for spectrin is associated with band 3 in human erythrocyte membranes — *V. Bennett and P.J. Stenbuck* 468

LETTERS
A precessing ring model for SS433 — *A. Amitai-Milchgrub, T. Piran and J. Shaham* 473
On the nature of SS433 — *G.W. Collins and G.H. Newsom* 474
Cool gaseous nebulae — *P.A. Shaver, R.X. McGee and S.R. Pottasch* 476
Measurements of the trigonometric parallax of pulsars — *M.J. Salter, A.G. Lyne and B. Anderson* 477
On the Pioneer 11 observation of the ring of Jupiter — *W.-H. Ip* 478
Compositional differences between Arctic aerosol and snow — *K.A. Rahn and R.J. McCaffrey* 479
The 20-yr cycle in Greenland ice core records — *W.H. Hibler and S.J. Johnsen* 481
Combined structural and chemical analysis of 3,800-Myr-old microfossils — *H.D. Pflug and H. Jaeschke-Boyer* 483
An early Cretaceous terrestrial crocodilian and the opening of the South Atlantic — *E. Buffetaut and P. Taquet* 486
Toxicity induced in the tobacco horn-worm (*Manduca sexta* L.) (Sphingidae, Lepidoptera) — *M. Rothschild, R. Aplin, J. Baker and N. Marsh* 487
Retinal site of transient tritanopia — *J. Mathee Valeton and D. van Norren* 488

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Immunisation of guinea pigs and cattle against ticks	*J.R. Allen and S.J. Humphreys*	491
Involvement of a gene on chromosome 9 in human fibroblast interferon production	*A. Meager, H. Graves, D.C. Burke and D.M. Swallow*	493
Sheep fetal haematopoietic cells produce adult haemoglobin when transplanted in the adult animal	*E.D. Zanjani, P.B. McGlave, A. Bhakthavathsalan and G. Stamatoyannopoulos*	495
Evidence of continuous evolutionary change in structures mediating adherence of lymphocytes to specialised venules	*E. Butcher, R. Scollay and I. Weissman*	496
Distribution of fibronectin in the ectoderm of gastrulating chick embryos	*D.R. Critchley, M.A. England, J. Wakely and R.O. Hynes*	498
Insulin-receptor antibodies mimic a late insulin effect	*E. Van Obberghen, P.M. Spooner, C.R. Kahn, S.S. Chernick, M.M. Garrison, F.A. Karlsson and C. Grunfeld*	500
ß-Adrenoreceptors determine affinity but not intrinsic activity of adenylate cyclase stimulants	*L.J. Pike, L.E. Limbird and R.J. Lefkowitz*	502
Acetylcholinesterase like that of skeletal muscle in smooth muscle reinnervated by a motor nerve	*N.C. Inestrosa, B. Mendez and J.V. Luco*	504
Aequorin luminescence during activation of single isolated smooth muscle cells	*F.S. Fay, H.H. Shlevin, W.C. Granger and S.R. Taylor*	506
Light-activated drug confirms a mechanism of ion channel blockade	*H.A. Lester, M.E. Krouse, M.M. Nass, N.H. Wassermann and B.F. Erlanger*	509
Occlusion of K ions in the unphosphorylated sodium pump	*L.A. Beaugé and I.M. Glynn*	510
Highly active cyclic and bicyclic somatostatin analogues of reduced ring size	*D.F. Veber, F.W. Holly, R.F. Nutt, S.J. Bergstrand, S.F. Brady, R. Hirschmann, M.S. Glitzer and R. Saperstein*	512
δ-Aminolaevulinic acid is a potent agonist for GABA autoreceptors	*M.J.W. Breennan and R.C. Cantrill*	514
Correlation of local stability of DNA during melting with environmental conditions	*J. Gabbarro-Arpa, P. Tougard and C. Reiss*	515
Environmental factors affecting the quantity of alcohol dehydrogenase in *Drosophila melanogaster*	*B. Clarke, R.G. Camfield, A.M. Galvin and C.R. Pitts*	517
Erratum and Corrigendum		518

BOOK REVIEWS

Caste and Ecology in the Social Insects (G.F. Oster and E.O. Wilson)	*Brian Charlesworth*	519
An Introduction to Affinity Chromatography (C.R. Lowe)	*P.D.G. Dean*	520
Dielectric and Electronic Properties of Biological Materials (R. Pethig)	*D.D. Eley*	520
Photochemistry of Small Molecules (Hideo Okabe)	*R.P. Wayne*	521
Asbestos. Vol.1: Properties, Applications and Hazards (L. Michaels and S.S. Chissick, editors)	*Charles E. Rossiter*	521
Arid-Land Ecosystems: Structure, Functioning and Management. Vol.1 (D.W. Goodall, R.A. Perry and K.M.W. Howes, editors)	*M.J. Chadwick*	522
The Study of Prosimian Behaviour (G.A. Doyle and R.D. Martin, editors)	*Alison Jolly*	522
Announcements		523
Newly on the market		ix

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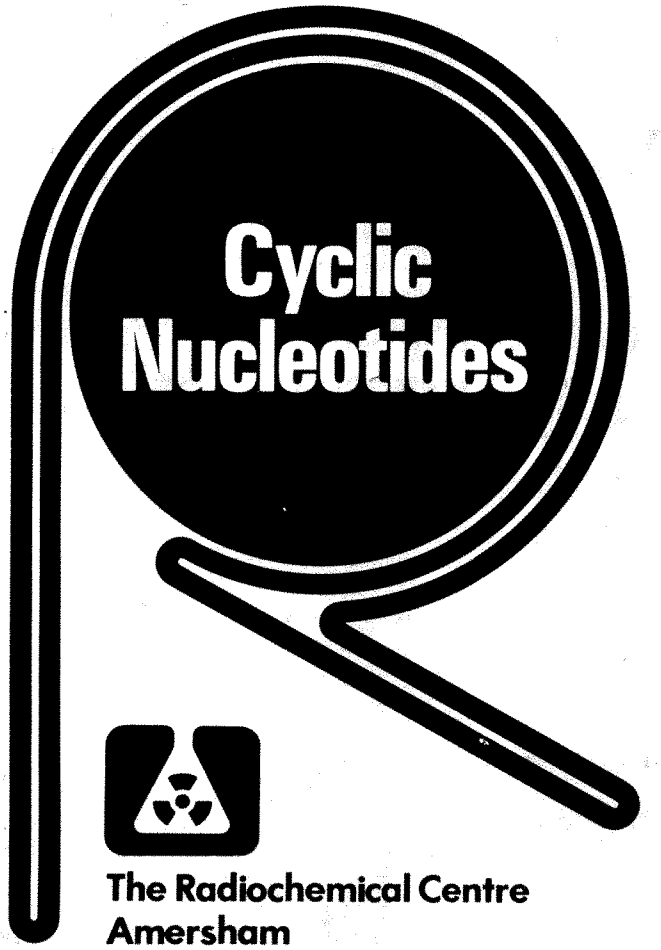
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Who participates . . . and how?

SYDNEY BRENNER: The discussion goes that the public should know and the public should decide and the public should participate in all the decisions on what research should or should not be pursued. And we are told that these are not decisions for scientists to take alone in laboratories. What I should like to hear spelt out is: how does the public actually come to participate in these decisions? I mean, we're talking about experimental politics rather than experimental biology here.

ANTHONY QUINTON: There's a saying here, isn't there, *sagesse oblige*. The trouble is that the general public is not in a position to make any sort of decision at all until it is told a great deal by scientists. I think one might reasonably say the existing institutional machinery for the public dissemination of socially interesting or socially relevant scientific activity is rather rudimentary and haphazard and improvisatory.

On the other hand, I don't think I'd like it to be the responsibility of a particular ministry, with civil servants putting in papers. It's just the sort of thing that a Quango might be rather good at doing, where you have a very substantial scientific representation, where scientists would come and talk about their work. A sort of conceptual, theoretical ombudsman in a way.

BBC Radio 3, reprinted in *The Listener*, 26 July 1979.

NIGEL FORMAN, one of the few Members of Parliament with a lively and continuing interest in science and technology, notes the demise of the Commons Select Committee on Science and Technology in this issue (page 443). With the disappearance of one valuable meeting point for scientists, administrators and 'the public', to be replaced by new department-orientated committees in which science is most unlikely to receive as consistent a hearing, it is reasonable to ask whether public understanding of science — or more particularly public understanding of the social issues raised by science — is unacceptably low, as might be inferred from Anthony Quniton's remarks.

What channels are there in Britain for keeping up the links? Obviously there are the printed and broadcast media, with quality ranging from very high to abysmal — but with, of course, relatively few opportunities for two-way communication. There is, sadly, no real British equivalent of the excellent serious American magazines able to devote substantial space to matters of public policy. There is the Royal Society, which has been trying, through its discussion meetings to introduce the occasional large scientific issue to a wide public — but which, too often, flounders on the uncompromisingly technical nature of its lecturers' contributions. There are the British Society for Social Responsibility in Science, the Royal Institution, the newly-formed Science and Technology Association, the Science Policy Foundation and the Council for Science and Society, each trying in distinctive ways to encourage scientists to think beyond the confining walls of their disciplines. There is the Parliamentary and Scientific Committee, an informal meeting point for parliamentarians and top scientists and engineers. There is, of course, a quango already in existence — the Genetic Manipulation Advisory Group, comprised not just of experts but of representtives of the public interest. There is the British Association for the Advancement of Science struggling against formidable odds to inform a wider public about science — including the social questions raised by science. And there is still some parliamentary select-committee activity, because the House of Lords keeps a rather effective eye on EEC science and environmental policy and may possibly broaden its interests in science and technology in response to the loss of the Commons committee.

But does all this activity add up to a scientific and technological community well aware of social issues? And does it produce a large pool of informed citizens capable of communicating easily with scientists and drawing them into debate on these issues? It does not. Part of the reason is undoubtedly that there are too many organisations covering the same sort of ground, looking to the same sources of financial support, calling on the same pool of people for their core support. But this is not the whole story. When scientists have gone out of their way in the past to discuss their work and its implications with intelligent non-scientists, they have too frequently found the response disappointing, both in terms of numbers who attend in the first place and those who are prepared to make the intellectual effort to stick with a subject after a first encounter.

So, with gloomy experiences in the recent past, there is a temptation to keep further efforts in the field to a low level and restricted to a limited number of people close to the interface. This would be a mistake. There is always scope for new initiatives, but they surely have to be done on a substantial scale and have enough long-term commitment that they do not capsize if one venture turns out to be a flop. The most obvious place in which to experiment is the Royal Society. It has adequate meeting rooms, a sizeable secretariat, connections in high places, easy access to a very wide range of scientists, prestige ... Regular, open meetings, not just in London, designed to bring scientists and non-scientists together are surely not beyond its capabilities and could play a major role in raising Britain's painfully low awareness of science. □

US Congress gives go-ahead to Third World institute

LEGISLATION establishing a new Institute for Scientific and Technological co-operation (ISTC) was approved in both houses of the US Congress last week, following the Senate's agreement to drop its previous opposition to the scheme. Although the budget of the ISTC still has to be agreed, the decision means that the US will be able to present the institute as a definite initiative to the United Nations Conference on Science and Technology for Development in Vienna.

Plans for the institute, first announced by President Carter last year and included in his budget request for 1980 presented to Congress in January, had been "derailed" by the Senate in June. The action took the form of an amendment to the Development Aid Authorisation Bill originally offered by Senator Deconcini, who objected to what he claimed was a "new federal bureaucracy" which would duplicate work already being done elsewhere. Meanwhile, the House of Representatives had approved the ISTC proposal, but recommended against the Administration's wishes, that the Peace Corps be made an autonomous agency within the newly-established international development cooperation agency.

The Administration, with a big political stake in the ISTC proposal, was able to watch the two decisions played off against each other during a series of conference meetings between delegates representing the two houses — the formal procedure by which differences are ironed out before legislation can be agreed. After three separate meetings, it was agreed to accept the House position approving the ISTC and the Senate position on the peace corps, thus satisfying the Administration on both counts.

The conference report, which was subsequently accepted by the full House and Senate, also stated that the amount of new money it was prepared to authorise for the ISTC should be cut from the $25 million in the President's request to $23.75 million. Initially the bulk of the institute's research funds will come from the transfer of research projects already underway within the Agency for International Development (AID). The precise budget will not be settled until both houses agree to foreign aid appropriations next month. The conference also agreed to accept the modifications which the House had previously made to the Administration's proposal for the ISTC. In particular, it has strengthened the role of the governing council so that it will have the right to review (although not, as some would have liked, to veto) all projects costing more than $200,000 or lasting more than two years. The new institute will have two main functions: to support research into topics of direct relevance to the developing countries and to help stimulate the development of research facilities within the developing countries.

David Dickson

Pakistan considers uranium enrichment

Pakistan has an ambitious nuclear power programme and, consequently, it is concerned that unless it acquires the capacity to enrich uranium locally, its energy supply will be highly vulnerable.

Two years ago, Canada backed out of the contract it had with Pakistan to provide fuel for KANUPP (the Karachi Nuclear Power Plant which it had already installed) over the issue of whether or not Pakistan was to acquire a reprocessing plant from France. "What do you expect us to do after the experience at KANUPP", said one Pakistani scientist. "If we had not developed the capability of fuelling KANUPP on our own, the 137 MW power plant would have been written-off".

Similarly, a 600 MW reactor being built at Chashma in north Pakistan, received a setback when France backed out of a deal to deliver a reprocessing plant to Pakistan after pressure from the US. Nevertheless, Pakistan's nuclear think-tank forsees many nuclear reactors of either the US light water type or the advanced gas-cooled type being built in the 1980s. Such reactors run on enriched uranium (3% or a little less). KANUPP, the only nuclear power reactor in the country at present, runs on natural uranium.

With the development of the centrifuge technique, uranium enrichment is no longer beyond the reach of the developing countries. Previously uranium enrichment had relied solely on gaseous diffusion. The situation, however, is now changing. In 1977, even the President of the US approved the gas-centrifuge technique and opposed the construction of a large and costly diffusion enrichment plant.

The centrifuge technique appears to have advantages over diffusion methods where the output of enriched uranium needed is small. A centrifuge complex can be built in stages and a start can be made with only a few hundred centrifuges.

Apart from the advantage that the centrifuge technique presents, Pakistan also possesses two other assets over other developing nations. It has about 500 nuclear scientists/engineers, and sizeable uranium deposits in the Dera Ghazi Khan district.

Azim Kidwai

HSE to review safety at Windscale

FOLLOWING the latest incident of a fire at Windscale where five workers suffered low level Cs^{137} contamination, the UK Health and Safety Executive has announced that it will conduct a review of the plant's safety arrangements. A review team consisting of Mr Fen Charlesworth, Senior Deputy Chief Inspector of the Nuclear Installations Inspectorate, Mr Bill Kenney of the Department of the Environment, Mr Stan Gronow from the HSE and a fourth independent specialist to be announced, will investigate safety management, safety surveillance and safety assessment procedures at the reprocessing plant.

The committee has taken the unusual step of announcing ahead of time that it will publish its findings. An HSE official told *Nature* that the large number of incidents at the plant and public concern about the plant's safety have made the HSE want to "let the public see what we are doing about Windscale". According to HSE records the Windscale plant has had 75 incidents since December 1976. Of these, three incidents in addition to the fire are considered major: a still unlocated leak in the B38 silo, a potentially explosive hydrogen build up in the same storage building, and a sump leaking 20,000 curies into the soil earlier this year.

The team will call independent consultants, chosen for specific expertise. They are not expected to include well-known critics of Windscale safety procedures such as Dr Michael Flood and Walt Patterson of Friends of the Earth.

The general investigation has pre-empted a review of the cause of the latest fire leaving numerous questions unanswered. The fire occurred when a fuel disassembly apparatus jammed in a decanning cave. The fire had to be put out manually and radiation escaped contaminating the five workers and causing fallout outside the building. The exact sequence of events is unknown and the questions raised by the incident are central to Windscale safety precautions. These include; how the fire began, why the installed sprinker system failed to put it out, why the fire burned for as long as 45 minutes, why the fire had to be put out with a fire hose by opening access holes in the roof of the cave, how the radiation escaped, whether the workers were wearing protective clothing and how they got exposed. British Nuclear Fuels has refused to comment on the questions. "We are not prepared to answer questions which will cut right across the internal inquiry presently underway," an official told *Nature*. When complete the report will be released to the local liaison committee.

Joe Schwartz

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Comecon tackles the energy crisis

Vera Rich explains how the Eastern bloc is coping with the rising price of oil

Recent oil price rises throughout Eastern Europe, coupled with Romania's unprecedented demand that tourists from other Comecon countries must purchase their petrol for hard currency, at first glance accords ill with Mr Kosygin's remarks at the Comecon summit at the end of June. In fact there is no contradiction. What Mr Kosygin promised for the next Five Year Plan (1981-86) was a 20% increase in energy supplied by the Soviet Union to the bloc, not specifically oil.

Commenting on Kosygin's speech, a TASS reporter quoted Dmitrii Mendeleev, discoverer of the periodic table, that "to use oil or banknotes for heating comes to about the same thing". While the West has based its economy on oil, he continued, the Soviet Union has "harmoniously" developed all fuel and energy branches of the economy: coal, shale, hydroelectricity and nuclear power, as well as the oil and gas fields.

Kosygin's statement of existing Soviet energy supplies to the Comecon allies is impressive: during this Five Year Plan, some 370 million tonnes of oil, 46 million tonnes of oil products, 88 million cubic metres of gas and 64 million kW-h of electricity. But the official communiques of the meeting stressed that from now on the extraction of oil and gas would continue to increase, but would be devoted primarily to the needs of the chemical industry. It seems likely that a major proportion of any increase in energy supplies from the Soviet Union will take the form of electricity. A massive plan to step up generating capacity in the USSR is already under way. From 1980, all increases in the European part of the USSR will be nuclear; east of the Urals, a network of thermal power stations will be brought into the grid, fired by lignite, of which there are abundant local supplies.

All Comecon countries, with the exception of the Soviet Union, are now net importers of petroleum. Even Romania, whose role as a former major oil producer is commemorated by an oil rig in the state coat of arms, has to import a certain proportion from the hard currency market. Although energy is one of the specific sectors selected for the "long-term target-orientated" Comecon integration programmes approved in 1976 (for the period 1975-1990), Soviet oil prices to the other Comecon countries are calculated from the average world price over the last five years and reassessed each year. Moreover, Soviet-exported oil accounts for only 80% of the other members' demands: the rest they must purchase on the world market.

Furthermore, the long-term availability of Soviet oil appears to be in some doubt. All official sources unequivocally repudiate the CIA predictions of an impending Soviet oil crisis. Shortfalls are attributed to the technical difficulties of bringing the East Siberian fields on-stream, and the consequent depletion of the West Siberian fields more rapidly than planned. Occasionally, too, "anti-social manifestations" among the oil workers may be blamed for a temporary deficit (*Bakiskii Rabochii* 1 June, 1979).



Russian pipeline: the oil will keep flowing, say Soviet officials

Nevertheless, nuclear power is seen as the mainstay of the future energy needs of the bloc, and the construction of nuclear power stations is a top priority in all member countries except Poland. Here the still abundant coal reserves make it possible to defer the introduction of nuclear power on a commercial scale, and the one nuclear power station so far scheduled (a joint venture with the Soviet Union) is seen, in the words of one energy expert, as "a pilot project for the Polish situation".

Socialist planning, it appears, leaves no room for an environmental lobby against nuclear power. A recent Hungarian publication stresses that "in the philosophy of socialism, environmental detriments may present no limitations to the welfare of society. The growing demands of society must be satisfied, since failing to do so would contravene the basic principles of socialism".

So far, only the dissident "Document 22" of the Charter-77 movement has dared to question the validity of the nuclear solution. By 1990, according to the latest agreement, the nuclear generating capacity of Comecon will have been raised from the present 15-18 million kW to 150 million kW, 37 million kW of which will be sited outside the Soviet Union.

Meanwhile, in spite of the agreed integration programme, the search for home-based fuel sources continues throughout the non-Soviet members of the bloc. Poland has a long-standing programme of coal mine modernisation, including a sophisticated research programme into the causes of mine-gas explosions. A country-wide geological survey for oil and gas resources has produced an impressive set of maps of the various strata, and is now proceeding to the field-work stage. No detailed interim results are available, although one geologist connected with the survey told *Nature*, last autumn that there were "positive indications". The survey is being continued offshore by an international consortium of Poland, East Germany and the Soviet Union.

Romania, too, is looking offshore. The new draft programme for research and development to be submitted to the forthcoming congress of the Romanian Communist Party stresses the need for offshore exploration and exploitation. Not only will this include mineral wealth, but the possibility of building a pilot wave-powered generating station is also under consideration. Full use of the hydroelectric potential of the Danube is a target for 1990, lignite and shale mining will be intensified, and a number of more exotic schemes — "biogas", the extraction of hydrogen from water, and the use of geothermal energy — are to be given serious consideration. By 1990 it is intended that the country should be self-supporting in energy, while the average energy consumed per 1000 lei of industrial output is intended to fall by at least 40%.

Czechoslovakia is looking closely at the home: modern flats, said the Director of the State Power Directorate recently, consume about 50% more heat than the norm, due to poor construction and installation. Yugoslavia (not a member of Comecon but closely linked with the bloc) has already decided not to build any further oil-burning power stations and to upgrade its neglected coal and lignite resources. Finland, likewise partially dependent on Soviet oil, is seriously considering the energy potentialities of scrub timber grown on marginal land. Hungary is showing a renewed interest in coal, and the latest updating of the UK-Hungarian Joint Commission on Technological Co-operation agreed last

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month includes a new cooperation project on coal technology. Bulgaria, while so far urging no major changes, is cautiously contributing to the energy debate by publishing statistics of kW-h of lighting saved this year by "summer time".

Meanwhile, East Germany, although claiming that, in spite of the harsh winter conditions, planned production of electric power was "achieved as a whole after the first six months", did not allow the temporary setback to the economy to be entirely attributed to meteorological conditions. In June, a result of "shortcomings in management, especially in the ministerial sector for coal and power", Klaus Seibold, the Minister of Energy, was dismissed from office. □

Spanish scientists have to fight for survival

Research in Spain is suffering from a lack of direction and funds. M Vicente, a Spanish biologist, reports on a recent meeting of scientists to discuss their plight and Emilio Munoz (below) reports on one ray of hope — the setting up of a new research ministry.

Uncertainty of direction, lack of promotion opportunities and no guarantee of long-term finance: these are the crucial problems that bedevil scientific research in Spain. They were given a public airing last month when biology and biomedical researchers met under the auspices of the CSIC (Consejo Superior de Investigaciones Cientificas). The scientists' intention was to test the reaction of the newly-created Ministry of Universities and Research (see below) to their complaints, and what emerged from the researchers amounted to a cry for survival.

The meeting had no difficulty agreeing that research policies were needed related to Spain's particular requirements. But they could not decide whether this was best approached through basic or applied research. One speaker said biology in Spain had concentrated on basic research for the past 15 years and all applied approaches had been treated as secondary. It was not surprising, he added, that there were no links between society and research and that, as a result, society was indifferent to the present scientific crisis. Other speakers defended basic research and argued that they were doing enough for society by teaching young scientists in the universities.

The second major theme of the meeting was that the country's scientific researchers were not only too few in number but also too old. The average age of Spain's scientific community was said to be around 48 and this restricted the job opportunities for young scientists. The number of young PhDs nearly doubled every four years but only one in five had a job related to his specialisation. Several causes were given for this situation, all with the same roots: the absence of long-term planning and the lack of interest in research.

Spain's scientific development during the 1960s happened at the wrong time and went in the wrong direction. The economy caught up with other developed countries, but largely because of imported foreign technology. A 10%-a-year increase in public sector research staff was intended to accompany economic growth but never came into effect. Researchers in the private sector are even fewer and their work is limited to quality control. All these problems have now been aggravated by economic difficulties, so that Spanish research is coming of age at the worst possible time.

For scientists, the finance situation could be the most worrying of all. Because of its lack of social impact, research is not in the official list of priorities. Half way through the present year, research groups are spending the money assigned to them last year. No official programme of grants has been issued yet for next year. Unless urgent measures are taken, and experience of Spanish bureaucracy makes it unlikely that they will be, most scientists will have to stop their work early in 1980. Even strong groups, such as those in the Molecular Biology Centre in Madrid.

A secret three-year government plan to finance research was drafted a year ago. Its text is not known, raising suspicions among scientists that it will channel money to the increasingly overcrowded universities in which research has been so difficult that it tends to be the activity of a few dilettantes. They are afraid that a decision to transfer them to academic jobs will irreversibly damage their research activities. □

Will the new research ministry fill the vacuum?

SPAIN has established a Ministry of Universities and Research — a move which has been welcomed by the scientific community. Scientists have felt that the lack of an effective government science policy has been paralysing efforts to build a new structure for research in Spain around the re-constituted Cosejo Superior de Investigaciones Cientificas (CSIC), and they hope the new ministry will fill the vacuum.

The Ministry of Universities and Research has been created by splitting the functions of the former Ministry of Education and Science, in which science had become submerged in the complicated problems of education. However, there are still fears that hopes for specific research proposals may again be disappointed. The government has not yet presented its research programme, so the reasons for the creation of the new ministry are unclear. And there is still a lack of communication between the scientific community and society. No national debate has taken place in parliament on this subject, although various sectors in the country have been demanding such a debate since 1977.

The need for a new ministry was established more clearly when the new statute of the CSIC was first put into practice. It was published in January 1978 and came into force the following June. It included a new system of government for the CSIC with the participation of collegiate bodies as advisory organs, and responsibility for the economic and scientific fields through the Scientific Council and Economic Council. This made real change in the CSIC a possibility. As with all the Spanish organisations during the time of the autocratic regime, CSIC had been conducted without any clear intent. However, the councils suffered many difficulties during their first months, due to a lack of scientific policy. The absence of any sign of a specific scientific policy has prevented a rational reorganisation of CSIC.

The reorganisation attempted by the CSIC so far has been mainly bureaucratic and administrative and is thus running the risk of becoming neither lively, dynamic nor relevant. However the Scientific Council of CSIC has made imaginative efforts to amend its organisation. A document on the general trends of scientific policy in CSIC has been elaborated, which considers as fundamental that the activity of CSIC be adjusted to the needs of Spanish society and to the manpower and material resources of CSIC itself. The budget for 1979 allows 100 pta. (less than US $2) per day per research worker for expenses of nonrecoverable material — and this represents a significant improvement over 1978!

Within the limited possibilities offered, CSIC has strived to overcome one of its main criticisms — its isolation from other research institutions. It is stressing agreements with universities and research institutions with the ultimate goal of resolving the existing academic power struggles. The Scientific Council of CSIC is trying to cooperate with the new Ministry of Universities and Research and recently asked for an interview with the minister with the aim of establishing contacts. □

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news in brief

Five French unions oppose CNRS reorganisation: Plans by the Giscard d'Estaing government to reorganise the Centre National de la Recherche Scientifique, France's national research council, have been met by opposition from five of the trade unions involved, according to recent reports in *Le Monde*. Mr Guy Hermier, a deputy from the Bouches-du-Rhône constituency, vice president of the national assembly and a member of the political bureau of the Communist Party, has called for a national debate on the future of the CNRS to be preceded by expensive consultations with the scientific community. In a letter to the Prime Minister, Hermier accused the government of giving up control of research to industry.

The reorganisation plan proposes a reduction in numbers of representatives on national CNRS committees, the addition of representatives from industry and the exclusion of technicians and administrators who are currently represented. The streamlining of the council and the involvement of representatives from outside the inner circles of the CNRS is seen by the government as an important move to decentralise research and to make results more accessible to the needs of French industry.

BSSRS criticises HSE on MMMFs: The Work Hazards Group of the British Society for Social Responsibility in Science has questioned a recent UK report on the hazards of mineral fibres used for insulation (19 July page 183). Although welcoming the tripartite (industry-trade union-government) report from the UK Health and Safety Executive which warns that "it would be prudent to regard these fibres with suspicion", the Work Hazards Group raises "considerable" objections to some of its conclusions. In particular the report is criticised for underplaying the risks to the domestic householder, 'minimising the risk of bronchitis due to high dust levels and inadequate dust-exposure and emission controls. Citing the known dangers of respirable dusts and fibres below 3.5 microns in diameter to the air sacs of the lungs, the Hazards Group criticises the classification of mineral dusts as nuisance dusts. "We know from asbestos that the small fibres cause pneumoniconiosis and mesotheliomas" says Dave Hayes of the Hazards Group. "And all mineral insulation materials contain these respirable fibres." More generally, the group is critical of the Department of Energy for promoting mineral fibres for the do-it-yourself home insulation market without "due regard for the hazards involved".

The Work Hazards Group recommends a sharp cutback in the production of small fibres (which it says are unneccesary for insulation), strict controls on home and industrial use of dust-causing insulation materials, notification to home owners that a "good" respirator should be used and that trade unions should keep their own medical records for workers at risk. Recommended substitute materials are insulation board, vermiculite and cellulose newsprint used with a good respirator. *Mineral Fibres. British Society for Social Responsibility in Science, 9 Poland St, London W1, 13p.*

Three Mile Island citizens panel dissolved: The shortest governmental advisory committee in many years was dissolved on 25 July without holding a single meeting. Four weeks ago, the Kemeny Commission which is investigating the accident at the Three Mile Island nuclear plant in Pennsylvania, appointed a nine member citizens advisory board to give it some "outside input". The Commission, apparently, got more than it bargained for when the panel demanded copies of all staff memoes, draft reports and work plans, access to all federal and industry documents subpoenaed by the Commission and depositions taken by the legal staff. In addition the panel demanded the opportunity to review lists of witnesses, to demand additional witnesses and to criticise and review the final report before it went to the White House. The Commission had envisaged the citizens committee taking a more passive role by answering nine questions put to it. "They wanted to be inside and we felt that was unacceptable" said Barbara Jorgenson, press officer of the Commission. At a meeting on 5 August the Commission voted to circulate the questions to the groups represented by the original nine panel members and to solicit suggestions and answers from an additional 200 public interest groups.

Life or death of an observatory: The Centre for Geodynamics and Astronomy, near Grasse in Southern France, was planned to co-ordinate all observational activities in the fields of positional astronomy and geophysics. The first interferometric star observations were obtained last year using a 30m baseline Michelson interferometer. But only 6.5 km away, a quarry and stone-crushing operation has created dust that will diffuse the night light from the coastal towns which will make very faint objects completely invisible. The large Schmidt optical telescope, installed last year, now might not be able to observe more than just a few percent of the interesting stars. Another problem is vibration from quarrying activities which disturbs high precision optical and long-baseline interferometric measurements. Appeals to the local authorities have failed and the CERGA has petitioned the appropriate ministry. But will the case be taken up in time, before the site is permanently ruined, by government bureaucracy?

From the staff of la Recherche



USSR higher education shows sharp regional discrepancies: A working note issued by the German Rectors Conference shows that higher education in the Western regions of the USSR continues to outstrip that in the other republics. The western republics lead in higher education with 200 students per 10,000 inhabitants. This is followed by the Baltic republics with 185, the Caucasian republics with 175 and the central Asian republics with 159.

The rate of increase of higher education also favours the western region with the number of students increasing relative to the population by a factor of 2.58 since 1966 while the Caucasian republics brought up the rear with an increase of only 1.06. The Rectors conclude that the discrepancies are small "considering that the Central Asian Republics can be regarded as developing areas." *CRE-INFORMATION No. 46. Standing Conference of Rectors and Vice-Chancellors of the European Universities.*

Solar activist to head research institute: The US Department of Energy has appointed Denis Hayes, a prominent leader of the environmentalist lobby and currently a senior research fellow at the Worldwatch Institute in Washington, as new director of the Solar Energy Research Institute (SERI) in Golden, Colorado. Hayes, one of the main organisers of last year's international sunday, will at the age of 34, become the youngest director of a national research laboratory. He succeeds the current director, Dr Paul Rappaport, who has headed the institute since it opened , and who will remain at the institute as distinguished scientist.

"My goal is to make SERI the most exciting and effective energy research organisation in the world," Hayes said last week. "The institute has a staff of first-rate analysts already thinking through the strategies and research requirements. Now its time to get those programmes moving".

Yoshimura has to leave Britain: Dr Tetz Yoshimura, the Japanese mathematician, has lost his fight to stay in Britain. He left for the continent earlier this week, a few hours ahead of a deportation order. Dr Yoshimura told *Nature* that he hopes to wait in "a country with less stringent immigration laws" for an invitation to a new academic appointment "somewhere in the third world".



'Always on tap, never on top'

Government scientists this week called off their industrial action after settlement of their prolonged pay dispute. **Joe Schwartz** reports on the scientists' attitudes towards the strike whose success took both union and management by surprise

"WE'VE ALWAYS been known as a non-militant union. I guess the Civil Service Department thought we would stay that way", declared a local committee member during the strike by the Institution of Professional Civil Servants. The 104,000 members of the IPCS amazed the public and fellow trade unionist with five weeks of action to back up their pay claim against the government.

To outsiders, the scientists and technologists in the UK's Civil Service are unlikely candidates for militant industrial action. Their union, one of seven in the Civil Service, only affiliated to the Trades Union Congress in 1976 by a vote of 57,000 to 38,000; the minority argued that trade union affiliation was "unprofessional". But strength of feeling built up through a series of skirmishes with their Civil Service bosses dating back to 1974. One member voiced the general feeling: "People think that civil servants are advisers to ministers. We're not. We're ordinary scientists and technologists who are sick and tired of being taken for a ride. Whenever there is money to be saved, the government tries to take it from the scientists".

With this growth of feeling, the only question earlier this year was how the IPCS's 25-member elected national executive and its full-time office staff of 30 would organise themselves for the annual pay talks with the Civil Service Department in April. "In fact, we organised very poorly," says Bill Brett, assistant general secretary of IPCS. Brett, a trade union organiser for 15 years in four different unions (including six years with Clive Jenkins of the Association of Scientific Technical and Managerial Staffs), expected that it would take a dramatic move on the part of the CSD to galvanise the membership. The CSD obliged. They refused to recognise a promise given the previous year that scientists' pay rises would be linked to civil servants' in the administrative grades. After granting 25%-45% to the administrators, the CSD offered only 18% to the scientists.

Scientists and technologists saw it as the culmination of what some alleged was a campaign in the CSD to downgrade the scientists. Tired of being "always on tap and never on top", they met in conference and instructed their leadership to organise industrial action.

The union drew up a battle plan of half-day strikes, working to rule, one-day demonstrations and the critical selective strikes that were to bring work in many government laboratories and defence establishments to a halt. The plan was put to the members at a series of meetings throughout the country. "We spoke to 50,000 members at about 25 evening meetings of from 500-2,000 people over a period of two weeks", says Brett. "We received an overwhelming mandate to go ahead."

For scientists at the Central Veterinary Laboratory in Weybridge, the strike call gave them the chance to use the experience gained two years earlier when an "informational picket" turned into a fully-fledged strike in support of a claim for an outer London pay allowance that had been granted to neighbouring laboratories.

The Weybridge laboratory does research on animal diseases. It is responsible for reviewing the quality and effectiveness of commercially produced drugs and it operates a P4 containment facility for diagnostic work on rabies. Work at the laboratory was brought to a halt when the local IPCS committee pulled out two of its supervisors from the laboratory's steam room. Members of the Transport and General Workers Union refused to work without supervision, and the laboratory's five high pressure boilers were shut down, cutting off heating, autoclaves and distilled water facilities. Eight more members were called out and a picket line set up which lorry drivers and post office workers refused to cross, thus halting the delivery of supplies and samples to the



Digging in: Striking scientists at the Laboratory of the Government Chemist in London combined three weeks of picketing with some voluntary community work, clearing up allotments for old people. Leaving one person on the picket line, the strikers kept themselves busy on a daily work brigade.

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laboratory.

The picket was not total, however. IPCS members decided they would give exemptions in cases of safety and public health. Thus, at Weybridge, operation of the P4 containment facility was guaranteed, as were deliveries of liquid nitrogen to maintain the laboratory's reference cultures of viruses, parasites and bacteria. Applications for exemptions went through the elected local committees; doubtful cases were passed on to the union executive committee.

At the Laboratory of the Government Chemist in London, a management request to allow gas deliveries through was refused. "All they wanted to do was to continue as usual. There was no question of safety", explained Arnold Pinder, secretary of the LGC section. Chemists at the LGC found that they too tried at first to carry on as usual. "Your first inclination is to keep working. You don't really want to strike and you look for ways to get around it. We had no chemicals, no water and no service. So we looked for other things to do", says Pinder.

A significant feature of the IPCS strike was the solidarity shown within the heterogeneous membership. Scientists stayed out on strike for three further weeks in support of the technologists after their own pay claim was settled. Again the Civil Service Department appears to have underestimated its adversary. "It was so obvious", says an LGC member. "First they split off the union with the least militant reputation and the most split membership. Then they tried to divide the scientists from the technologists, and then they tried to settle with the younger scientists at the expense of senior staff by offering the first two steps of the rise only." The tactic unified rather than divided the membership. "The CSD did at a stroke what the IPCS has been trying to do for years."

The IPCS action achieved a number of specific results and one important general result. The scientists are to set the same pay rise as their administrative colleagues this year. The technologists, however, feel they have a basic principal to settle about their status and comparability. This is now to go to a conciliation procedure.

But most importantly, IPCS members have realised that they can exercise effective industrial muscle. It has surprised both union and management. Researchers are reluctant to strike and have difficulty in seeing how withdrawal of their labour can be effective. Scientists in the IPCS have learned how to do it. This will change the complexion of all future pay negotiations. As Arnold Pinder put it: "We don't like to strike. But no one will listen to you unless you strike. You have to show your power before they'll listen." □

Ireland, land of priests . . . and engineers

Robin McKie compares the high status of the Irish engineer with the low status of engineers in Britain

THE realisation that Britain's manufacturing industry is far behind our competitors in terms of flexibility, sophistication and output has been forced upon the nation in the wake of its decline as a major commercial and financial force. And certainly any understanding of the problems which afflict the country's technological capabilities requires some form of detailed comparison with the performances of our closest competitors.

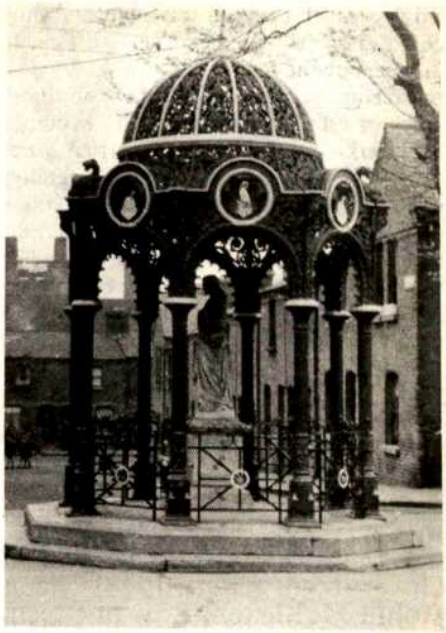
Indeed, in the case of our nearest English-speaking neighbours, the Irish, the contrast is striking — and decidedly unflattering. Last year the gross national product of Ireland expanded by 5.5%, almost twice the European average; manufacturing investment increased by 20% and manufactured exports rose by more than 20%. This year, industrial output is expected to rise by 11% — compared with 2.25% in the UK! This has been achieved by attracting a vast amount of foreign investment, mainly by permitting companies to make tax-free export profits until 1990.

It may allow large amounts of money to slip out of Ireland, but it has also succeeded in attracting more than 750 manufacturers — including major microelectronics, drug and oil companies — in the past 20 years, produced fixed assests worth £1500 million and in 1977 alone saw the creation of 24,000 new jobs.

In a sense, these measures were forced upon Ireland — a nation which has never been rich or prosperous. However, it is hoped that eventually the resulting financial regeneration and accompanying technological infrastructures will help transform the country into a major independent industrial force. And certainly "the Irish miracle" represents a remarkable achievement and indicates the country's critical recognition of the importance of high technology.

This last factor reflects the influence of scientists and engineers in Ireland and their importance within industry and government. In Britain, the engineer and technologist is all too often relegated to a minor, supervisory role inside a company, while his Irish counterpart wallows in high status and recognition.

As Professor P Leahy, dean of engineering at University College Dublin, put it: "Over here, an engineer is looked upon as someone like a doctor or a priest".



Religious shrine in Dublin — also a city of developing industry

And a more striking illustration is provided by the fact that the chief executives of Ireland's broadcasting corporation, transport system, electricity board and many of its investment banks were all originally engineers.

This obvious high achievement rate has had the effect of encouraging the very best students to take up engineering. For instance, in 1977, 15% of entrants to University College Dublin's engineering school had scored 30 or more points in the national school leaving examinations of Ireland. This compared with 7% of those going into medicine, 0.72% into law, 0.41% into science and 0.19% into arts.

This is a remarkable contrast to Britain where often the lowest qualified students form the largest proportion of engineering students. For instance in the period 1973-75, a total of 42% of students taking engineering at universities had worse than the equivalent of three Cs at A level. In science the proportion was 34%, in medicine 26%, and in social, administration and business studies, only 24%.

For the Irish, this concentration of talent has resulted in engineering schools becoming the prime recruiting grounds for chartered accountancy firms and investment banks who sell employment as a major route into management. (Indeed, many of the academic staff at UCD's faculty of commerce also have engineering first degrees.) And in this way an appreciation of technological matters becomes embedded in the fabric of Irish management and industry.

However, the reason for the original high status of engineers in Ireland is less easily defined, although it is closely connected with the industrial growth of the country and related educational routes.

In Britain, a rapid industrial revolution

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led to a quick demand for engineers who could not be supplied in sufficient numbers by the few existing university departments. So most rose from shop floor workers and craftsmen to become engineers — a process that carried little prestige. But in Ireland — which generally escaped the rigours of industrial revolution — most engineers were civil engineers, with university degrees, who provided roads, bridges and other services for the community and carried a corresponding high status.

Indeed the pervading nature of the differing prestiges of Irish and British engineers is explained by the fact that in 1958, 95% of those achieving engineering status in Ireland did so through university degrees. In Britain, the proportion was still only 39%, the rest achieving chartered status via part-time exams set by the professional engineering institutions. Only in recent years has Britain approached Irish levels for university educated engineers. For instance, in 1974, the figure had reached 90% in the UK, and 93% in Ireland.

Thus a picture can be seen of an original, well-established prestige for engineers in Ireland, leading constantly to better students and eventually to an even higher status. And certainly a thorough confidence in its engineers and technologists, combined with its recent industrial re-birth, indicates a strong financial future for Ireland.

It is then all the more vital to have the right quality of technical manpower to sustain this industrial growth. As Matt O'Donovan, of the Institute of Engineers of Ireland, stated: "It is important that we are training not just for existing industry but to initiate new industry".

It is this last capability that is particularly absent in Britain at present and which must be restored through the proposals of the Finniston committee of inquiry into the UK's manufacturing industry. However it is hard to see how it can find a way to cut through effectively the damaging spiral of engineers' low status, producing poorer levels of student intakes and eventual graduates, and resulting in even lower status for engineers. □

How industry lost an enthusiastic engineer

Robin McKie tells how one technologist's enthusiasm and ambition turned to disillusionment

For Barry Francis, engineering was his only choice of career since the age of nine. It seemed an obvious selection for a youth fired by an early enthusiasm for constructing crystal radio sets and who eventually ended up building hi-fi systems as a hobby.

So after he took O and A levels at Goring Hall, Sussex, he entered Middlesex Polytechnic for his main engineering course and later transferred to Manchester University for a postgraduate diploma in semiconductor electronics. Then in 1969, Barry moved to industry where he began work as a research engineer for Hawker Siddeley at Hatfield.

And almost immediately disillusion set in. "I discovered rather quickly that I would not be pushing back the boundaries of discovery every week. Instead I found myself working on projects that would not come to fruition for 10 to 15 years", he said.

Although the work — designing special instrument displays for vertical take-off jets — provided reasonably fulfilling employment in a limited fashion, it was also clear that the aircraft construction industry was far from healthy. So, with no immediate job satisfaction and no obvious career path, Barry left Hawker Siddeley after only 18 months and moved into mainstream engineering.

At Control Systems, Uxbridge, he was appointed a senior research engineer jointly in charge of a project team of 15 technicians, programmers and engineers who were designing a small business computer to control payroll, invoicing and other office chores for companies of up to 100 employees.

Sadly, although the work again was reasonably fulfilling, disillusion quickly set in once more. A particular problem lay with the firm's controlling company which eventually seemed to lose interest in the project and the team found itself increasingly pushed lower and lower on lists of priorities.

However, more importantly, Barry believes that although the project was a resonably modest undertaking, it required more in terms of human resources than the company could then provide.

These experiences, plus involvement as a writer for engineering journals, led 34-year-old Barry to the view that a major flaw in UK industry is its amateurishness. This is not the particular fault of its exponents but there can be little doubt, he feels, that British scientists, technologists and engineers have a poor feeling for industry.

And a major portion of the blame must lie with UK universities and polytechnics. "My own education did not in any way prepare me for industry's requirements. There was no design, management or marketing training — and these are crucial for you cannot just build a piece of equipment in isolation without knowing if there is a need or a sales potential for it. Really, at university and college, we were just being prepared as glorified technicians".

Generally Britain can be seen as a nation that cobbles things together to produce a solution under pressure. Often budget constraints can help to find more effective production methods but all too frequently initiatives are lost to other countries who are capable of more adventurous and stylish marketing and design systems.

After his two depressing experiences in inudstry, Barry left to be a writer and associate editor for various engineering magazines before later moving on to his present occupation as a public relations consultant. And from his story it is hard to avoid the view that British industry, because of its very make-up, lost someone of ambition and drive who is now unlikely to return. "In a sense I cannot move back at the moment without having to make a big financial sacrifice. In fact, if I went into industry again, I reckon my income would be halved."

This view has often been endorsed by various studies of engineers' conditions in industry. For instance, Peter Lawrence, of Southampton University's engineering department, recently produced a paper which showed that salaries in the UK were greatly below the levels of those in Germany. In 1976, the average salary for an engineer in the UK was £5510 while in Germany it was £12,119 — and at that time the cost of living was only 36.4% higher than in Britain.

Similar discrepencies in awards exist in most other European countries, including Ireland. As Professor Scanlon, head of University College Dublin's electrical engineering department put it: "I can give more to a PhD student in Ireland than industry gives as a salary in the UK".

Of course poor salaries reflect the low status of engineers in Britain — a view backed by Barry Francis. "Not enough bright kids are going into engineering", he said. "They see lawyers and doctors living well and decide to follow them.

"However once you have put this right and have raised the prestige of engineers, the bright children will start coming in, and then we'll eventually get better British products and an improved industrial performance".

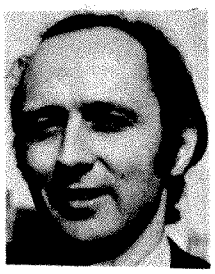
But even if conditions were improved, there would still be difficulties in attracting the return of former engineers such as Barry Francis. All too often, these people find their conditions and trainings badly out of date and discover there is no system for updating engineers in recent technological developments. Proposals — such as one-month university courses — are now being considered by the Finniston committee and the Science Research Council is also set to launch a trial project on these lines.

Only when a total package of proposals on these lines is implemented can Britain then hope to attract the talent and ability needed to restore its technological and industrial strength. □

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Sadly missed: a key contributor to science policy



Nigel Forman MP mourns the passing of the House of Commons Select Committee on Science and Technology

IT IS not just those of us that had the good fortune to serve on the Select Committee on Science and Technology who regret its demise. There must be many others outside the House of Commons who recognise the valuable contribution which it made to a wider understanding within Parliament of certain aspects of science and technology and to the quality of some government decision-making.

Since its establishment in January 1967, the Select Committee investigated a wide spectrum of subjects, ranging from nuclear power to coastal pollution and from carbon fibres to computers. It was chaired and guided by two distinguished politicians, Arthur Palmer from 1967-70 and again from 1974-79 and the late Airey Neave from 1970-74. There was also a sensible degree of continuity in its relatively small but enthusiastic membership.

It is hard to say exactly how much influence was wielded by the committee, because government departments are not in the habit of revealing to the public the various stages of their decision-making procedures and in our constituion ministers claim sole responsibility for the decisions which are actually taken. However, the record shows that the committee's 1969 report on the UK nuclear power industry which proposed the establishment of an Atomic Energy Board, was eventually followed by the Atomic Energy Authority Act 1971; that the 1971 report on population policy which called for the establishment in government of a special population office, was followed by the creation of the Population Panel; and that the 1974 report on energy conservation was eventually followed in December 1977 by a substantial, if belated, package of energy conservation measures which included some of the recommendations made by the committee.

In other cases, although the government of the day initially reacted in a defensive, even hostile, way to some of the committee's recommendations, subsequent events proved them right or led government to reconsider its earlier policy response. One example is provided by the way in which the last government's initially negative response to the committee's advocacy of alternative sources of energy in its 1977 report was eventually transformed into a more positive view of the need at least to explore the technical possibilities in this area and to do so with the support of adequate R&D funding. Another example is the way in which the Departments of Environment and Transport eventually came to accept the need for the Planning and Transport Research Advisory Committee to include in its terms of reference and its annual report a specific account of R&D work on advanced ground transport, something which had been rejected by government in its initial response of August 1974.

As for the work in progress at the time of the committee's demise, it is impossible to tell yet whether or not the uncompleted investigations into recombinant DNA research and developments in small passenger car engine technology will be pursued by the relevant "departmental" Select Committees which are to take its place. With any luck this will happen and a great deal of useful evidence will not have been wasted. Both these investigations exemplify the tendency of the Committee latterly to balance its work on short-term subjects — such as filament and discharge lamps or the lessons of the Eleni V disaster — with more important long-term work on emerging technologies for the future which may have significant benefits and costs for our society in the 1980s and beyond.

Now that the committee is no longer in existence, it is possible to form a clearer assessment of just what will be missed by Parliament, the press and the general public. Members of the House of Commons will miss the opportunities which the committee provided for consideration of important scientific and technological issues which by dint of their scope and complexity, are bound to transcend departmental boundaries. Recombinant DNA research and the wide-ranging implications of innovation, R&D in Japanese science-based industry are two typical and important examples.

Journalists will miss the platform which the committee provided for the public airing of important questions which are not otherwise debated with any frequency or in any depth in the normal proceedings of Parliament, whether in the Chamber or in legislative Standing Committees. The general public will be deprived of a useful Parliamentary mechanism for encouraging at least some of their elected representatives to look beyond the inevitably short-term horizons of contemporary politics. This last consequence is perhaps the most severe blow, since it is now more important than ever to preserve and, if necessary, create institutional mechanisms which allow arguments about social time preference and encourage the broadest possible cost-benefit analysis to be undertaken across the whole spectrum of contemporary science and technology.

Of course, in many ways the influence of the committee will live on through its *alumni*, like Neil Macfarlane, the junior minister with responsibility for science, who now find themselves in influential positions in government, and through its example in seeking to tackle a wide range of scientific and technological issues which will not go away just because it no longer exists. Furthermore, it is not as if there are not other laudable institutions of these issues. There is the well-established Parliamentary and Scientific Committee which has played a valuable role since its inception soon after the Second World War. There is also the Council for Science and Society which deserves to find continued support for the useful work which it does in attempting to bring scientists and laymen into a state of greater mutual understanding.

However, the fact remains that the peculiar dual role of the Select Committee as an educator of Members of Parliament and an investigator of issues which would not otherwise be the subject of Parliamentary scrutiny, will be difficult to ascribe to any one of the "departmental" Select Committees within the new system recently endorsed by the House of Commons. It must, therefore, become an important objective of those concerned with these matters in the new Parliament to meet this requirement by other means. □

Select committee considers GMAG

THE final report of the Select Committee on Science and Technology, released last week, is the result of the committee's recent investigations into the "public safety and public policy" issues of recombinant DNA.

In a brief summing-up of the evidence taken, the report expresses concern at the way in which the Genetic Manipulation Advisory Group handles notifications of industry's intentions to work with recombinant DNA. Under present arrangements, members of GMAG with potential industrial interests and those who have not signed a confidentiality agreement, withdraw from discussion of notifications by industry. The Select Committee disapproves that an advisory body "set up to safeguard both workers and the general public, should have apparently first and second class members". The report also expresses "surprise" that the Department of Education and Science (DES), not the Department of Health and Social Security (DHSS) is responsible for GMAG. It is concerned that the strong position of UK researchers and companies should not be hampered by unnecessary regulation. International safety standards also "appear to be desirable", it says.

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correspondence

Mongolian science

SIR, — In connection with the interview with me, by Vera Rich in *Nature* (28 June, page 754) I would like to ask you to publish the following explanation.

When Vera Rich visited me in Warsaw in October 1978, she was astonished to learn from me that there is a Museum and Palaeontological Laboratory in Ulan Bator, with a group of competent Palaeontologists, some of a very high standard.

I was shocked when I read what she published in *Nature* some 9 months later — an article with ironical remarks about the scientific relationships between the CMEA countries (Comecon in Vera Rich's wording) and about Soviet science; and containing misleading information, for example that R. Barsbold's artile (28 June, page 792) is based on materials from the Polish-Mongolian expeditions, while in fact it describes the specimens collected by the Soviet-Mongolian expeditions.

The unacceptable side of her article is the perjorative and condescending tone in which she writes about Mongolian science and scientists. As her article is written in a form of an interview with me, it makes me look disloyal to the people with whom I have been co-operating for years. I have never described my Mongolian colleagues in a derogatory way. What I have done was the opposite, but this apparently did not fit Vera Rich's ideas about Mongolia.

As a result of this interview co-operation between Polish and Mongolian palaeontology may be impaired. Is this really the aim of *Nature's* policy?

Yours faithfully,

ZOFIA KIELAN-JAWOROWSKA

Academy of Sciences Institute of Palaeobiology, Warsaw, Poland.

Readers concerned that we may have seriously slighted Mongolian scientists are urged to read Vera Rich's original article. Ed.

Alpha-fetoprotein screening on a US regional basis

SIR — The future of pregnancy alpha-fetoprotein (AFP) serum screening in the United States (5 July, page 6) is dependent upon decisions from the political as well as the scientific communities. Intense pressure has been applied to the Food and Drug Administration (FDA) to restrict test reagent kit licensure to carefully monitored regional programme. Few such programmes currently exist, due to lack of financial support from major funding sources such as the Department of Health, Education and Welfare, thus placing the FDA in a difficult position.

A number of private and commercial laboratories in this country have produced their own reagents and now offer the AFP test for pregnancy. Such testing is legal and is not covered by FDA restrictions. In the absence of coordinated action by federal funding and licensing agents such testing will proliferate and its quality will be unpredicatable. Equally important, the ability to interpret and follow up properly on test results will be generally poor, especially in the absence of an integrated patient service programme. These issues have been extensively discussed at the 1977 and 1978 Scarborough Conferences.

Pilot programmes in Nassau County, New York and in Maine have demonstratred beyond doubt that AFP testing in pregnancy can be carried out successfully on a regional basis within the structure of the US health care system. There is no question that such testing, properly applied, represents a major step forward in the identification and management of a variety of high-risk pregnancies, including major malformations (e.g. anencephaly, spina bifida, omphalocele, congenital nephrosis), twins, molar pregnancies, missed abortions, fetal demise, and pregnancies at high risk for premature delivery. If the full potential for this testing is to be realized, it must be actively supported and carefully executed.

Towards that end, plans are being made for a third Scarborough Conference to be held in June 1980, and emphasis is to be placed on the process of regionalisation. We hope that government agencies will have taken purposeful and positive action by that time; representatives from that sector will be invited to participate.

Yours faithfully,

JAMES E. HADDOW
EDWARD M. KLOZA

Foundation for Blood Research, Scarborough, Maine, US.

Recombinant DNA and induced tumours

SIR, — In the editorial (7 June, page 461), in which you call on the US Congress to act on recombinant DNA legislation (in itself a questionable intervention by *Nature*), you base your recommendation, in part, on risk experiments which "have indicated hitherto unanticipated potential hazards (such as the ability of DNA strands to induce tumours in laboratory animals)". Since the "risk experiments" presumably referred to were from our laboratories, we would like to comment.

As part of the polyoma risk assessment experiment (*Science* **203**, 883 and 887; 1979), which was designed to monitor the possible transfer of potentially infectious DNA out of *E. coli* and into susceptible mammalian cells, we inoculated baby hamsters with polyoma virus DNA (Israel *et al.*, *J. Virol.* **29**, 990; 1979) and with polyoma DNA contained in recombinant molecules (Israel *et al.*, *Science*, in the press). In brief, the two types of polyoma DNA showed comparable oncogenicity; when contained in the *E. coli* K-12 host, which was the experimental system, the recombinant DNA did not induce any tumours.

First, it seems misleading to state that "DNA strands" can induce tumours, without the qualification that you are referring to DNA from a tumour virus. Second, this finding would have been "unanticipated" only by persons who both know little or nothing of tumour virology, and who did not even read the material. Tumourigenesis and cell transformation by DNAs from various tumour viruses, including polyoma virus, have been reported many times over the past 19 years; these findings are widely known, and are well referenced in our paper in the *Journal of Virology*, as well as in the publications cited there.

Since the example cited by *Nature* has no substance, we must wonder if there are any "unanticipated potential hazards" at all. Indeed, the 31 May issue (page 360) quotes one of us as saying "I do not know of a single piece of new data that has indicated that K-12 recombinant DNA research [meaning those experiments not prohibited by the US guidelines] could generate a biohazard." This remains our view, despite *Nature's* careless assertion to the contrary.

Major recombinant DNA policy recommendations based on uncritically accepted misstatements made by uninformed persons are only too familiar, but one would expect *Nature* to employ higher standards.

Yours faithfully,

WALLACE P. ROWE
MALCOLM A. MARTIN

National Institute of Allergy and Infectious Diseases, Maryland, US.

Preparing oral rehydration salt solutions

SIR, — Even if packets of oral renydration salts are available (29 March, page 389), are illiterate village people capable of preparing the correct concentration of solution? If not, then they must be helped — in high concentrations the salts could be hazardous and in low concentrations they are useless. The ingredients, brown sugar, sugar, salt and sodium bicarbonate are available in most rural areas; but the 'pinch', spoon and drinking glasses are so variable that they cannot be reliably used for measuring.

The Indonesian spoon mentioned in the article is suitable for measuring the solids, but how is the fluid measured? The recommended beer bottle is not available in Muslim countries. I would, therefore, like to suggest that the UNICEF oralyte packet should contain a plastic bag, with a marked narrow neck like a bottle and instructions for measuring the fluid, and that the relevant agencies distribute cheap half-litre drinking glasses in remote villages where the UNICEF packets are not available. Three indentations should be made on the bottom of the glass: one for measuring each of sugar, salt and sodium bicarbonate. The level of the water required should be marked on the glass and instructions together with the limitations of oral rehydration should be engraved on it in local language.

The glasses should be distributed free or at a subsidised cost. They will usually be used for drinking water.

Yours faithfully,

MOSLEMUDDIN KHAN

International Centre for Diarrhoeal Disease Research, Bangladesh.

Erratum

In Norman Dombey's article (26 July, page 270) the sentence "So in a fast reactor of the design envisaged in CFR1 or Superphenix . . . there will be no overall negative Doppler coefficient" should conclude" . . . there will be an overall negative Doppler coefficient".

news and views

Hypercycles and the origin of life

from John Maynard Smith

PERHAPS the most difficult step to explain in the origin of life is that from the replication of molecules (RNA for example) in the absence of specific proteins, to the appearance of polymerases and other proteins involved in the replication of RNA and themselves coded for by that RNA. Suppose we start with a population of replicating RNA molecules. Without specific enzymes the accuracy of replication is low and hence the length of RNA which could be precisely replicated small. Before replication can be reasonably accurate, there must as a minimum be a specific polymerase, as well as synthetases and tRNAs, which in turn implies an RNA genome of considerable length. Thus, even if one supposes an initially very limited set of codons, one cannot have accurate replication without a length of RNA, say, 2,000 or more base pairs, and one cannot have that much RNA without accurate replication. This is the central problem discussed in a series of papers by Manfred Eigen and Peter Schuster (*Naturwissenschaften* **64**, 541; 1977; **65**, 7; 1978; **65**, 341; 1978), proposing the 'hypercycle' as a necessary intermediate stage.

First, imagine a population of replicating RNA molecules, lacking genetic recombination, but with a certain 'error' or 'mutation' rate per base replication. Very roughly, if more than one mutation occurred per molecular replication, the population would come to consist of a random collection of sequences. But if less than one mutation occurred per replication, and if one sequence was 'fitter' than others in the sense of being more stable and/or more easily replicated, then a population would arise whose sequences were centred around this optimal one. Such a population Eigen and Schuster call a molecular 'quasi-species'. If the mutation rate per replication was much less than one, and if fitness differences were large, most molecules would have the optimal sequence. As the mutation rate increased, the proportion of the population with the optimal sequence would fall, until a critical mutation rate was reached, above which sequences would be random.

The replication of RNA molecules in the absence of specific replicases has not yet been achieved in the laboratory; it may initially have depended on the presence of nonspecific random sequence polypeptides. At this stage, the error rate would depend solely on the energy levels of base pairing between G and C, and between A and U, and would be such that the maximum length of a quasi-species would be 10–100 base pairs; even this length would require the RNA to be G–C rich. In contrast, RNA replication by the enzyme coded for by the RNA phage Qβ is accurate enough to permit a genome of 1,000-10,000 base pairs, which would be sufficient to code for a primitive protein-synthesising machinery. The next stage in the evolution of accuracy of replication, achieved by prokaryotes, was the recognition and correction of mismatches in DNA replication, a process which depends on recognising which is the old strand and which the new. With such proof-correcting, a genome of at least 10^6 base pairs, and perhaps 10^8 - 10^9 base pairs, can be maintained. It is the gap between the 10–100 bases, without specific enzymes, and the 1,000–10,000 bases, with specific enzymes, that Eigen and Schuster aim to bridge.

To understand their proposal, imagine that you wish to replicate the message GOD SAVE THE QUEEN. Counting 5 bits per letter, this is a total of 75 bits, and a maximum of 25 bits per word. Supposed the error rate was, say, 1/50 per bit. You start with a population of messages, copy each of them with that error rate, and then discard half of the population, applying a rule of selection so that messages with many errors are more likely to be discarded. Despite this 'natural selection', the population would steadily accumulate errors. The mutation rate is too high.

Suppose, therefore, that after each replication you selected word by word, discarding half the quasi-species GOD, and similarly for each other word. Since no word contains as many as 50 bits, you could now maintain a meaningful message. However, there is an important reason why this is *not* an adequate model of the replication of a set of RNA molecules. Thus, to maintain complete messages you would have to ensure in each generation that you selected equal numbers (or approximately so) of each word. But suppose the words competed, as RNA molecules would compete for substrates. Then before long only one quasi-species

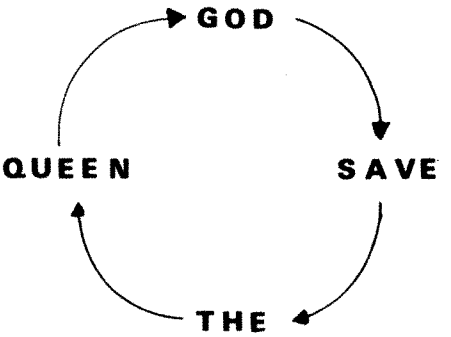


Fig. 1

Hence if the words are strung together into a single selected message (analogous to a single RNA molecule), the message is too long to replicate; if they are replicated separately, competition between the words destroys the message. You can escape from this dilemma by arranging the words in a 'hypercycle', as in Fig. 1. In this cycle, the *rate* (not the accuracy) of replication of SAVE is increased by the number of GODs, of THE by the number of SAVEs, of QUEEN by the number of THEs, and, coming full circle, of GOD by the number of QUEENs. Each word, or quasi-species, is selected independently, according to its own fitness. This is a hypercycle. It can be shown that only by linking together a set of replicating quasi-species in this cyclical way can the stability of the whole be maintained.

A possible way in which a simple 'two-word' hypercycle might be realised in molecular terms is shown in Fig. 2. (Eigen and Schuster would not insist on this particular realisation; it is intended only to show the kind of thing they have in mind). It is necessary to describe a cycle in some detail, so as to be able to discuss the difficulty which arises in explaining the further evolution of such cycles. I_1 and I_2 would remain — GOD only, or THE only, if short words replicate faster.
are two RNA quasi-species. Both the + and — strands must be good replicators. RNA molecules have a phenotype, because they fold up, and folding patterns affect both survival (resistance to hydrolysis) and replication rate. Since both + and - strands must replicate, they are likely to have similar folding patterns. Further, I_1 and I_2 may be descendants of the same ancestral quasi-species, in which case they will resemble one another.

In this particular realisation, the

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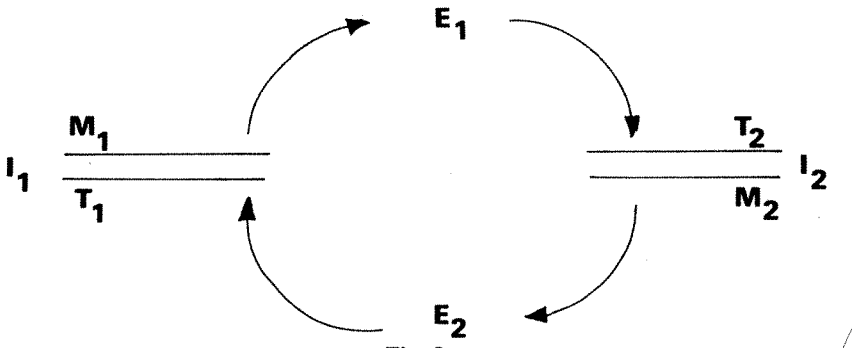


Fig. 2

— strands T_1 and T_2 are supposed to be 'adapters' or precursors of tRNA, coupling with specific amino acids, and having an anticodon loop. The + strands M_1 and M_2 are 'messengers', composed of only two kinds of codon, and coding for two proteins E_1 and E_2 which act as replicases for I_2 and I_1 respectively. Thus the model assumes that some kind of translation is possible without synthetases or ribosomes, and that the code is arbitrary from the start (that is, that there is no chemical constraint on which triplet codes for which amino acid). It is not an essential feature of the argument that messenger and transfer molecules be the + and — strands of the same quasi-species, but if they were not, then a hypercycle of more than two elements would be needed.

One difficulty in conceiving how such a cycle might arise is that it requires that each of two 'messengers' (or of more than two in the case of a longer cycle) should programme a replicase for the other: Eigen and Schuster argue that this becomes less implausible if it is remembered that I_1 and I_2 may be descended from members of the same molecular quasi-species. Initially, the members of a quasi-species would be sufficiently alike to share the same replicase. Hypercyclic organisation could then arise by the gradual differentiation of a single quasi-species into two or more.

I now digress to give the authors' views on the origin of the code. They argue that primitive RNA molecules must have been G–C rich (for reasons already mentioned). Further, in the absence of ribosomes the message must have been one which could only be read 'in frame' (Crick *et al. Origins of Life*, 7, 389; 1976). These two conditions imply that the earliest messages consisted of strings either of GNC codons or CNG codons, where N stands for any base. The authors use an argument based on wobble to prefer the former codon type. Hence, from arguments based on the stability, replication and translation of nucleic acids, they conclude that the first two codons were GGC and GCC, followed by GAC and GUC. This conclusion agrees nicely with the likely abundances of amino acids in the primitive oceans. By far the most abundant amino acids in simulated prebiotic synthesis (reviewed by Miller and Orgel, *The Origins of Life on Earth*, Prentice-Hall, 1973) are glycine and alanine (today coded by GGC and GCC), the next commonest being aspartic acid (GAC) and valine (GUC).

Returning to the main theme, the following question arises. Given that a hypercycle ensures the accurate replication of a larger total quantity of information, how will it evolve further? Consider the particular realisation in Fig. 2. There are three kinds of mutation which might occur in I_1 (and a similar set in I_2):

(1) Mutations making I_1 better (or worse) at replicating, for example by becoming a better target for E_2. (I_1 might also become a better target for E_1; if this process went too far the hypercycle would be disrupted).

(2) Mutations making E_1 a better (or worse) replicator of I_2.

(3) Mutations making T_1 a better adapter.

If there is not compartmentalisation, only mutations of type (1) would be incorporated by selection. Each quasi-species in the hypercycle would evolve independently. There would be no selection favouring mutations of types (2) and (3), although such mutations would be needed before the speed and accuracy of replication would improve sufficiently to permit the genetic information to be united in a single 'chromosome'. There is a natural analogy here to an ecosystem. Imagine an ecosystem consisting of grass, antelopes and lions. The more grass there is, the more rapid is the multiplication of antelopes. Similarly, antelopes encourage the multiplication of lions, and (by some stretch of the imagination) lions, by fertilising the ground, encourage the growth of grass. The point of the analogy is this. Natural selection will favour mutations in grass which increase the fitness of individual grass plants, but will not favour changes in grass making it more edible to antelopes.

How then can a hypercycle evolve characteristics which favour the growth of the cycle as a whole, rather than merely its constituent parts? So long as there is no compartmentalisation, it cannot. For natural selection to act, there must be individuals. In the present context, this seems to require that the compartments of a hypercycle be enclosed in a membrane to form a proto-cell. If these proto-cells grew and divided – by some kind of budding or fission – at a rate which increased with the growth rate of the enclosed hypercycle, then selection would favour mutations making the hypercycle more efficient. In the example of Fig. 2, mutations of types (2) and (3) would be favoured.

Clearly, these papers raise more problems than they solve. Their merit is that they put in sharp terms the problem raised by the relatively inaccurate replication of nucleic acids, and, in the hypercycle, they suggest a way in which a relatively complex structure could be replicated despite this inaccuracy. □

More twists and turns to recombination

from a Correspondent

CURRENT understanding of the molecular basis of homologous DNA recombination has evolved from analyses of the progeny of fungal and bacterial genetical crosses, the isolation of mutants deficient in some or all of the steps, electron microscopy of DNA intermediates generated *in vivo* or *in vitro* and most recently from biochemical studies of the proteins involved acting on specific substrates. The integration and transposition of IS elements, transposons and bacteriophage Mu into bacterial chromosomes has also stimulated interest in non-homologous recombination. The five EMBO recombination workshops have reflected this evolution and the latest one contained a fascinating mixture*.

Genetical analysis of fungal tetrads and octads provided support for the Meselson–Radding recombination model, which proposes initiation by invasion of a single DNA strand from one duplex into a second one, resulting in asymmetrical hybrid DNA. Symmetrical hybrid DNA can then be generated by the Siegel–Alberts isomerisation. The pattern of gene conversion and postmeiotic segregation at the b_2 locus of *Ascobolus* is consistent with asymmetrical initiation of recombination from a preferred site outside the locus followed by a transition to a symmetrical structure, with the probability of repair of mismatched bases in the hybrid DNA decreasing with increasing distance from the initiation site (J. L. Rossignol, Université de Paris-Sud, Orsay). The positions of strand crossing-over and hybrid DNA during recombination at the *arg4* locus of yeast is, however, contrary to the model's predictions and evidence for symmetrical hybrid in yeast is lacking (S. Fogel, University of California, Berkeley). Although the direction of correction can be random in fungi, there was agreement that correction to the invading strand information is strongly preferred in yeast (Fogel; P. Hastings, University of

*The fifth EMBO Workshop on DNA Recombination, was held at Nethybridge, Scotland from 25-30 June, 1979.

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Edmonton, Edmonton) and in *Sordaria*, correction may influence the likelihood of nearby cross-overs (H. Sang, Harvard University).

Repair of mismatched bases when artificial heteroduplex λ DNA molecules transfect wild-type hosts was explored (M. Radman, Université Libre de Bruxelles, Brussels). Correction occurs on the unmethylated strand, an observation of importance for correction of non-complementary bases inserted during DNA replication when parental and nascent strands are differentially methylated. During replication in *dam* mutants (adenine methylation blocked), correction can occur on both strands, perhaps leading to overlapping recombinogenic excision tracts, explaining the hyper-Rec phenotype of these mutants. Transfection of the heteroduplexes into mutator *mut* H, L and S hosts results in the loss of preferential correction. Furthermore, λ crosses under non-replicating conditions revealed that crossing-over between widely spaced markers was similar in wild-type and *mut*S hosts, whereas mismatch repair in the λ *P* gene was reduced 10-fold in the latter host, suggesting that mismatch repair is specifically blocked in these mutator strains.

Chi mutations in λ confer large plaque size on *red*$^-\gamma^-$ mutants of λ infecting *rec*A proficient hosts and also stimulate recombination in their vicinity in the λ genome. They also occur naturally in λ, *Escherichia coli* and yeast. It was suggested that the basis of their strong directionality of action might lie in the directional packaging of λ DNA and that during attempted packaging, they might resolve recombination intermediates, leading to exclusion of one of the possible products. From limited sequence data, the number of nucleotides common to all chi sequences is not yet certain. It could be as high as 23, which would not be expected to occur randomly at the frequency observed in yeast, suggesting the exciting possibility that they might be universal recombinators (F. Stahl, University of Oregon, Eugene).

The development of *in vitro* recombination systems, retarded by the lack of a versatile assay, makes the purification and characterisation of the proteins involved possible. A rapid filter-binding assay has resolved several peaks of DNA circle fusion activity after DEAE chromatography of *E. coli* extracts and one has been purified further. It is, however, present in equal amounts in both *rec*A$^-$ and wild-type extracts and so its relationship to *rec*A dependent recombination *in vivo* is obscure (D. Dressler, Harvard University). A different approach assays T7 DNA recombination *in vitro* and two pathways have been delineated. In one, purified gene 6 exonuclease catalyses recombination between two DNA duplexes or between one duplex and single strands of another. The renaturing activity of the T7 single-stranded DNA binding protein is also required (P. Sadowski, University of Toronto).

The *rec*A protein of *E. coli* is the subject of intense study because the cloning of the gene enables large amounts of the protein to be purified. It is a DNA-dependent ATPase, unwinds DNA and catalyses the insertion of homologous linear single-stranded DNA into superhelical or linear duplex DNA to form D-loops, a putative early stage in recombination (C. Radding, Yale University). On the basis of studies using ATP and a non-hydrolysable analogue, the mechanism proposed was ATP-dependent binding to homologous single strands (without ATP hydrolysis), the resulting complex unwinding the duplex DNA and annealing the single strands into the duplex, accompanied by ATP hydrolysis. Perhaps similar success will follow the cloning of the *rec*F gene (A. J. Clarke, University of California, Berkeley). Recombination promoted by the λ *int* gene, which ensures the integration of λ DNA into a specific site in the host chromosome, can also be accomplished *in vitro*. The purified *int* protein has now been shown to be a topoisomerase (H. Nash, National Institutes of Health, Bethesda). It resembles eukaryotic topoisomerases and does not require attachment sites in its substrate for this activity, but its preferred binding to the phage attachment site and left attachment site may focus its activity at the specific region needed for λ integration and excision. Thus, whereas it was envisaged that recombination would require several enzymes acting sequentially, the biochemistry suggests that rather simple, but multi-functional ones can accomplish at least some initial events on their own.

The integration and transposition of IS sequences, transposons and bacteriophage Mu involves specialised or non-homologous recombination, and transposition is non-reciprocal because they are inserted into a recipient without their loss from the donor. Thus DNA synthesis is an integral part of transposition. Partial deletions in Tn*3* were used to analyse transposon functions required for transposition (D. Sherratt, University of Sussex). Some deletions blocked transposition at an intermediate stage where a Tn*3*-containing plasmid was physically linked to a recipient plasmid. In some cases such intermediates were resolved by the host *rec*A system which was, however, less efficient than the transposon's own system. A sequence within 250 base pairs of a gene coding for a 19,000 molecular weight protein was also mapped which is required in *cis* to resolve cointegrates or Tn*3* dimers in a plasmid. A search for *E.coli* mutants reducing IS*1*-mediated deletion formation in the *gal* operon revealed a trans-acting locus named *del* (P. Nevers, University of Freiburg). From further studies, it was concluded that *del*$^+$ might be a natural IS*1* sequence linked to a strong promotor acting in trans to boost IS*1* functions at a second insertion site in the *gal* operon.

Bacteriophage Mu shares many properties with transposons, such as a requirement for replication to transfer Mu from one site to another (A. Bukhari, Cold Spring Habor Laboratory, New York). Also Mu can transpose from one plasmid to another equally efficiently in *rec*A$^+$ and *rec*A$^-$ hosts, but in the latter, cointegrates are detected which can be resolved by the *rec*A$^+$ system. Two Mu deletion mutants also form cointegrates, but the one with deleted early genes is unable to transpose into the host chromosome (D. Leach, University of Sussex). When Mu is induced, the *G* region of about 50% of the progeny is inverted, preventing growth of Mu on *E. coli*. This puzzle has been resolved because this loss of infectivity is matched by a gain in ability to infect other bacteria. *G* inversion apparently activates different *S* genes, coding for host specific phage tail fibres, by way of a promotor at the left of *G*. Inversion also increased read-through of a flanking gene *mom* which antagonises host-specific modification of Mu DNA so that phage with a new host range are less likely to have their DNA restricted.

Transposon-like sequences may also exist in yeast. The *his*4-912 mutation maps outside structural genes, is polar but is not a nonsense, frameshift or deletion and revertants are unstable and cause chromosomal rearrangements. Restriction fragments containing the region from *his*4-912, a revertant and wild-type decrease in length in that order, the revertant being longer than the wild-type by 250 base pairs, a sequence found in many yeast restriction fragments. It was suggested that *his*4-912 contains a transposable element in the promotor which is excised in the revertant leaving a 250 base pair terminal repeat (G. Roeder, University of Toronto). The 'Cassette Model' for control of the mating type in yeast is also consistent with a transposition-like mechanism. Cloning of the α and A mating type alleles indicates that the former is 200 base pairs longer, but the degree of internal homology between them remains to be determined (A. Klar, Cold Spring Harbor Laboratory).

The possible involvement of recombination in carcinogenesis was proposed by Radman and A. Kinsella. TPA, a strong promotor of carcinogenesis, causes a large increase in sister chromatid exchanges in cultured CHO cells. They propose that carcinogenesis is initiated by mutations which remain silent because of the presence of intact DNA on the homologous chromosome. If recombination occurs, perhaps stimulated by TPA, there is an opportunity for the loss of the wild-type gene, permitting expression of the carcinogenic mutation. Their proposal was strengthened by the finding that the anti-tumour agent antipain, prevents sister chromatid exchange. □

Structure and control of phosphorylase

from C.C.F. Blake

IT is perhaps not really surprising that the promise that X-ray crystallography can make a vital contribution to the understanding of enzyme regulation, by being able to define the various structural states through which the activities of control enzymes are regulated, is taking a long time to be completely fulfilled. The enzymes by their very nature are large, their conformational states can be various, and can differ from one another to such an extent that a number of crystal forms may have to be analysed separately (if they can be obtained at all). Of the four regulatory enzymes whose X-ray study is well advanced: glycogen phosphorylase, hexokinase, phosphofructokinase and glyceraldehyde phosphate dehydrogenase (all members of the glycolytic pathway), phosphorylase is under the most elaborate control. This suggests that the analysis of its structural and regulatory properties now under way by Fletterick and Madsen in Edmonton, and Johnson in Oxford will be especially valuable in providing information on the regulation of metabolism.

Glycogen phosphorylase is, of course, the classic example of hormonal control of a metabolic enzyme through the mediation of the adenyl cyclase system. Phosphorylase *b* is converted to phosphorylase *a* by the phosphorylation of Ser 14 by a specific kinase; a reaction that is reversed by a specific phosphatase. This hormonal regulation is linked to further control by the levels of a number of metabolites, including AMP, ATP and glucose-6-phosphate. Phosphorylase *b* requires AMP for activity and is allosterically inhibited by ATP and glucose-6-phosphate. In resting muscle the levels of these metabolites are such that this form of the enzyme is inactive. The conversion of the *b*-form to the *a*-form results in an enzyme that no longer requires AMP for activity and is not subject to allosteric inhibition. However, it is strongly inhibited by glucose, for which it is regarded as a receptor in the liver, and which promotes the conversion of the *a*-form to the *b*-form by the phosphatase. In addition phosphorylase is in intimate physical association with the glycogen particles, and this association also seems to affect the enzymic activity.

X-ray studies are being carried out on crystals of phosphorylase *b* grown in the presence of its activator IMP at 3 Å resolution (Johnson *et al. Proc. 11th FEBS Mtg.* **42**, 185; 1978; Weber *et al. Nature*, **274**, 288; 1978) and of phosphorylase *a* in the presence of its inhibitor glucose at 3 Å resolution (Fletterick *et al. J. biol. Chem.* **251**, 6142; 1976) which have now been extended to 2.5 Å resolution (Sprang & Fletterick, *J. molec. Biol.* **131**, *523;* 1979). It was extremely valuable that the complete sequence of the 841 residues of rabbit muscle phosphorylase was determined by Neurath's group (Titani *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 4762; 1977) at the time when the electron density maps were being interpreted. The 97,400 molecular weight subunit of phosphorylase has its polypeptide chain organised largely into alternating α- and β- structures, like all the other glycolytic enzymes whose structures are known. The folding of the polypeptide chain divides the large subunit into three domains: an N-terminal domain of about 320 residues, a central domain of 160 residues, and a C-terminal domain of about 360 residues. These three domains are located at the apices of a roughly equilateral triangle giving the subunit a compact shape. The interface of the functional dimers in the crystals is formed by the N-terminal domains and includes two large projections, known as the 'tower' and the 'cap' that extend beyond the molecular two-fold symmetry axis to make extensive contacts with the other subunit. A most striking feature of the subunit is the location of the active site at the bottom of a deep, narrow cavity near the centre of the subunit at the point where the three domains come together. The pyridoxal phosphate, whose presence was so long a mystery, is bound as a Schiff's base to Lys 679 close to the active site in apparent confirmation of proposals that it is directly involved in the catalytic process. In phosphorylase *a*, the glucose inhibitor is bound in the active site.

Rather surprisingly the crystals of phosphorylase *a* and *b* are closely isomorphous, suggesting no large structural difference between them. Unfortunately no detailed comparisons of the two structures have yet been made, but it seems that a major difference lies in the N-terminal region which in the *b*-form is disordered, but becomes ordered in the *a*-form because the phosphate group attached to Ser 14 makes an ionic interaction with Arg 69 located on the N-terminal helix. Soaking experiments with substrates, activators and inhibitors have revealed further binding sites (Kasvisky *et al. J. biol. Chem.* **253**, 1290, 3343; 1978; Johnson *et al.* unpublished). The allosteric activator site lies on the N-terminal domain at the subunit interface. AMP and ATP located in this site make contact with residues from both subunits. Maltotriose and maltoheptose bind at a site on the central domain, which has consequently been termed the glycogen storage domain, but do not seem to bind at the active site. Finally, AMP, ATP and the inhibitors caffeine and inosine, bind near the active site in a position that probably marks the allosteric inhibitor site.

Despite the success in locating these five ligand binding sites, the only evidence for associated conformational change has been obtained from crystals of phosphorylase *a* in the presence of the virtual substrate, 5-thio-α-D-glucopyranosyl phosphate, and maltoheptose (Madsen *et al. J. biol. Chem.* **253**, 9097; 1978). To prevent the disintegration of the crystals that usually accompanies attempts to bind substrate, they were lightly cross-linked with glutaraldehyde and allowed to anneal after the addition of the substrate analogue. Difference maps at low resolution revealed that the segments in the N-terminal half of the molecule had undergone conformational change, possibly representative of the R → T transition. The most intriguing aspect of these conformational changes is that they appear to link together the five major ligand binding sites. These structural changes seem to bear obvious relationships to the known homotropic and heterotropic cooperativities of the enzyme.

The work on phosphorylase is an exemplar of X-ray studies on enzymes, regulatory or not, both in its successes and difficulties. As is usual the structural results are clear, and revealing. The division of the molecule into three domains of specialised function is characteristic, as is the location of the active site at the triple domain interface. The domain/subunit geometry lays the groundplan for regulation by defining, broadly, the structural pathways of communication between the various control sites and the active sites. However, as is also usual, the definition of function, that is how the communication actually works, seems more problematic even though in this case some low resolution results have been obtained with phosphorylase *a*. This is because the structural changes induced by ligand binding may be partly, or wholly, inhibited by the crystal lattice. Ideally separate structural analyses of crystals representing each stable structural state are required, but that may not be possible because certain states may not crystallise. Function is always the most difficult aspect of X-ray studies, and one that requires much further effort. □

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Erratum

IN the article 'The Oceanography of Fjords (*News and Views* **280**, 273; 1979) the figure legend is incorrect. The upper figure represents the laboratory simulation and the lower the tidally-generated flow in a fjord, and not as stated.

Theoretical difficulties with the binary pulsar?

from Malcolm MacCallum

THE agreement of the rate of change of period of the binary pulsar with the usual quadrupole formula for gravitational radiation, reported by J. H. Taylor, L. A. Fowler, and P. M. McCulloch (*Nature* **277**, 437; 1979), has been hailed as a triumph for general relativity. Unfortunately it has been known to specialists for some time that the theoretical predictions are themselves open to question.

J. Ehlers, A. Rosenblum, J. N. Goldberg and P. Havas drew attention to the unsatisfactory nature of all existing derivations of the formulae in an article in the *Astrophysical Journal* in 1976 (**208**, L77). Concerning these arguments, Taylor *et al.* remark that "our data suggest that any such inaccuracy is not very large", but there is of course the more intriguing possibility that the observations disagree with the correct general-relativistic formula. Indeed, what some specialists fear most is that the usual formula will prove correct to better than 10%, with the consequence that any future objections to non-rigorous arguments in relativistic astrophysics and cosmology will be dismissed as merely pedantic quibbles.

In deriving the standard formula, L. D. Landau and E. M. Lifschitz (in *The Classical Theory of Fields* third edition, Pergamon, 1971) wrote "In principle, all the calculations are completely analogous to those which we carried out for electromagnetic waves." It is this analogy which has given the quadrupole formula its strong intuitive appeal to physicists, but it also emphasises the underlying reasons for some of the experts' doubts, namely that it uses an essentially linear approximation in a flat space-time background when the full theory is non-linear and uses curved spaces.

Since 1976 renewed efforts have been made to reach an agreed alternative to (or confirmation of ?) the usual formula, based on more sophisticated approximations, and the Gregynog meeting* provided an opportunity to review progress. Some of the most illuminating results reported arose from model calculations using scaler waves in one dimension (whose relevance is accentuated by a recent preprint from J. M. Stewart (University of Cambridge) showing that perturbations of algebraically special space-times — which include most of the physically interesting cases — can be represented in terms of scaler potentials).

At great distances from the sources, the field is usually assumed to propagate along flat space light rays and to be expandable in inverse powers of the radial distance r. Stewart, speaking about definitions of mass at infinity, reminded the audience that, however small the mass involved, light rays in the field of a spherical body (a Schwarzschild metric) would diverge logarithmically from those of the corresponding flat space. M. Walker (Max-Planck-Institute, Munich) reported an example in which logarithmic terms appear in the field in the far past.

One way of relating the field to the sources is through a retarded Green's function integral. In relativity, however, such retarded solutions are not simply related to natural choices of boundary condition, such as 'no incoming radiation in the far past' or 'only outgoing radiation in the far future'. Walker gave an example in which the retarded solution involved incoming radiation in the far past. B. Schmidt (Max-Planck-Institute, Munich) presented a string and spring example in which the amount of incoming radiation affected the damping rate, and speculated, amusingly, that the binary pulsar measurements might mean only that if general relativity is true, the pulsar is receiving no incoming radiation. Schutz proposed avoiding the boundary conditions by assuming that at an initial instant the field external to the system, being unknown, should be taken to be zero — the average of all possible values.

The dynamics of the source has to take account of the reaction from the field. Schutz showed that in his approach the usual radiation reaction expression acquired an extra term, which he related to the need to compensate for coordinate choice effects. More detailed examinations of the internal dynamics of the sources usually follow one of two approximation schemes.

The first of these is the 'fast motion' approximation. D. Christodoulou (Max-Planck-Institute, Munich) reported on some rigorous estimates of errors in this scheme. A. Rosenblum (Temple University, Philadelphia) reported that the well-known discrepant result of Havas and Goldberg (that two bodies in a binary system spiral away from, not towards, each other) was due to neglect of non-linear terms comparable with those retained, and presented his own scattering calculations, which gave about double the result predicted by the quadrupole formula. He hopes to extend these calculations to bound systems.

The second method is the 'slow motion' technique, which involves expansion in powers of a small parameter e, essentially the ratio of a characteristic velocity in the system to the velocity of light. The calculations introduce terms in er, and are therefore valid only in a limited region. The boundary conditions for the slow motion approximation should be supplied by an outer, wave zone, solution, which itself must use the light rays of the curved space, and not a flat space approximation. Both Dixon and J. L. Anderson (Stevens Institute, Hoboken, New Jersey) have carried out model calculations of this type, joining the inner and outer regions by the method of matched asymptotic expansions. This method was introduced to relativity by W.J. Burke (*J. Math. Phys.* **12**, 401; 1971), but has been familiar in other contexts for some time, and is even known to be rigorous in certain limited circumstances. Because their calculations differed in detail, Dixon and Anderson, although in broad agreement, emphasised different aspects of the results.

Dixon's model calculation showed, contrary to some expectations, that slow motion expansions can include effects arising from tail terms of a retarded Green's function (crudely, effects propagating at a speed less than that of light), and that radiative effects can appear in even as well as odd orders in the expansion. Both authors found that the matching introduced terms in the inner region logarithmic in e. Anderson noted that approximations good for one feature may be bad for another (in his example, the damping and phase respectively), though I understand that at the subsequent Dublin meeting ('Current Problems in General Relativity', 2-6 July, 1979) P. Parker (Syracuse University) suggested that this could be overcome using results by Hörmander (*Acta Math.* **127**, 79; 1971).

The problem of what to approximate recurred in a final discussion excellently led by Stewart. It was agreed that intermediate concepts like energy (awkward to define in the full theory) and radiation reaction force should be avoided if possible, and attention focused on directly measurable quantities (like the binary pulsar period changes). Care would be needed to avoid tailoring approximations to produce the 'right' results, so that as many observable effects as possible should be included.

Stewart concluded the meeting by announcing a competition for a model problem, with an unambiguous numerical solution, which could act as a testbed for the different approximation schemes. It should have as many features of the full problem as possible, for example, non-linear waves, retarded coupling to a source oscillator, and back-reaction on the source. Perhaps when such a problem has been found, and solved, we will know how to tackle the binary pulsar question. Then we can decide whether the observed behaviour presents difficulties for the theory, or whether the present doubts will prove only 'theoretical' in the worse sense.

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*The third Gregynog Relativity Workshop was held at Gregynog, Newtown, Wales, on 25-28 June, 1979, under the title 'Gravitational Radiation Theory'. The organisers were B. Schutz (University College, Cardiff), G. Dixon (Churchill College, Cambridge) and M. Walker (Max-Planck-Institute, Munich).

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Minding the psi's and q's

from Roger Cashmore

THE European Physical Society's International Conference on High Energy Physics* was held in Geneva this year — the 25th anniversary of CERN. Much of the data presented originated from accelerators within Europe, from the CERN SPS and ISR together with a substantial contribution from the new e^+e^- storage ring PETRA at DESY.

Much of the conference was devoted to reports on the physics of both the new quarks, (charm and bottom — c,b) and the old quarks (up, down and strange — u,d,s) as well as searches for the expected quark (top — t). First the charm quark. Within the charmonium model of the $c\bar{c}$ meson states, the claimed pseudoscalar states ($^1S_0$ states), the κ ($\sim$2830) and κ ($\sim$3450) have long been a problem. They possessed masses, widths and M1 electromagnetic transition rates in conflict with predictions from charmonium. In the crystal ball experiment at SPEAR, an experiment particularly designed to study the electromagnetic transitions from the ψ' and ψ mesons using high resolution NaI crystals, no evidence has been found for these states at the claimed mass values, although all of the other intermediate states have been observed (the $^3P_{0,1,2}$ states — the $\chi(3400)$, $\chi(3510)$ and $\chi(3550)$). Thus the embarrassing problem of having states of the grossly wrong properties has been removed but replaced by the lesser problem of the whereabouts of these mesons, vital in the $c\bar{c}$ picture. In a separate experiment at SPEAR, using the Mark II detector, data has been taken on the D^0 and $\bar{D}^0$ mesons at the ψ'' (3772), a copious source of these particles. With the high statistics obtained the first observations of the Cabbibo suppressed $D \to \pi\pi$ and $K\bar{K}$ decays have been made which are consistent with 6 quark extensions of the GIM model of incorporating new quarks into the weak and electromagnetic gauge theories.

Until this conference evidence for charmed baryons has been scant, the only observations being in neutrino experiments and one photoproduction experiment. Three experiments from the ISR reported the observation of narrow hadronic states consistent with the earlier observations. These states have appreciable diffractive production cross sections ($\sigma.B \sim 1\mu b$, where B is the decay mode branching fraction) while the more prominent decay mode of the lightest of these baryons, the Λ_c^+ ($\sim$2255) appears to be to $K^-p\pi^+$ although the $\Lambda^0\pi^+\pi^+\pi^-$ decay is observed. This conclusion is supported by evidence from SPEAR that the $p+\bar{p}$ yield increases more dramatically than the $\Lambda+\bar{\Lambda}$ yield in the vicinity of 5.0 GeV, the region where charmed baryon production is expected to occur.

*Held on 27 June-4 July.

Probably the most exciting prospect at the conference was the suggestion of a meson containing a bottom (b) quark. The b quark is expected to decay preferentially to a c quark and it has been surmised that this c quark might appear a fraction of the time within a ψ meson together with at least one 'strange' particle. A group (Indiana/Imperial College/Saclay/Southampton collaboration) studying ψ production in $\sim$150 GeV π^-p collisions at the CERN SPS looked at the $\psi K\pi$ final states and discovered an excess of events ($\sim$4 standard deviations) concentrated in a single 40 MeV bin at a mass of $\sim$5.3 GeV. From a knowledge of the Υ state, assumed to be a $b\bar{b}$ meson, the mass of a meson containing a bottom quark can be estimated and falls in this vicinity. Clearly further experimental results are eagerly awaited in the hope of confirming this exciting possibility.

Finally, an expected new quark, the t quark, has not yet been revealed in e^+e^- annihilation experiments at PETRA. Thus the mass of this quark must be greater than 13.5 GeV and means that the new accelerator must be stretched to its present limit of 16 GeV in the hope of discovering it.

Nearly all known mesons and baryons can be accommodated in models of hadrons in which mesons are $q\bar{q}$ states and baryons are qqq states, leading to the puzzle of the absence of resonances composed of greater numbers of quarks, for example $q\bar{q}q\bar{q}$ or $qqqq\bar{q}$. A few narrow structures observed in $\bar{p}p$ annihilations had been the best candidates for such states but unfortunately in a recent $\bar{p}p$ experiment at Brookhaven National Laboratory no evidence was found for the existence of one of the best of these candidates, the S(1935). Thus the puzzle remains as one of the most important questions of hadron physics.

The problem of permanently confining quarks within hadrons has apparently yet to be solved despite the tremendous amount of theoretical effort directed towards QCD (quantum chromodynamics — the theory of coloured quark and gluon interactions), a possible theory of the strong interaction. The experimental evidence for jets of particles, the remains of original quarks, was beautifully demonstrated in the new results from PETRA. However the evidence for the gluons which are essential in this picture remains tentative. Data presented on the decay of the Υ (9.45) is clearly best fitted with a model which describes its decay by way of three gluons, while in the high energy e^+e^- annihilation the evidence for a third jet (a gluon bremsstrahlung from one of the quarks) was tantalising. The existence of this effect together with a quantitative confirmation of the QCD predictions will only be obtained with the acquisition of much more data during the coming year. Results were also presented on the variation of the moments of the structure functions in deep inelastic νN and μN scattering, the 'classic' testing ground of QCD to date. Once again these were consistent with the predictions of QCD but fall short of a definitive proof, since other explanations are possible.

Results from all the high energy physics experiments studying the weak neutral current are now consistent with a value of $\sin^2\theta_w = 0.23$, the one parameter in the Weinberg-Salam model for a gauge theory of the weak and electromagnetic interactions. The only area in which there is any conflict with this value lies in atomic physics experiments studying parity violation in thallium and bismuth atoms. However here there are direct conflicts between the various experiments and a further sequence of experiments is clearly required before this situation is resolved.

There remains the unification of the weak, electromagnetic and strong interactions. The currently most popular models imply a 'desert' from the mass region of the W and Z intermediate bosons ($\sim$100 GeV) to the mass at which the various interactions have a similar strength — the grand unification mass ($\sim 10^{15}$ GeV). However A. Salam, in his summary, pointed out that this 'desert' was model-dependent and indeed other models would lead to a proliferation of particles in this region — only experiment will reveal the correct path. This represented a clear challenge to the experimental high energy physicists to understand better the properties of the particles of today and furthermore to achieve these higher energies as soon as possible with the accelerators of the future, the $\bar{p}p$ collider at CERN and, one hopes, LEP. □

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Naming names

IN a paper published in this issue of *Nature* (page 468) V. Bennett and P.J. Stenbuck describe how a red cell membrane protein is linked to the cytoskeletal spectrin through the membrane Band 2.1 protein. Bennett and Stenbuck have recently rechristened Band 2.1 protein ankyrin (Greek, anchor). But beware. The self-same protein has, by others, been rechristened nexin (Latin, to bind) and syndein (Greek, to bind). Perplexingly, the work that led to all three christenings was initiated in one laboratory, that of Professor D. Branton at Harvard.

This etymological discordance is hardly edifying. Perhaps the younger generation should devote less time to scouring dictionaries and reflect instead on other traditions. After all there was a time when the leader of an expedition could expect to have his (or even her) name enshrined in any new species discovered by juniors.

So, by way of revitalising an old tradition, I hereby propose that the names ankyrin, nexin and syndein be cast aside in favour of brantonin (from, if you must, the words branch — a subdivision — and ton — a large quantity). International Commissions on Nomenclature please note. **P.N.**

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review articles

Evidence for the quantum nature of light

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A unique property predicted by the quantum theory of light is the phenomenon of photon antibunching. Recent theoretical predictions and experimental observations of photon antibunching in resonance fluorescence from a two-level atom are reviewed.

THE advent of photon correlation experiments, as pioneered by Hanbury-Brown and Twist[1], began a new era in the field of optics. The experiments of classical optics involved a measurement of the first-order correlation function of the electromagnetic field and as such involved only the interference between the probability amplitudes of a single photon. Phenomena such as diffraction, Young's interference experiment and spectral measurements may be categorised as being in the domain of one photon or linear optics. Photon correlation experiments represent a fundamental deviation from these experiments as they involve the interference between different photons and as such are in the realm of nonlinear optics. The quantum-mechanical interpretation of the Hanbury-Brown Twiss effect was given by Glauber[2]. However, adequate explanations of the Hanbury-Brown Twiss effect and related photon correlation experiments have been given which invoke a classical description of a fluctuating electromagnetic field. However, Glauber[2] pointed out that photon correlation experiments offer the possibility of observing a uniquely quantum-mechanical effect—namely photon antibunching. We describe here the recent prediction by Carmichael and Walls[3] and observation by Kimble, Dagenais and Mandel[4–6] and G. Leuchs, M. Rateike and H. Walther (personal communication) of this unique quantum-mechanical effect in photon correlation experiments of fluorescent light from a two-level atom. This marks a dramatic change from all previous photon correlation experiments since the photon antibunching observed cannot be explained on the basis of a classical description of the radiation field. We begin with a brief outline of events leading up to the present investigations.

The quantum theory of light

The quantum theory of light beginning with Planck[7] and Einstein[8] played a central part in the development of quantum theory during this century. As the quantum theory was developed a sophisticated theory for the interaction of photons and electrons namely quantum electrodynamics evolved. Amongst the predictions of quantum electrodynamics was that the emission of light from an atom would experience a small shift away from the resonance line of the atom. The experimental observation of this shift, known as the Lamb shift, came as a major triumph for the quantum theory of light[9]. However, for many experiments in optics especially in the field of physical optics the classical picture of light as propagating waves of electromagnetic radiation was adequate to describe the observations. The idea developed that it was not necessary to quantise the light field but only necessary to quantise the atoms and thus describe their interaction by a semiclassical theory. Modifications of this concept such as neoclassical radiation theory also developed. These theories can explain phenomena such as the photoelectric effect, spontaneous emission and even give estimates of the Lamb shift. (A discussion on the present status of such theories as an alternative to quantum electrodynamics is given in ref. 10.) An early attempt to find quantum effects in a physical optics experiment was made by Taylor[11] who performed Young's interference experiment at very low intensities such that on the average only one photon was incident on the screen at a time. Integrating over a long detection time Taylor obtained an interference pattern as predicted by classical wave theory with no evidence of any unique quantum effects. Taylor's experiment may also be explained on the basis of the quantum theory which interprets it as the interference of the quantum-mechanical probability amplitudes for the photon to go through one slit or the other[12,13]. Essentially the experiments of physical optics were in the regime of one photon or linear optics. We need to go outside the experiments of one photon optics in order to detect any uniquely quantum-mechanical effects.

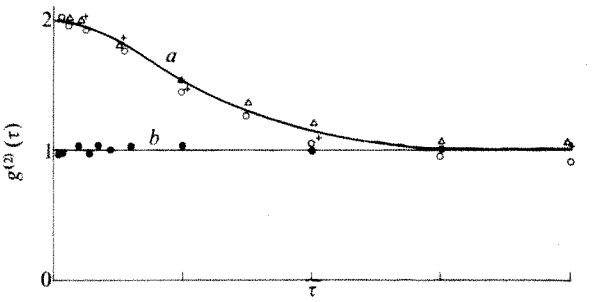


Fig. 1 Second order correlation function $g^{(2)}(\tau)$ for: *a*, thermal light; *b*, coherent light. Experimental points from Arrechi *et al.*[17].

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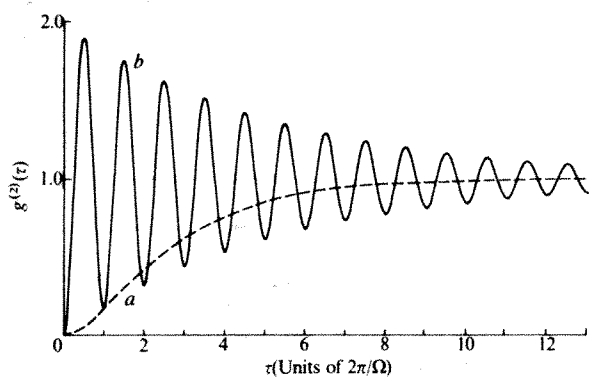


Fig. 2 Second order correlation function $g^{(2)}(\tau)$ for fluorescent light from a two level atom: *a*, low driving field intensity $\Omega \ll \gamma$; *b*, high driving field intensity $\Omega \gg \gamma$. Theoretical predictions from Carmichael and Walls[3].

Photon correlation experiments

The first experiment outside the domain of one photon optics was performed by Hanbury-Brown and Twiss in 1956. They performed an intensity correlation experiment, that is a measurement of $\langle I(t)I(t+\tau)\rangle$. Although the original experiment involved the analogue correlation of photocurrents, later experiments used photon counters and digital correlators[14–18]. In essence these experiments measure the joint photocount probability of detecting the arrival of a photon at time t and another photon at time $t+\tau$. The measurements made in this experiment may be described in terms of the second-order correlation function of the radiation field introduced by Glauber[2] in his formulation of optical coherence theory. This is defined as

$$g^{(2)}(\tau) = \frac{\langle E^{(-)}(t)E^{(-)}(t+\tau)E^{(+)}(t+\tau)E^{(+)}(t)\rangle}{(\langle E^{(-)}(t)E^{(+)}(t)\rangle)^2} \qquad (1)$$

where $E^{(+)}(t)$ and $E^{(-)}(t)$ are the positive and negative frequency components of the electromagnetic field respectively.

The result of a photon correlation measurement of $g^{(2)}(\tau)$ for a thermal light source using photomultipliers and digital correlators is shown in Fig. 1 curve *a*. We see that the joint counting rate for zero time delay is twice the coincidence rate for large time delays τ. That is, there is a tendency for the photons to arrive in pairs, or a photon-bunching effect. The decay time of the correlations is given by the inverse bandwidth of the light source.

If instead of a thermal light source the experiment is performed with a highly stabilised laser source one obtains a constant correlation function as shown in Fig. 1 curve *b*. This result holds even if the laser and thermal light source have the same bandwidth. Hence there is some fundamental difference between a laser and a thermal light source which may not be apparent in the first-order correlation function but which is manifest in the second-order correlation function. This effect may be understood by considering the photon statistics of different light fields.

Quantum theory of photon correlations

We shall attempt in this section to give a quantum theoretic interpretation of photon correlation experiments. For simplicity we shall concentrate on an interpretation of $g^{(2)}(0)$ which enables us to restrict our attention to a single mode field. For a single mode field we may write $g^{(2)}(0)$ in the form

$$g^{(2)}(0) = 1 + \frac{\sigma^2 - \bar{n}}{\bar{n}^2} \qquad (2)$$

where $\bar{n}$ and σ^2 are the mean and variance respectively of the photon number distribution. Thermal light has a power law photon number distribution with variance $\sigma^2 = \bar{n}^2 + \bar{n}$ which gives $g^{(2)}(0) = 2$. Coherent light on the other hand as produced by a highly stabilised laser has a poissonian photon number distribution[19–21] with $\sigma^2 = \bar{n}$ leading to $g^{(2)}(0) = 1$. This clearly yields the results shown in Fig. 1 for $g^{(2)}(0)$. To include the decay time of the correlation function one must consider many modes. For a field which has a photon number distribution narrower than a Poisson ($\sigma^2 < \bar{n}$) it is possible in principle to obtain a $g^{(2)}(0) < 1$. That is the photon coincidence rate for zero time delay is less than that for large time delays τ. This is the opposite effect to the photon bunching observed for thermal light and has been called photon antibunching. This represents a reduction in the photon number fluctuations below that of a poissonian distribution. The extreme case of photon antibunching would be a light beam where the photons arrive evenly spaced. For a single mode field this is equivalent to having a fixed photon number N which gives $g^{(2)}(0) = 1 - 1/N$.

Let us now consider a classical description of the electromagnetic field. We consider a field described by a fluctuating amplitude E. These fluctuations are taken into account by introducing a probability distribution $P(E)$ for the complex field amplitude. A calculation of $g^{(2)}(0)$ yields

$$g^{(2)}(0) - 1 = \frac{\int P(E)(|E|^2 - \langle|E|^2\rangle)^2 \, d^2E}{(\langle|E|^2\rangle)^2} \qquad (3)$$

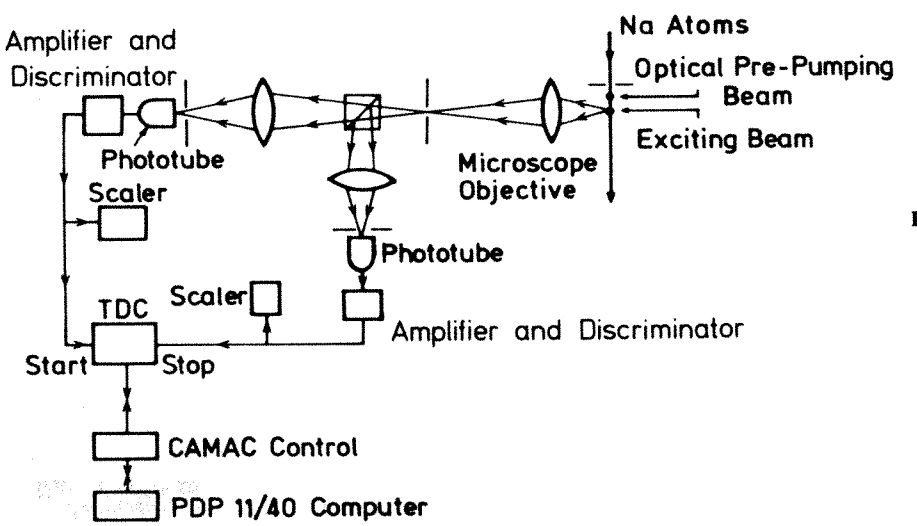


Fig. 3 Experimental arrangement used by Kimble *et al.*[4].

For a thermal field which has a gaussian distribution for $P(E)$ we recover $g^{(2)}(0)=2$ whereas for a coherent field with a stabilised amplitude $P(E)$ is a delta function and hence $g^{(2)}(0)=1$. Hence the Hanbury-Brown and Twiss effect for chaotic fields and indeed for the coherent laser field is adequately described by classical theory. However, since the right hand side of equation (3) is a positive semi-definite quantity, we see that classical theory requires $g^{(2)}(0)\geqslant 1$ and hence does not allow photon antibunching.

The formulation of the quantum theory closest in formal appearance to the classical theory involves the diagonal expansion of the density operator of the radiation field in terms of coherent states[2,22],

$$\rho=\int P(\mathscr{E})|\mathscr{E}\rangle\langle\mathscr{E}|\,\mathrm{d}^2\mathscr{E} \tag{4}$$

where the coherent states $|\mathscr{E}\rangle$ are eigenstates of the positive frequency part of the electromagnetic field

$$E^{(+)}|\mathscr{E}\rangle=\mathscr{E}|\mathscr{E}\rangle \tag{5}$$

with eigenvalue $\mathscr{E}$. The function $P(\mathscr{E})$ does not have the character of a probability distribution since for certain nonclassical states of the radiation field it may be negative or highly singular.

A calculation of $g^{(2)}(0)$ using the P representation yields

$$g^{(2)}(0)-1=\frac{\int P(\mathscr{E})(|\mathscr{E}|^2-\langle|\mathscr{E}|^2\rangle)^2 d^2\mathscr{E}}{\langle|\mathscr{E}|^2\rangle^2} \tag{6}$$

This appears similar in form to the classical expression of equation (3), however, since $P(\mathscr{E})$ may for certain fields which have no classical description take on negative values then $g^{(2)}(0)$ may be less than unity in quantum theory.

While this particular feature of quantum theory was recognised it remained to find a light field which exhibited the property of photon antibunching. It was suggested that certain processes of nonlinear optics for example sub-second harmonic generation[23–26] and two-photon absorption[27–33] would exhibit photon antibunching. To date there has been no experimental verification of these predictions. It was resonance fluorescence from a two-level atom that led to the first experimental observation of photon antibunching.

Resonance fluorescence from a two-level atom

The topic of resonance fluorescence from a two-level atom has been the subject of considerable theoretical and experimental investigation. For weak incident fields the light is coherently scattered, whereas for strong incident fields when the Rabi frequency Ω ($\Omega=2\kappa\mathscr{E}/h$ where $\mathscr{E}$ is the driving field amplitude and κ is the atomic dipole matrix element) exceeds the Einstein A coefficient γ of the atom the spectrum of the scattered light splits into three peaks with the two sidebands displaced from the central peak by the Rabi frequency. This spectrum first predicted by Mollow[34] was verified in detail by the experiments of Wu *et al.*[35] and Hartig *et al.*[36] following an earlier experiment of Schuda *et al.*[37]. These experiments used an atomic beam of sodium atoms which were optically pumped to prepare a pure two-level system. These atoms were then subjected to irradiation from a highly stabilised dye laser tuned to resonance with the atomic transition. This system was to prove of further interest to physicists.

Carmichael and Walls[3] predicted that the second-order correlation function of the light emitted by a single atom undergoing resonance fluorescence would exhibit the property of photon antibunching. This was confirmed in subsequent calculations by Cohen-Tannoudji[38] and Kimble and Mandel[39]. The results obtained by Carmichael and Walls for the second order correlation function $g^{(2)}(\tau)$ in the steady state are

$$g^{(2)}(\tau)=\left[1-\exp\left(\frac{-\gamma\tau}{2}\right)\right]^2 \quad \Omega\ll\gamma \tag{7}$$

$$g^{(2)}(\tau)=\left[1-\exp\left(\frac{-3\gamma}{4}\tau\right)\cos\Omega\tau\right] \quad \Omega\gg\gamma \tag{8}$$

in the limiting cases of very weak and very strong driving fields. The behaviour of $g^{(2)}(\tau)$ is plotted in Fig. 2. This system clearly displays the property of photon antibunching since in both limits $g^{(2)}(\tau)$ starts at zero. For low-intensity driving fields $g^{(2)}(\tau)$ rises monotonically to a background value of unity, whereas for high driving field intensities $g^{(2)}(\tau)$ rises above unity before reaching a steady state value of unity by damped oscillations.

This behaviour can be understood as follows. A measurement of $g^{(2)}(\tau)$ records the joint probability for the arrival of a photon at time $t=0$ and the arrival of a photon at time $t=\tau$. Consider now the driven two-level atom as our source of photons. The detection of a fluorescent photon prepares the atom in its ground state since it has just emitted this photon. The probability of seeing a second photon at $\tau=0$ is zero since the atom cannot re-radiate from the ground state. One must allow some time to elapse so that these may be a finite probability for the atom to be

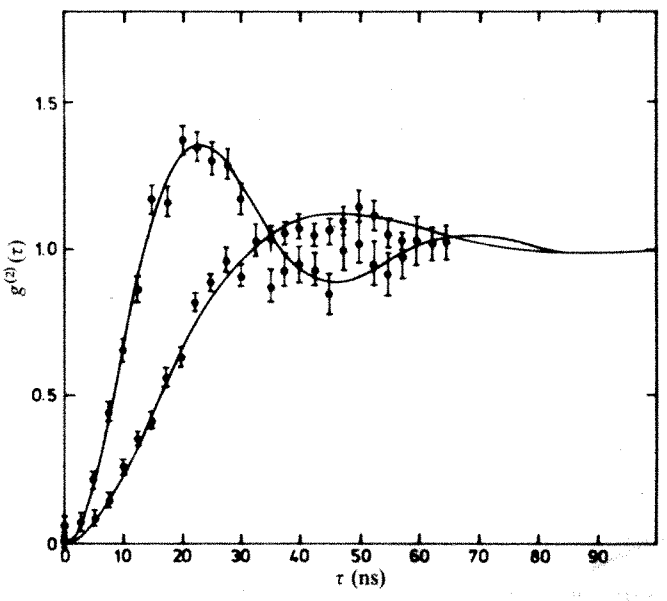


Fig. 4 Photon correlation measurements of fluorescent light from sodium. Experimental results obtained by Dagenais and Mandel compared with theory (solid line). ●, $\Omega/\gamma=2.2$; ○, $\Omega/\gamma=1.1$.

in the excited state and hence a finite probability for the emission of a second photon. In fact $g^{(2)}(\tau)$ is proportional to the probability that the atom will be in its excited state at time τ given that it was initially in the ground state.

Thus a single atom undergoing resonance fluorescence became a candidate for observing photon antibunching. However, the results (equations (7), (8)) hold only for resonance fluorescence from a single atom. If photons from many atoms contribute to the signal detected then one gets interference effects and the antibunching is diminished and for a large number of atoms lost entirely. In fact for a large number of independently contributing atoms one finds $g^{(2)}(\tau)=2$ in agreement with the central limit theorem[3].

It is clear therefore that an experiment of high sensitivity was necessary if measurements were to be made on the second order correlation function of light from a single atom. Such an experiment was performed by Kimble, Dagenais and Mandel[4]. A sketch of their experimental setup is shown in Fig. 3. In a similar configuration to the experiments which measured the spectrum they used an atomic beam of sodium atoms optically pumped to prepare a pure two-level system. The atomic beam was irradiated at right angles with a highly stabilised dye laser tuned on resonance with the $3P_{3/2}$, $F=3$, $M_F=2$ to $3^2S_{1/2}$, $F=2$, $M_F=2$ transition in sodium. The intensity of the atomic

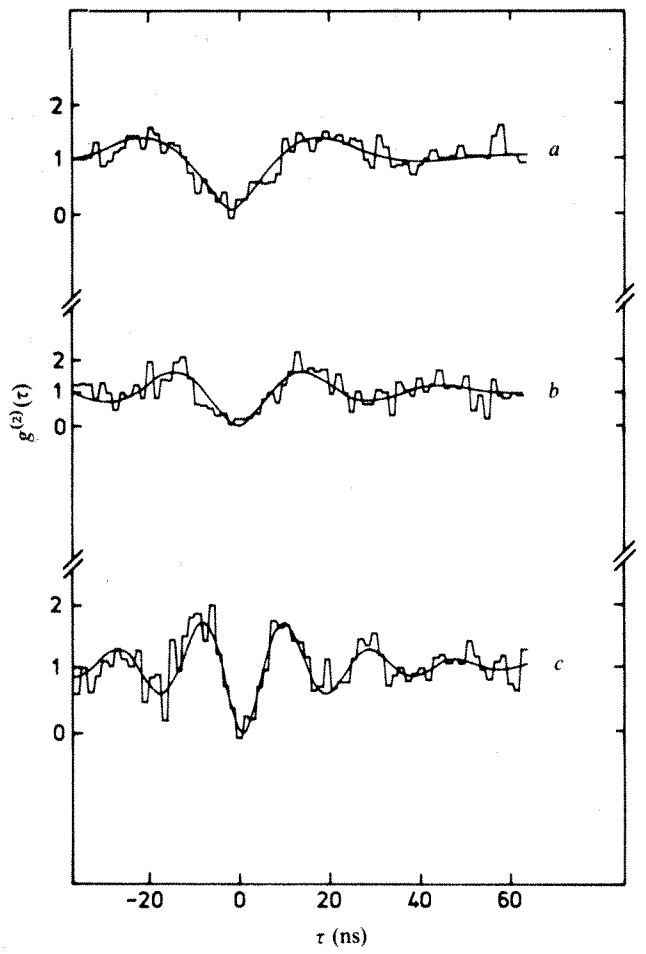


Fig. 5 Photon correlation measurements of fluorescent light from sodium. Experimental points adapted from the results of Leuchs *et al.* (personal communication) compared with theory. *a*, $\Omega/\gamma = 2.2$; *b*, $\Omega/\gamma = 4.3$; *c*, $\Omega/\gamma = 5.7$.

beam was reduced so that on the average no more than one atom is present in the observation region at a time. The fluorescent light from a small observation volume is observed in a direction orthogonal to both the atomic and laser beams.

The fluorescent light in this direction is divided into two equal parts by a beam splitter and the arrival of photons in each beam is detected by two photomultipliers. The pulses from the two detectors are fed to the start and stop inputs of a time to digital converter (TDC) where the time intervals τ between start and stop pulses are digitised in units of 0.5 ns and stored. The number of events $n(\tau)$ stored at address τ is therefore a measure of the joint photoelectric detection probability density which equals $\eta^2 g^{(2)}(\tau)$ where η is the detector efficiency. The initial results obtained by Kimble *et al.*[4] showed the initial positive slope of $g^{(2)}(\tau)$ characteristic of photon antibunching but starting with $g^{(2)}(0) = 1$ rather than zero.

The reason for this disagreement with the theory was pointed out by Jakeman *et al.*[40] who attributed it to number fluctuations in the atomic beam. Though the atomic beam density is such that on the average only one atom is in the observation volume during a correlation time, there are poissonian fluctuations about this mean value so that at times there could be two or more atoms in the observation volume. Thus in the atomic beam experiment one observes the photon antibunching superimposed on the poissonian number fluctuations of the atoms. A calculation including the atomic number fluctuations was carried out by Carmichael *et al.*[41] Similar calculations were made by Kimble *et al.*[5] who obtained very good agreement with experimental results. Some recent experimental results of Dagenais and Mandel[6] are shown in Fig. 4, where when the effects of atomic number fluctuations are taken into account excellent agreement is obtained with the theory. A photon correlation experiment on resonance fluorescence from a beam of sodium atoms has been recently performed by Leuchs *et al.* (personal communication). The results of this experiment are shown in Fig. 5, where with compensation for the atomic number fluctuations excellent agreement is found with the theory. These experiments show clear evidence for the existence of photon antibunching and thus verify the predictions of the quantum theory of light.

Discussion

It could still be argued that one has not observed light with the antibunching property but only inferred from the experimental observation that the fluorescent light from a single atom must possess the antibunching property. This is due to the finite correlation at $\tau = 0$ arising from the number fluctuations of the atoms. It would be useful, therefore, to consider ways of improving experimental methods. Recently advances have been made towards isolating a single atom in an ion trap[42]. This suggests the possibility of performing resonance fluorescence on a single stationary atom which should radiate light with the antibunching character. In addition there are the suggested systems in nonlinear optics for example, sub-second harmonic generation[23–26] and two-photon absorption[27–33] where photon antibunching has been predicted. These experiments involve the nonlinear coupling of electromagnetic waves through a medium which may be in the solid state thereby eliminating problems due to atomic number fluctuations.

Although minor refinements may be possible the experiments performed by Kimble *et al.*[4–6] and Leuchs *et al.* (personal communication) represent a fundamental contribution to our understanding of the nature of light. For the first time photon correlation experiments have detected an effect which is a direct manifestation of the quantum nature of light.

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Population biology of infectious diseases: Part II

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In the first part of this two-part article (Nature **280**, *361–367*), *mathematical models of directly transmitted microparasitic infections were developed, taking explicit account of the dynamics of the host population. The discussion is now extended to both microparasites (viruses, bacteria and protozoa) and macroparasites (helminths and arthropods), transmitted either directly or indirectly via one or more intermediate hosts. Consideration is given to the relation between the ecology and evolution of the transmission processes and the overall dynamics, and to the mechanisms that can produce cyclic patterns, or multiple stable states, in the levels of infection in the host population.*

IN the first part of this article[1] we considered the dynamics of microparasitic infections with direct transmission between hosts. We now extend the discussion to other kinds of parasites and transmission processes, and examine the general relations between population behaviour and parasite life cycle structure. The conclusions are broadly similar to those in the first part[1], but there are interesting similarities and differences both in the mathematical structure and in the biological conclusions.

We then give a brief discussion of general evolutionary trends, and end with a survey of the main mechanisms that can produce cyclic patterns, or multiple stable states, in the levels of infection in the host population.

Life cycle structure and disease dynamics

Macroparasites with direct life cycles tend to produce persistent infections, with the host harbouring populations of parasites for long periods, due to continual reinfections. Among many examples are the hookworm species of man, *Ancylostoma duodenale* and *Necator americanus* (see Table 1); in endemic areas the prevalence of these infections may approach[2] 100%. For such systems, the pathogenicity to the host, the rate of production of transmission stages of the parasite and any resistance of the host to further infection all typically depend on the number of parasites present in a given host. A crude division of the host population into susceptible, infected and immune classes is therefore not helpful, and a detailed description of the dynamics needs to deal with the full probability distribution of parasites within the host population[3–6] (that is, with the number of hosts harbouring i parasites $N(i)$, where $i = 0, 1, 2, \ldots$). Figure 1, which is to be compared with Fig. 3 of the first part of this article[1], depicts the essential structure of such models.

It is often useful to simplify these models by making a phenomenological assumption about the statistical distribution of parasites among hosts[3,7–10] (or even, occasionally, by making assumptions that permit this distribution to be deduced theoretically[11,12]). A usual phenomenological assumption is that the parasite distribution is a negative binomial[3,7–10,13,14], with the parameter k providing an inverse measure of the degree of parasite 'clumping' or overdispersion within the host population; the limit $k \to \infty$ corresponds to the parasites being distributed in an independently random or Poisson form, while very small k corresponds to very high clumping. It is then possible to use such statistical moments of the $N(i)$ distribution as the total host population ($N = \sum_i N(i)$), the number of uninfected hosts ($X = N(0)$), the total parasite population ($P = \sum_i iN(i)$), and the mean parasite burden per host ($m = P/N$). In this way, models of the kind depicted in Fig. 1 can be brought into correspondence with the coarser models of the kind discussed in Part I (see Fig. 3 of Part I)[1].

The most detailed study of this type[3,4] draws on a synoptic collection of data for direct life cycle parasites (mainly helminths), and describes the dynamics in terms of three differential equations, for the number of hosts N, parasites P, and free-living infective stages w:

$$dN/dt = (a-b)N - \alpha P \quad (1)$$

$$dP/dt = \beta wN - (\mu + b + \alpha)P - \alpha(k+1)P^2/(kN) \quad (2)$$

$$dw/dt = \lambda P - cw - \beta wN \quad (3)$$

Here the birth and death rates a and b are as defined in the first part[1] of this article, as is the transmission parameter β (hosts acquire individual adult parasites at a rate proportional to the number of contacts between hosts and parasite infective stages, βwN). The parasite-induced host death rate (or, equivalently, depression of the birth rate) is taken to be linearly proportional to the parasite burden in a given host, at a rate α per parasite. The parasites are distributed as a negative binomial with parameter k; μ is the natural mortality rate of adult parasites; λ is the rate of production of infective stages by an adult parasite; and c is the death rate of these infective stages. The biological underpinning of these equations, and their dynamical behaviour, have been expounded in detail elsewhere[3,4].

A rough understanding of the relation between this system of equations for typical macroparasites with direct transmission, and the earlier set of equations (8)–(10) of Part I for directly transmitted microparasites, can be obtained as follows. First, note that the lifespan of the free-living infective stages is usually much shorter than that of the host and the adult parasite (compare Table 1). Thus the set of differential equations can be decoupled, by assuming the 'short lived' infective stages are adjusted essentially instantaneously to their equilibrium level ($dw/dt = 0$) for any given value of N and P. This gives

$$dN/dt = rN - \alpha P \quad (4)$$

$$dP/dt = \frac{\lambda NP}{H_0 + N} - (\mu + b + \alpha)P - \frac{\alpha(k+1)P^2}{kN} \quad (5)$$

(where $r = a - b$ and $H_0 = c/\beta$). Second, a phase-plane analysis now lays bare the properties of this pair of equations.

Three patterns of dynamical behaviour are possible[3,4]. (1) If

$$\lambda - (\mu + b + \alpha) > r(k+1)/k \quad (6)$$

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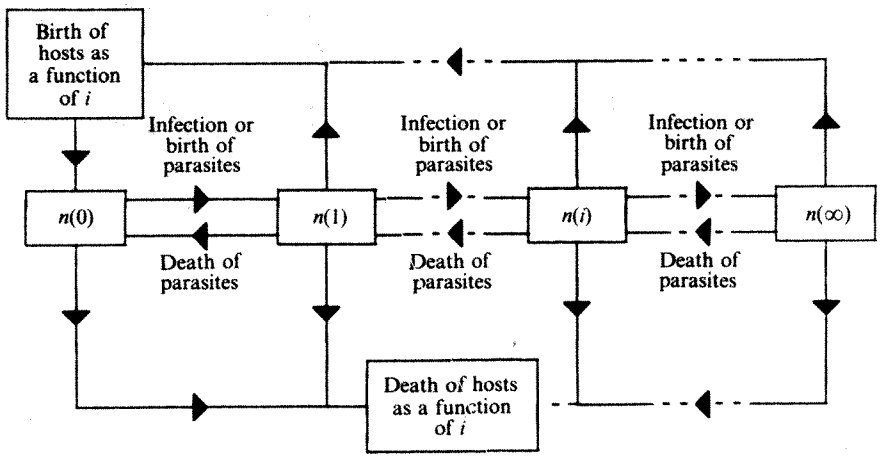


Fig. 1 Diagrammatic flow chart for a directly transmitted infection, based on a model with compartments for the number of hosts, $N(i)$, harbouring i parasites ($i=0, 1, 2, \ldots$). The model has a structure similar to, but obviously more complex than, that of Fig. of 3 Part I[1].

the parasite regulates the host population to a stable equilibrium value. The average parasite burden per host settles to

$$m = r/\alpha \tag{7}$$

(2) If Equation (6) is not satisfied, but

$$\lambda - (\mu + b + \alpha) > 0 \tag{8}$$

the host population continues to grow exponentially, but at a rate

$$\rho = r - [\lambda - (\mu + b + \alpha)][k/(k+1)] \tag{9}$$

This is less than the disease-free rate, r. In this case, the mean parasite burden in the exponentially growing host population settles to the value

$$m \to (r - \rho)/\alpha \tag{10}$$

In either event, if the host population is initially below the value

$$N_T = \frac{H_0(\mu + b + \alpha)}{\lambda - (\mu + b + \alpha)} \tag{11}$$

the parasite cannot become established ($dP/dt < 0$). However, as long as equation (8) is satisfied, the host population will grow exponentially (at the rate r) until this threshold value N_T is exceeded, whereupon the infection will become established, either regulating the host population or at least slowing its growth rate. Furthermore, in view of the large values for the reproductive output λ of most helminth parasites, N_T will typically be relatively small. This expectation of commonly finding direct life cycle helminth infections persisting in low density host populations is borne out by the evidence[15,16].

(3) Finally, if λ is so small that equation (8) is not satisfied, the infection can never become established (N_T is negative!).

The similarities between cases (1) and (2) here, and the results displayed in Fig. 4 of Part I, are striking. In particular, for measures of the prevalence of infection, notice the exact formal equivalence between equation (15) of Part I and equation (7), and between equation (17) of Part I and equation (10). A dissimilarity is that whereas the ability of a microparasitic infection to regulate its host population essentially depends on its pathogenicity α exceeding the host population growth rate r (weighted by rates of recovery, loss of immunity and so on: see Table 1 of Part I), for a macroparasite it is its net reproductive ability, $\lambda - (\mu + b + \alpha)$, that plays a central role (λ is the 'birth rate', while μ, b and α are the natural parasite, natural host and parasite-induced host death rates). The macroparasitic infection can never persist if this effective net reproductive rate is not positive (equation (8)). The parasite will regulate the host population, or merely slow its growth, depending on whether this effective net reproductive rate $\lambda - (\mu + b + \alpha)$ is, or is not, greater than the host reproductive rate r, weighted by a factor $(k+1)/k$ to allow for the clumped distribution of parasites. Thus equation (6) is for these directly transmitted macroparasites the analogue of the microparasite equation (13) in Part I[1].

Table 1 Expected lifespans of the host and parasitic stages involved in the life cycle of *Schistosoma mansoni* and *Ancylostoma duodenale*

	Population	Lifespan (yr)
S. mansoni (refs 24, 29, 82)	Man (primary host)	50.00
	Adult parasite	5.00
	Infected snails (intermediate host)	0.10
	Cercariae	0.003
	Miracidia	0.0009
A. duodenale (ref. 2)	Man	50.0
	Adult parasite	1.0
	Free-living infective stage	0.1

Indirect life cycles constitute another qualitatively different kind of complication, arising when the life cycle of the parasite involves one or more intermediate hosts. This happens for both microparasites (for example, the arthropod-borne viruses or arboviruses such as yellow fever or Rocky Mountain spotted fever; the protozoan malaria species) and macroparasites (for example, schistosomes, the filarial worms causing onchocerciasis, and other roundworms and flatworms that involve dipteran, molluscan and other intermediate hosts). Malaria and schistosomiasis in human populations are the two parasites whose transmission cycles have been most fully studied and each enjoys its own independent and growing literature, both empirical and theoretical (see Table 2). Their basic dynamical character is, however, in many respects common to all parasites with indirect life cycles.

If we adopt the approach of equations (8)–(10) and Fig. 3 discussed in part I, namely dividing the host population into susceptible, infected and immune categories, we will in the simplest case have a system of six differential equations: three for the primary host (alternatively referred to as the definitive host, or final host) classes X, Y, Z; three for the intermediate host populations X', Y', Z'. All existing models, however, assume the total populations of both primary host ($N = X + Y + Z$) and the intermediate host ($N' = X' + Y' + Z'$) are constant, unaffected by the dynamics of the disease. This reduces the system to four equations. If, furthermore, immunity is either ignored or handled by specific assumptions about 'superinfection', the Z and Z' classes are effectively removed to give two coupled differential equations for the number of primary hosts Y, and of intermediate vectors Y', that are infected. This, in essence, is the source of the classic Ross-Macdonald[17,18] malaria equations, the Nasell–Hirsch[19] schistosomiasis model, and the Dietz[20] arbovirus equations.

These equations have been subjected to various kinds of more refined treatment, including age structure[20–24], immunity and 'superinfection'[17,21,23–26] and the use of several immunological categories of hosts[26] (intermediate between Fig. 3 of Part I and Fig. 1 of Part II). However, essentially all the existing work on

indirectly transmitted parasites retains the assumption that the populations of host and intermediate vector are constant, not dynamically involved with the infection. Analysis of such models reveals threshold relations[17,20,21,27,28] between N and N', analogous to but more complicated than the N_T of the direct life cycle models. If N and N' lie below the threshold combination, the disease cannot be maintained.

For many human, and other animal, infections by parasites with indirect life cycles, what is needed is a theory in which the populations of primary and intermediate hosts are affected, and possibly even determined, by the presence of the infection. While it may often be reasonable to treat a human primary host population as roughly constant, we believe that cases where intermediate host populations are unaffected by the prevalence levels of the infection will be the exception rather than the rule[29]. There is no formal problem in extending our dynamic models of either the 'microparasite' kind of equations (8)–(10), (Part I) or the 'macroparasite' kind of equations (1)–(3) (Part II), to encompass the added complication of one or more intermediate vector populations. Space forbids a full exposition of the emergent properties, but the main trends are indicated in the following section.

Time scales and transmission terms

A full model for an indirectly transmitted parasite might include not only dynamical descriptions of the prevalence of infection in primary and intermediate host populations, but also additional differential equations (analogous to equation (3)) for the free-living transmission stages that carry the parasite from primary to intermediate host, and back again. For example, for schistosomiasis we could add a differential equation describing the miracidial stage (man to snail), and another for the cercarial stage (snail to man), to the usual equations for infection levels in the human and snail populations[30]. The reason this is not commonly done can be seen from Table 1; the dynamics of the free-living stages takes place on a time scale so much shorter than the other time scales in the system that miracidial and cercarial populations can be assumed to have the equilibrium values appropriate to the prevailing conditions among human and snail populations. In just this way, we collapsed the three-equation system (1)–(3) to the two equations (4), (5).

This technique of using biological insights about the time scales of various infection processes can be used to make further rough but useful approximations. For example, the time scales for processes (such as mortality rates) within the intermediate host population are typically significantly shorter than those in the primary host. Again, Table 1 testifies to this. Accordingly, we can assume that the numbers of susceptible, infected and immune intermediate hosts are adjusted to have the equilibrium values ($dX'/dt = 0$, and so on) appropriate to the current levels of infection in the primary hosts. In this way, parasitic infections with indirect life cycles can be approximately brought to a form similar to that of equations (8)–(10) in Part I for direct life cycles[1].

As a concrete example, consider a grossly oversimplified model for malaria, in which 'superinfection'[17,25,26], and mosquito latency[17,21] (and immunity[20,26]), are ignored. Assume also the total mosquito population is constant; $N' = X' + Y' =$ constant. The populations of infected humans Y, and mosquitos Y', then obey

$$dY/dt = \beta' Y' X - (b + \alpha + v)Y \quad (12)$$

$$dY'/dt = \beta Y(N' - Y') - (b' + \alpha' + v')Y' \quad (13)$$

Here β, b, α and v (plain for humans, primed for mosquitos) have their previous meanings; conventionally, most infected humans are assumed to recover ($v \gg \alpha, b$), and most infected mosquitos to die at a rate largely unaffected by the infection ($b' \gg \alpha', v'$). The assumption that mosquito processes happen on a relatively fast time scale enables Y' in equation (12) to be determined by setting $dY'/dt = 0$ in equation (13), leading to

$$dY/dt = Y[(\beta\beta' N' X)/(b' + \alpha' + v' + \beta Y) - (b + \alpha + v)] \quad (14)$$

This is exactly of the form for a directly transmitted infection (equation 9 of Part I), except that the simple transmission coefficient β has been replaced by the more complicated factor $\beta\beta' N'/(b' + \alpha' + v' + \beta Y)$. Similarly, the Nasell–Hirsch two equation model[19] for prevalence of schistosomiasis among humans and snails can be collapsed to Macdonald's[27,28] single equation for prevalence in the human population.

Conversely, for humans the total population is often growing on a time scale that is long compared to the relevant time scales of even persistent infections. This is why the total population can be treated as a constant in most epidemiological models. The approximation, whereby the dynamics of the prevalence (Y/N) and of the total population (N) are decoupled, can often be useful in discussing the transmission cycle of the infection, even though the long-term growth or regulation of the host population is affected by the presence of the infection.

Table 2 uses these ideas to attempt to give a schematic account of the relations among some of the many models, of differing degrees of complexity, that are to be found in the literature.

Saturation of transmission terms. The transmission terms obtained in equation (14) by 'collapsing out' the mosquito dynamics of equation (13), and in equation (5) by collapsing equation (3) for the free-living infective stages of the parasite, manifest a feature that is common to all such approximate representations of complex transmission processes[3,4,14,31,32]. Essentially, the simple term βXY for direct transmission

Table 2 Schematic representation of relationships between various kinds of models for parasitic infections, based on relative time scales of population processes

	Host population(s) constant	Host population(s) a dynamic variable
In considering the dynamics of infection, only one species is involved (for example, the host species).	**Direct life cycles** Classical epidemiological models (refs 21, 56, 65, 67, 83–84, 87). Models for the dynamics of a parasite population within a host population of fixed size (refs 5, 88, 89). **Indirect life cycles**. Models for schistosomiasis (refs 28, 34, 90) and for malaria (refs 17, 18, 21), considering only the dynamics within the human host.	**Direct life cycles**. Models similar to those for classical epidemiology, but with total host population a dynamic variable, determined by birth and death processes (refs 35, 91 and this review). **Direct and indirect life cycles**. Dynamics of models in the compartment below but with all populations but the primary host 'collapsed out' (this review).
In considering the dynamics of infections, two or more species are involved (e.g., primary *and* intermediate host, or host *and* parasite population).	**Direct life cycles**. Models similar to the classical epidemiological equations, but including the dynamics of free-living infective stages (ref. 35). **Indirect life cycles.** Models for schistosomiasis (refs 19, 34, 92), malaria (refs 17, 21, 93) and arbovirus infections (ref. 20) in which both human hosts and intermediate vectors are considered. Models of schistosomiasis where humans, snails, miracidiae and cercariae are all considered (ref. 30).	**Direct life cycles.** Models similar to classical epidemiology, but with host population and free living infective stages both included as dynamic variables (ref. 35). Models for dynamics of host parasite systems (refs 3, 4, 7, 8, 14, 35), sometimes with dynamic aspects of free-living infective stages also included (refs 3, 4). **Indirect life cycles.** Any of the models in the compartment to the left, but with the total host populations treated as dynamic variables (this review).

between susceptible and infected people or βNw for direct transmission between hosts and free-living infective stages are replaced by expressions of the general form $AXY/(1+\nu Y)$ or $ANP/(1+\nu N)$, respectively. In the limit when, for example, νY is small, the expression has the familiar form, proportional to X and Y. But it can be that νY becomes significant compared to unity, whereupon the transmission term saturates to a value (AX/ν) proportional only to X. Such saturation effects can be important in diminishing the ability of the parasite to regulate its host population[3,14].

Ecology of the transmission process. Further complications can arise from the ecological nature of the individual links in the transmission process.

For infections that are communicated directly, the assumption that the net rate is proportional to the number of susceptibles and to the number of infectives is clearly reasonable for many diseases, and strikingly successful in explaining the mouse pox and mouse pasteurellosis data[1]. But for sexually transmitted diseases, for example, this is only plausible in a population that is astonishingly promiscuous and sexually active. In a society whose members typically have only a small number, η, of sexual partners (independent of the absolute population size), the rate at which an infected person propagates the infection is proportional not to the total number of susceptibles, but to η times the probability that a given person is a susceptible; that is, βXY is to be replaced[21] by $\beta\eta XY/N$. Under these very simple assumptions, the condition for maintenance of such diseases is $\beta\eta > (b+\alpha+\upsilon)$, independent of the population size. In reality, a more careful treatment of the distribution of degrees of sexual activity within the population is needed[33], but the fact remains that infections of this sort are relatively easy to maintain in low density populations.

More broadly, biological insights into the relative time scales associated with the various phases of indirect life cycles enable us to discuss the prevalance of infection in the primary host population by retaining equations (8) and (10) for X and Z, in Part I of this article, but replacing equation (9) with the more general expression

$$dY/dt = Y[h-(a+b+\upsilon)] \tag{15}$$

The transmission term is here denoted by h (Ross' 'happenings'[18]), and the threshold condition for the disease to increase upon introduction at low levels is clearly that $h > (\alpha+b+\upsilon)$ in the limit $Y \to 0$. For the simple circumstances of the indirect life cycle that led to equation (14) above, this requirement comes down to the threshold criterion[20,21]

$$NN' > \frac{(\alpha+b+\upsilon)(\alpha'+b'+\upsilon')}{\beta\beta'} \tag{16}$$

Note that a large population N' of intermediate vectors can enable the disease to persist, even when the primary host population N is small.

However, for malaria and many other infections borne by biting arthropods, the intermediate vector tends to make a fixed number of bites per week, independent of the number of primary hosts available to feed on. Thus the transmission rate from infected arthropods to people (and from infected people back to susceptible arthropods) is proportional to the biting rate ω times the probability that a given human is susceptible (or infected), and not simply proportional to the number of susceptible (or infected) people. That is, in equations (12) and (13), β and β' are to be replaced by ω/N. The threshold condition (16) is accordingly modified to[17,18,20]

$$\frac{N'}{N} > \frac{(\alpha+b+\upsilon)(\alpha'+b'+\upsilon')}{\omega^2} \tag{17}$$

Note that latency effects have been neglected here, although they can be important in infections with indirect life cycles, and they certainly modify threshold conditions significantly for malaria[17] and schistosomiasis[29]. Infections with intermediate vectors of this character are relatively easy to maintain at low population densities of the primary host, provided only that the ratio of intermediate to primary hosts is sufficiently high. Indeed, equation (17) suggests the infection is actually easier to maintain at low host population levels; the mosquito or other intermediate host population N' is, however, typically dependent on the primary host for blood meals or the like, so that things are not as simple as they might seem. (A more general discussion, from which the threshold relations (16) and (17) emerge as limiting cases, has been given by Dietz[20]).

Yet another form of complication enters with parasites that have sexual stages, yet can have low densities, in a host. Schistosomiasis is one such example[10,19,27,28,34]. At high levels of prevalence of the infection in the human population, people tend to have worm burdens such that most adult female schistosomes are mated, and the circumstances leading to equation (16) are well approximated. But at low levels of prevalence, it can be that the average female is not mated, which tends to require that the transmission link from snail to man be counted twice in considering the overall cycle, thus giving complicated threshold conditions (very roughly of the form $N[N']^2 >$ constant[19,34]).

Finally, note that (apart from the laboratory experiments on mice[1]) in all our models the host population either is regulated to some stable value by the disease, or else it grows exponentially. In practice, other constraints, set by resources, predators or the like, will eventually limit population growth. Such biological realities can be included in all our models, by introducing a logistic constraint (at a 'carrying capacity' K) in the growth of the disease-free population[35]. The resulting situation, for both direct and indirect parasite life cycles, is similar to that illustrated in Figs 1*e*, *f* and 2*b* in Part I, with the host population depressed below its disease-free level K, provided the parasite-induced host mortality α is not too large[1]. Too small an α leads to relatively little depression of the host population; too large an α renders the disease unable to persist, and the host population remains at K; maximum parasite-induced depression of the host population is attained for intermediate levels of pathogenicity[1,36]. This broad statement glosses over many intricacies that can arise (R.M.M. and R.M.A., in preparation), particularly with indirect life cycles when the intermediate vector has a constant biting rate (producing threshold conditions such as equation (17) in simpler models), but the gist is true.

Population parameters and evolutionary trends

Any discussion of the relations among the population parameters that characterize an infectious disease must ultimately take account of the evolutionary pressures on both hosts and parasites. Population dynamics is always confounded by population genetics.

For example, even if we assume no genetic change in the parasite, its action on the host will select for individuals with reduced susceptibility to the disease. For this reason alone, the pathogenicity of the parasite will tend to decrease through evolutionary time. Conspicuous examples are provided by the presence of the sickling gene (and other blood-group phenomena) in regions where malaria is endemic[37], and by the history of myxomatosis in rabbit populations in Australia[38]. An interesting theoretical discussion has been given by Gillespie[39].

Selective forces also act strongly on the parasites[40,41]. As we have seen, the persistence of a disease is facilitated by low pathogenicity and by long duration of infection[1]. Countervailing forces can, however, act to increase the virulence of an infectious disease; increased pathogenicity may often be associated with enhanced rates of production (within the host) of the parasite's transmission stages[38,42,43].

The regulatory potential of an infectious disease will, therefore, typically change as time goes by. A parasite may stably regulate its host population during their early association. But,

as selective pressures reduce the average susceptibility of the hosts, such regulatory effects will tend to wane. Eventually, the host population may escape being controlled by the parasitic infection.

Because the generation times of most hosts are several orders of magnitude longer than those of their parasites, it is tempting to conclude that selection acts more rapidly on the parasites. However, the way parasitic infections act within host populations makes it likely that the parasites force the pace of host evolution to keep in step with, or even ahead of, their own evolution.

Among the recondite variety of strategies that parasites have evolved for persistence and transmission, some general trends can be discovered. For example, many parasitic species traverse links in community food webs by virtue of predator–prey associations between primary and intermediate hosts. Such associations, which include biting arthropods feeding on vertebrates, have played an important part in the evolution of complex life cycles. The high transmission efficiency β of these links suggest the threshold host populations for maintenance of such parasites will be low (see equations (16) and (17)). Consequently, we expect indirect life cycles to predominate among parasitic infections of hosts that exist at low density.

In contrast, directly transmitted microparasites that require high host densities in order to persist should be more commonly associated with animals that exhibit herd or shoaling behaviour, or breed in large colonies. Empirical evidence in support of these ideas comes from the abundance of directly transmitted viral and bacterial infections within modern human societies[42,44], large herds of ungulates[45], breeding colonies of sea birds[46,47], and the social insects[48,49]. Those diseases with direct life cycles that do persist within low density host populations should possess distinctive characteristics, such as long-lived infective stages[50,51], failure to induce lasting immunity[33,52–53], or ability to persist within the host for very long times[54].

Another trend to be noted is that highly pathogenic species usually exist, if at all, at low levels of prevalence (see equations (15) and (17) in Part I and equations (7) and (10) in Part II). An example is the digenean parasite *Haematolaechus colaradensis* whose primary host is frogs, but which has a transmission pathway involving first snails, and then dragonflies, as intermediate vectors on the way to the next frog. The prevalence among frogs is high, 60–70%, and the parasite is long-lived and has very low pathogenicity; in dragonflies the fluke induces moderate mortality, and has 30–40% prevalence; while in snails it is highly pathogenic but appears to have only about 5–10% prevalence[55]. These broad patterns, which are often found in helminths with life cycles involving two or three host species, are summarized schematically in Table 3.

Cyclic patterns of disease prevalence

Annual or other cycles in the prevalence of infection are often observed, and can arise in at least three distinct ways.

First, for many short-lived viral and bacterial infections in human populations, there is a propensity for the steady, endemic level of prevalence of infection to be attained by damped oscillations. Particularly if this equilibrium prevalence is low, it is possible for stochastic fluctuations in the number of people infected at the minimum of the cycle to, in effect, keep the cycle 'pumped' and prevent it from damping to equilibrium. This interplay between demographic stochasticity and an inherent propensity to weakly damped oscillations is essentially the mechanism proposed in the classic work of Bartlett[21,56–59] to account for cyclic patterns in the prevalence of measles and other viral infections in large cities. Gurney and Nisbet[60] have proposed a similar mechanism as an explanation for predator–prey cycles.

Second, time dependence in any of the population parameters may, in principle, produce cyclic variations in infection. In particular, seasonal variation in the transmission coefficient is important in setting temporal patterns for many parasitic infections, and may often be central for human viral infections[44,61–65]. The mechanisms underlying the seasonality in the transmission rates are poorly understood, but for human viruses the main causes are probably climatic (temperature and humidity) effects influencing survival and dispersal of transmission stages, and seasonal changes in social behaviour[66,67] (children returning to school after the long summer vacation). The seasonal cycles characteristic of the prevalence of measles, chicken pox, poliomyelitis and mumps in large cities could arise in this way[21,44,65].

Annual periodicity in transmission rates can, moreover, produce complicated nonseasonal cycles in the prevalence of infection. Yorke and co-workers[44,63,65], and Dietz[64], have cogently argued that such a mechanism is responsible for the regular biennial cycle, alternating between years of high and low incidence, for measles in New York City between 1948 and 1964; in the same city, mumps and chicken pox showed clear annual cycles. The explanation of Yorke *et al.* is to the contrary of the conventional explanation of these non-seasonal cycles in terms of demographic stochasticity, as described above. Their model is essentially the set of deterministic equations (1)–(3) in Part I, with an assumed constant number of new susceptibles appearing each year, life-long immunity, and with the system enriched by the inclusion of a brief incubation period during which infected hosts are not infective. The basic feature is that the transmission coefficient $\beta(t)$ varies seasonally with a 1 yr period. Within a narrow window of parameter values, the number of infected people can show biennial peaks (Fig. 2*a*) similar to those for measles in New York City. This window separates highly transmissable diseases which produce an epidemic with eventual fade-out, from the diseases with low transmission efficiency which give rise to endemic seasonal patterns of infection (Fig. 2*b*), as usually shown by mumps and chicken pox.

Third, various kinds of nonlinearities in the transmission terms may produce stable limit cycles, whose periods depend on the population parameters and will rarely be seasonal. People familiar with the ease whereby stable limit cycles arise in predator–prey models may be surprised to learn the structure of most host–parasite models is such that stable cycles do not easily occur. However, they can be produced without excessive contrivance. One simple example is to take the basic equations (8)–(10) of part I, and introduce the possibility of saturation in the transmission by replacing βXY by $\lambda XY/(H_0+X)$. Such a modification can arise naturally[4,35], in the manner of the analogous expression in equations (5) and (14), if the term is thought of as deriving from the 'collapsed' dynamics of a free-living infective stage. This system can now exhibit stable limit cycles for a specific range of parameter values (corresponding to λ neither too small nor too large). One such stable cycle is illustrated in Fig. 2*c*. In general, however, little is yet agreed about the kinds of biological processes that can generate nonseasonal patterns of disease prevalence.

Table 3 Some population characteristics of diseases caused by indirectly transmitted helminths

Host	Pathogenicity of parasite	Prevalence of infection within host population	Expected life span of host (inversely related to time scaled dynamics)
Final	Low	High	Long
Second intermediate	Medium	Medium	Medium
First intermediate	High	Low	Short

Multiple stable states of disease prevalence

A growing number of empirical and theoretical studies suggest that many natural assemblies of plants and animals can have a multiplicity of alternative stable states[68]. Once two or more stable statesare possible, the actual state the system settles into

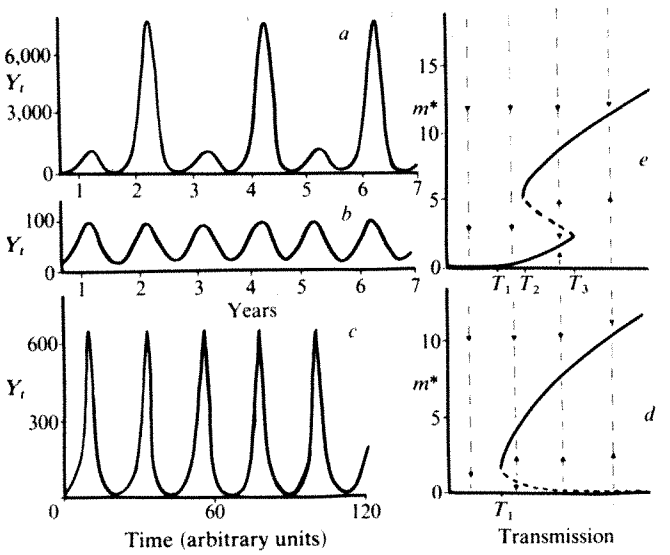


Fig. 2 *a*, Simulations of recurrent outbreaks of measles in New York City, showing biennial peaks superimposed on an underlying seasonal cycle (from London and Yorke[44]). *b*, Simulations[44,63] of recurrent outbreaks of mumps in New York City, with simple seasonal peaks, annually. *c*, Simple limit cycle behaviour generated by the model described in the text. *d*, The transmission threshold, alternative stable states, and 'breakpoint' phenomena that arise in simple models for the transmission dynamics of schistosomiasis[10,19,27,28,34]; the features are as discussed in the text. *e*, Transmission threshold and alternative stable states arising in a model for directly transmitted helminth infections, where it is assumed that the pathogenicity of the disease is related to the nutritional state of the host[35]. The graph shows the mean equilibrium burden of parasites per host, m^*, as a function of a parameter, T, representing transmission efficiency. The infection cannot persist below a threshold value T_1; between T_1 and T_2 there is a unique low level of disease endemicity; between T_2 and T_3 two stable levels of prevalence may occur, one high and the other low, separated by a breakpoint (the dashed line); above T_3 there is again a unique equilibrium level, corresponding to high average parasite burdens per host. The arrows indicate the stable state to which the system will sw from a given initial value.

depends on the initial conditions. The system will tend to recover its original configuration if subject to small disturbance, but sufficiently severe perturbations are liable to precipitate it into an alternative state in a different region of the dynamical landscape.

The nonlinearities in population models for parasitic infections can generate such multiple states by three principal mechanisms: worm pairing for sexual reproduction in the primary host; nonlinearities associated with the transmission from primary to intermediate host, or vice versa (mosquitos biting man for malaria, or predatory primary hosts consuming infected intermediate-host prey); parasite pathogenicity dependent on the nutritional state of the host.

The first and most fully studied of these categories arises for many helminth infections with indirect life cycles, such as schistosomes[10,19,27,28,34]. It serves to exemplify the phenomenon. As portrayed in Fig. 2*e*, the equilibrium value of the mean parasite burden per human host (m) will be zero if the rate of transmission (T) from snail to man is below the threshold value T_1. Above this threshold, two alternative stable states occur, one of endemic infection ($m > 0$), the other of parasite absence ($m = 0$). The basic reason is that at low levels of m the female worms are unlikely to be mated, so that the disease cannot be maintained, even though the transmission parameters are such as to permit its endemicity if introduced at high values of m. The two stable states (valley bottoms in the dynamical landscape) are separated by a 'breakpoint' (watershed), indicated by the dashed line in Fig. 2*e*; disturbances severe enough to transgress the breakpoint will carry the system from one state to the other.

These threshold and breakpoint concepts are of obvious importance to epidemiologists concerned with disease eradication[10,27,28].

Of special importance are the effects that can arise from the now widely recognised fact that the impact of an infection is often related to the nutritional state of the host[69–75]. Broadly speaking, malnourished hosts have lowered immunological competence, and are less able to withstand the onslaught of infection[76–78]. The effective pathogenicity of a parasite therefore tends to increase as host density rises to a level where competition for available food resources is severe[79,80]. Given certain reasonable assumptions[35] about the exact relation between pathogenicity (α) and host density (N), two stable states may occur for a given set of rate parameters. The outcome of such a model[35], for a directly transmitted helminth infection, is shown in Fig. 2*d*. Both states reflect stable endemic disease: one equilibrium is characterized by high host density and low worm burdens; the other by low host density (severely depressed by the disease) and high average burdens of parasites. As for the schistosome model of Fig. 2*e*, the two states are separated by a breakpoint or unstable equilibrium.

The discontinuous switch from low to high levels of infection, following a disturbance severe enough to cross the breakpoint, will show up as an apparent 'epidemic' outbreak of disease, typically producing many host deaths. Interestingly, many documented accounts of disease outbreaks are for host populations at high densities, where stress induced by overcrowding or malnutrition is present[71,72]. It is very likely that such outbreaks are to be explained[35] by the alternative stable states produced by close links between pathogenicity and nutrition or stress, rather than by the commonly accepted hypothesis of enhanced transmission with high density populations[81].

Parasitic infections with very complex life cycles may possess more than two stable states, particularly if predator-prey links are involved in the transmission from one host to the next, as is the case for many helminth parasites. There is a desperate paucity of data, from field or laboratory, bearing on these general points.

Conclusion

This two-part article has blended some new theoretical studies and new analysis of existing laboratory data with a review and synthesis of past and present models for the overall transmission dynamics of parasitic infections. We have defined 'parasite' broadly to include viruses, bacteria and protozoans along with the more conventional helminth and arthropod parasites, and we have concentrated attention upon the circumstances under which the infection may significantly alter the growth rate of its host population.

Some of the theoretical conclusions can be pleasingly supported by hard data, while others remain more speculative. On the whole, our main goal is to help elevate the study of host–parasite population dynamics to its proper place in ecological thinking; parasites (broadly defined) are probably at least as important as the more usually-studied predators and insect parasitoids in regulating natural populations.

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articles

Radio studies of the double QSO, 0957+561A, B

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The radio source 0957+561 has at least four components. Two coincide with the optical QSOs, which is in accordance with the hypothesis that the QSOs are images of a single object due to a gravitational lens. There are details of spectra and structure which are more difficult to reconcile with the hypothesis.

THE pair of QSOs 0957+561A, B have been shown to have remarkably similar optical characteristics by Walsh, Carswell and Weymann[1] and they suggest that the QSOs may be two images of the same object formed by a gravitational lens. The detailed properties of the radio source are clearly of great interest, and we present here a radio map and other observations discussed in the context of a gravitational lens.

Observations

The total flux density of the radio source has been measured at various frequencies summarised in Table 1. The data are consistent with a flux density $S \propto \nu^{-0.65}$. The two observations at λ 6 cm suggest the possibility of variability.

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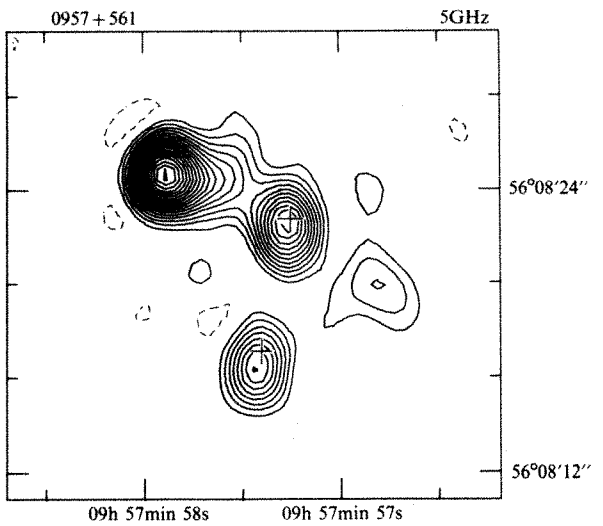


Fig. 1 5-GHz map with 5-km telescope using normal aperture grading. The contour interval is 4 mJy for an unresolved source. Negative contours are shown as dashed lines and the zero contour is omitted. +, Positions of the optical QSOs.

To obtain the 5 GHz map the source was observed with the Cambridge 5-km telescope for three 12-h periods during the period 22–29 May 1979, twice to map the Stokes parameters $I-Q$ and once to map U and Q. The two observations of $I-Q$ show only differences consistent with noise, atmospheric fluctuations, and so on. The mean of the two sets of data is presented in Figs 1 and 2. Figure 1 shows the map produced using the standard truncated gaussian grading function (falling to 0.3 at the largest spacing) with a beamwidth 2″ in RA by 2.4″ in dec. The map in Fig. 2 was produced using uniform aperture grading with a 1.75″×2.11″ beam but rather larger sidelobe responses. Note that the declination scale in Fig. 2 is compressed by sin δ so that the beam appears circular on this projection; this shows more clearly whether components are extended.

The positions, flux densities and angular sizes of the components are summarised in Table 2, which also includes the positions of the optical objects A and B for comparison.

The polarisation observations show no polarised flux >10 mJy, except for component B, which shows a marginally significant value of 11 mJy at position angle 90°.

Observations were made at 408 MHz in April–May 1979 with interferometers consisting of (1) the MK IA telescope of Jodrell Bank and a 25-m telescope at Knockin (67 km south-west of Jodrell Bank), and (2) MK IA and a 25-m telescope at Defford (127 km south of Jodrell Bank). In each case the source was tracked continuously for 24 h. Data were also taken simultaneously on a compact source, 0954+556 (4C55.17) within the primary beam. The latter was used as a reference to reduce phase fluctuations due to the troposphere and ionosphere using the technique described by Peckham[2]. The data were transformed and 'cleaned' to produce a map with a synthesised beam of FWHM 0.9″. The map suffers limitations because of incomplete coverage of the u–v plane, and residual phase fluctuations. In particular, the incomplete coverage means that components extended on a scale ≥4″ will not be detected.

The maximum correlated flux density is 900 mJy, corresponding to a visibility of ~0.6. Details of the various components definitely detected are given in Table 3. The flux densities are subject to a possible scale error of ±25% and a random error of ~50 mJy. At 408 MHz the NE component is the dominant one and its extension is very similar to that seen at 5 GHz. The ratio of the integrated flux densities at these two frequencies has spectral index α_{408}^{5000} of −0.65, similar to the overall spectrum of the source. It is not possible to say whether component W is extended, but again $\alpha_{408}^{5000}=-0.65$ although the error in this value is rather large. The spectrum of component A appears to be significantly flatter than either NE or W. In addition to the components in Table 3, there is a possible component which may be extended ~2″–3″, centred east of component B. There is no definite detection of component B itself.

Observations were also made at 1,612 MHz between MK IA and Knockin and at 1,667 MHz between MK IA and Defford, again for 24 h at each baseline. Because of the narrower primary beam it was not possible to observe 0957+561 and the reference 0954+556 simultaneously. Instead each was observed for alternate 10-min intervals throughout the 24 h. The data were combined into a 'clean' map with a synthesised beam of FWHM 0.2″, and components extended on a scale ≥1″ cannot be detected. The maximum correlated flux density was 190 mJy corresponding to a visibility of the order ~0.4. Because of the poorer signal-to-noise ratio and the inability to observe the reference source simultaneously, the phase fluctuations were worse than at 408 MHz and the map correspondingly degraded. The apparent flux density of components is reduced by an unknown factor. A compact feature with extension <0.2″ was, nevertheless, clearly detected in component A. There were also probably detections of components W and B; both were apparently extended and this makes comparison of flux densities with A very difficult.

Table 1 Spectral data for 0957+561

Frequency (MHz)	Flux density (mJy)	Error (mJy)	Epoch
151	2,200	200	1978*
408	1,450	150	1979.4†
966	560	150	1976.8§
1,407	450	40	1979.4†
2,695	360	15	1976.7‖
4,995	230	20	1979.4¶
5,000	310	27	1975.5‖

Flux densities from the following telescopes: * Cambridge 151 MHz (R. Saunders, personal communication); † Cambridge One-Mile; § Jodrell Bank 278-m interferometer; ‖ NRAO 300-ft (R. W. Porcas, personal communication); ¶ Cambridge 5-km.

Table 2 Details of components on 5 GHz map; positions of optical QSOs are included for comparison

Component	RA	Dec (1950.0)	Flux density (mJy)	Size (arc s)
W	09 h 57 min 56.81 s	+56° 08′ 19.8″	25	1.5–2
A radio	57.27	22.5	48	1
optical	57.25	22.7		
B radio	57.43	16.4	37	1.5
optical	57.40	17.1		
NE	57.90	24.7	120	~3

Discussion

The structure of 0957+561 is unusually complex, and unlike any other single radio source we know. We consider first the relevance of the observations to the gravitational lens hypothesis. The most important prediction of the theory is that a point object should give rise to point images which have identical spectra apart from possible extinction along the differing paths of propagation. In the case of a point mass causing the gravitational deflection there should be two images, but if an extended mass such as a galaxy causes the deflection, then multiple images are possible (see ref. 3 and references therein). Radio components A and B in Figs 1 and 2 are coincident with the optical objects within the errors of measurement. Like the optical QSOs they are comparable in flux density. However, a detailed comparison of radio and optical properties presents some apparent discrepancies with the theory.

Flux ratio. Walsh *et al.*[1] pointed out that in the blue the flux density of QSO B is ~0.7 that of QSO A but in the red they are

equal. They suggested that a possible cause is extinction by dust at the redshift of the QSO ($z = 1.4$). If so then the intrinsic flux from B should be greater than that from A, the ratio being ~1.5. While this factor is somewhat uncertain, it should definitely be >1 (ref. 4). According to Table 2, the ratio at 5 GHz is ~0.8. If the gravitational lens hypothesis is to accommodate this discrepancy, then (1) the source must vary at either radio or optical wavelengths, or both, or (2) an alternative expanation to the dust hypothesis must be found to account for the optical colour difference. In connection with (1), we note that the two images should follow identical light curves with a relative delay of several months to years depending on the precise geometry of the paths. The two observations at 5 GHz in Table 1 indicate a change in total flux density of 80 ± 35 mJy over 4 yr. While some reservation must be made about this apparent variation because the observations were made by very different techniques, clearly a variation of this magnitude could reconcile the observed flux densities. Changes should be observable in 5 GHz maps fairly quickly, and regular observations are planned for this purpose.

Radio spectral differences. This is an extension of the previous point. The failure to detect component B at 408 MHz suggests the flux ratio (B/A) is ≤ 0.5, which is less than that at 5 GHz or in the optical region. Again, in principle, this could be accounted for by differences in variation at different frequencies; indeed, variable radio sources do in fact normally show differences in the pattern of variation at different frequencies. However, variation of this magnitude at 408 MHz is very unusual. The errors in the 408 MHz data at present require this point to be treated with caution, but if further observations confirm it then it may be difficult to sustain the gravitational lens hypothesis. We hope to make improved long-baseline observations at 408 MHz shortly.

Extension of the components. Figure 2 suggests that component A is <1″ in extent but B is extended ~1.5″ along the line joining A and B. This is incompatible with the simple picture of two images of a point object. We may be seeing unresolved multiple images produced because the mass causing the deflection is extended. The direction of extension is as expected for such an effect. The optical image should then also be extended; available observations do not enable this to be seen. Higher resolution observations at both radio and optical wavelengths would clearly be desirable.

So far we have considered only the components A and B close to the optical QSOs. We now consider the components NE and W. Suppose we are in fact seeing images formed by a gravitational lens. Where are the true positions of the 'objects' and where are the second images? Figure 3 shows a possible geometry based purely on the radio observations.

The theory of image formation by a point deflecting mass can be found in, for example, Refsdal[5] and Press and Gunn[6]. Here we quote some results which are useful in interpreting observations and for constructions such as Fig. 3. On the celestial sphere, let M be the projection of the deflecting mass and O the projection of the 'true' position of a point object (the position in the absence of M). Then point images O′, O″ are formed on the extension of the line OM; the former is on the opposite side of O from M, the latter on the opposite side of M from O. Let the angles MO, MO′, MO″ and O′O″ be β, α_1, α_2 and α respectively. Clearly, $\alpha = \alpha_1 + \alpha_2$. Then the following relations hold:

Flux ratio of O′ and O″ $= F'/F'' = \alpha_1^2/\alpha_2^2$

$$4\alpha_1\alpha_2 = \alpha^2 - \beta^2 = \alpha_0^2 = \text{constant independent of data}$$

$$\alpha_1 = \alpha_2 + \beta$$

The flux ratio and angular separation of A and B with these equations enable Fig. 3 to be constructed.

The second images of components NE and W are in fact too faint to be detected as separate components (less than 1 contour on Fig. 1). Note that they lie in the region of the northwards extension of component B, which may provide an alternative explanation of the extension. The true source then consists of three components: a flat spectrum one giving rise to images A and B (and the optical images) and two steep spectrum ones giving rise to images NE and W. Three-component structure of radio sources is quite common with a flat spectrum object coincident with the optical object and two steep spectrum lobes on opposite sides. In Fig. 3 the true position of the flat spectrum component is not on the line joining the two steep spectrum components. However, though unusual, this configuration is by no means unknown, see for example 3C270.1 and 3C275.1 (ref. 7).

If we are not seeing the result of a gravitational lens, then since component A lies on the line joining NE and W it is likely that these three components constitute a single radio source of a common type. Then we must assume component B is independently a radio source of very nearly the same flux density as A. This then adds another coincidence to those of the emission spectra and absorption spectra described by Walsh *et al.*[1] and by Weymann *et al.*[8].

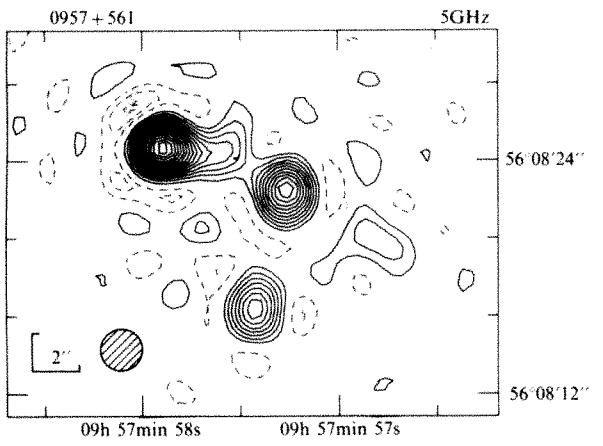


Fig. 2 5-GHz map with uniform aperture grading and declination scale compressed by sin δ.

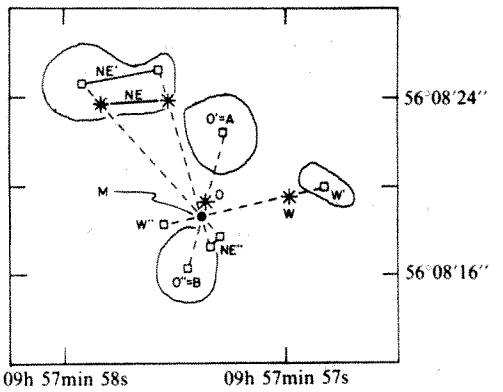


Fig. 3 Possible geometry of the source and image formed by gravitational lens. M is a point deflecting mass; W, O and NE are true positions of 'object' components; W′, W″, O′ = A, O″ = B and NE′, NE″ are the images. The position of M is determined purely from the radio flux density ratio of components A and B.

Table 3 Details of components detected at 408 MHz

Component	RA	Dec (1950.0)	Flux density (mJy)	Size (arc s)
W	09 h 57 min 56.7 s	+56° 08′ 19″	140	—
A	57.36	22.6	120	—
NE	57.89	24.9	600	3

Finally on the gravitational lens hypothesis we consider the possibility that the massive object causing the deflection is itself a radio source. While the mass required depends on the distance of the object, it is likely to be in the range of the most massive elliptical galaxies. Statistics are very sparse for such objects, but using the bivariate luminosity function of Auriemma *et al.*[9] as a guide, the probability that the galaxy may have an associated radio source of flux density 25 mJy at 5 GHz is ~0.005. Thus it is unlikely that either component W or NE is associated with the lens itself.

Conclusions

The most important feature of the radio data is that there are radio components coincident with the optical QSOs and with comparable flux density at 5 GHz where the structure is best determined. This is consistent with the gravitational lens hypothesis, which would have been ruled out if, for example, only one QSO had a coincident radio source. However, there are details which may or may not be reconciled with the hypothesis. More stringent tests, the most crucial being to study the variations of flux from A and B, must now be made to distinguish between the gravitational lens and other hypotheses.

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The amino acid sequence of the α-chain of human fibrinogen

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The amino acid sequence of the human fibrinogen α-chain reveals a structure that can be divided into three zones of unique amino acid composition. The middle of these contains the two primary α-chain cross-linking acceptor sites and consists of a remarkable series of internal duplications.

THE α-chain is the largest of the three non-identical chains that compose the human fibrinogen molecule $(\alpha_2 \beta_2 \gamma_2)$[1]. It contains 610 residues and has a calculated molecular weight of 66,125. Unlike the β- and γ-chains, it does not contain covalently bound carbohydrate[2,3]. More than 90% of the α-chain amino acid sequence has previously been reported[4–10]. In this article we report the complete amino acid sequence (Fig. 1) and comment on a variety of structural and evolutionary features revealed by these determinations. The completion of the sequence was accomplished by the isolation of an 84-residue peptide from a staphylococcal protease digest of the largest α-chain cyanogen bromide fragment. A summary of the data used to obtain the sequence is depicted in Fig. 2.

Distribution of amino acids

A linear residue-by-residue plot of the amino acid sequence of the human fibrinogen α-chain indicates that the structure can be divided into three distinct zones of approximately 200 residues, each of which is readily distinguishable on the basis of amino acid composition alone (Fig. 3). We do not know whether these zones are equivalent to independently folding domains. The regions are designated ZN, ZM and ZC and correspond roughly to the amino-terminal third (ZN), the middle third (ZM), and the carboxy-terminal third (ZC), respectively. A short interzonal sequence between zones ZN and ZM has been designated IZ (Fig. 4). In zone ZN, the first 194 residues from the amino-terminus, the amino acid composition is well balanced except for a very long proline-free stretch and a notable deficiency of glycine residues. This part of the α-chain is linked to the β- and γ-chains by two sets of unique six-cysteine combinations which we have called disulphide rings[11]. The regions between these delineating rings are thought to be three-stranded compound α-helices of the sort known as coiled coils[11]. Indeed, computer-generated conformational prediction schemes[12] indicate that virtually all the α-helical content of the α-chain occurs in zone ZN (Fig. 4).

Zone ZM (middle section) is composed of residues 240–424. More than half the 185 residues in this section are glycine, serine, proline or threonine, and, with the exception of tryptophan, there are few amino acids with nonpolar side chains. The tryptophan exception is extraordinary in that 7 of the α-chain's 10 tryptophan residues occur in this otherwise

```
  1  A D S G E G D F L A E G G G V R G P R V V E R H Q S A C K D
 31  S D W P F C S D E D W N Y K C P S G C R M K G L I D E V N Q
 61  D F T N R I N K L K N S L F E Y Q K N N K D S H S L T T N I
 91  M E I L R G D F S S A N N R D N T Y N R V S E D L R S R I E
121  V L K R K V I Q K V Q H I Q L L Q K N V R A Q L V D M K R L
151  E V D I D I K I R S C R G S C S R A L A R E V D L K N Y E D
181  Q Q K Q L E Q V I A K D L L P S R D R Q H L P L I K M K P V
211  P N L V P G N F K S Q L Q K V P P E W K A L T D M P Q M R M
241  E L E R P G G N E I T R G G S T S Y G T G S E T E S P R N P
271  S S A G S W N S G S S G P G S T G N R N P G S S G T G S G A
301  T W K P G S S G P G S T G S W N S G S S G T G S T G N Q* N P
331  G S P R P G S T G T W N P G S S E R G S A G H W T S E S S V
361  S G S T G Q* W H S E S G S F R P D S P G S G N A R P N D P N
391  W G T F E E V S G N V S P G T R R E Y H T E K L V T S K G D
421  K E L R T G K E K V T S G S T T T T R R S C S K T V T K T V
451  I G P D G H K E V T K E V V T S E D G S D C P E A M D L G T
481  L S G I G T L D G F R H R H P D E A A F F D T A S T G K T F
511  P G F F S P M L G E F V S E T E S R G S E S G I F T N T K E
541  S S S H H P G I A E F P S R G K S S S Y S K Q F T S S T S Y
571  N R G D S T F E S K S Y K M A D E A G S E A D H E G T H S T
601  K R G H A K S R P V
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Fig. 1 Complete amino acid sequence of the α-chain of human fibrinogen.

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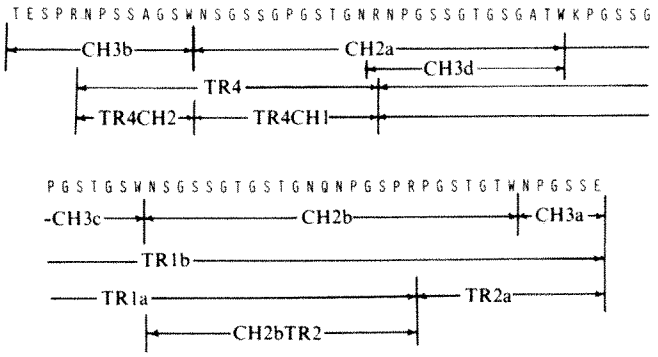


Fig. 2 Summary of data used to deduce the sequence of an 84-residue peptide isolated from a staphylococcal protease digest of the largest α-chain cyanogen bromide fragment. TR = trypsin; CH = chymotrypsin. The sequences of the various subpeptides were determined by procedures reported in our earlier papers. Full experimental details will be presented elsewhere.

exceedingly polar zone. Moreover, the tryptophan residues occur with a definite periodicity reflecting an extended series of 13-residue homology repeats (Fig. 5). The homology repeats, which can also be depicted as a set of 39-residue duplications, have been evaluated by the computer conformation programs and assessed as being predominantly random coil with some contribution from reverse turns. A prototype sequence, derived from a count of the most frequent residues at each position of the 13-residue segments, was also evaluated and found to be a random coil with regularly interspersed turns (Fig. 5).

The carboxy-terminal zone (ZC; residues 425–610) is also rich in serine, threonine and glycine, but in addition, this section contains a large number of amino acids with nonpolar side chains. Although there are no tryptophan residues, more than a quarter of the 186 residues are categorised as nonpolar. A single interchain disulphide loop connects residues 442 and 472. As in the case of ZM, the major conformational prediction is for random coil, although a short segment of α-helix is predicted for the region 583–591.

Distribution of charges

Approximately one-quarter of the α-chain's 610 residues are charged at neutral *p*H, the net charge being about +6 (Table 1). This net positive charge is partly offset by the fractional occurrence of phosphoserine residues that have not been included in the estimate. Moreover, each of the major zones is effectively neutral, compensating numbers of positive and negative charges being present throughout. An exception occurs in the 45-residue interzonal segment IZ, where basic residues (neglecting the single histidine) outnumber acidic residues by eight to three. Zone ZM is not only effectively neutral, but it also bears fewer charges generally than the other two major segments; only one-sixth of its side chains are charged at neutral *p*H (Table 1).

Although we have commented on the general charge distribution of the entire fibrinogen molecule elsewhere[13], it is useful to summarise some of those observations here (Table 2). Thus, the net charge on the three non-identical chains from fibrinogen as estimated from their amino acid sequences is in complete agreement with their observed behaviour on CM-cellulose chromatography, the most negatively charged γ-chain eluting first, followed by the β-chain, which is less negative, and finally, by the α-chain with its net positive charge. Moreover, the sum of the estimates (Table 2) indicates that at *p*H 7.3 the parent fibrinogen molecule bears a net charge of about −20. When considered domain by domain, it seems that almost half of this negativity is localised in the central domain (net charge −8) and that a charge reversal occurs centrally on removal of the two pairs of negatively charged fibrinopeptides (A and B) by thrombin. The terminal domains (compare fragments D) are also negative (−4 each), whereas the combined α-chain protuberances (ZM and ZC) are only very slightly negative (−1 each).

Susceptibility to proteases

The susceptibility of the α-chain to proteases—especially as the chain exists in the native fibrinogen molecule—provides certain insights into its domainal structure. In particular, the 45-residue

Table 1 Distribution of charges in α-chain by zone

Zone	Residues	Lys Arg	His	Glu Asp	Approximate net charge*	% Charged residues
ZN	1–194	33	3	32	+2	(34%)
IZ	195–239	8	1	3	+5	(24%)
ZM	240–424	15	3	16	0	(16%)
ZC	425–610	23	8	26	−1	(27%)
Total chain	1–610	79	15	77	(+5)	(26%)

*Calculated for *p*H 7.3; histidine *p*K taken as 6.7. The estimate does not include contributions from fractionally occurring phosphoserines.

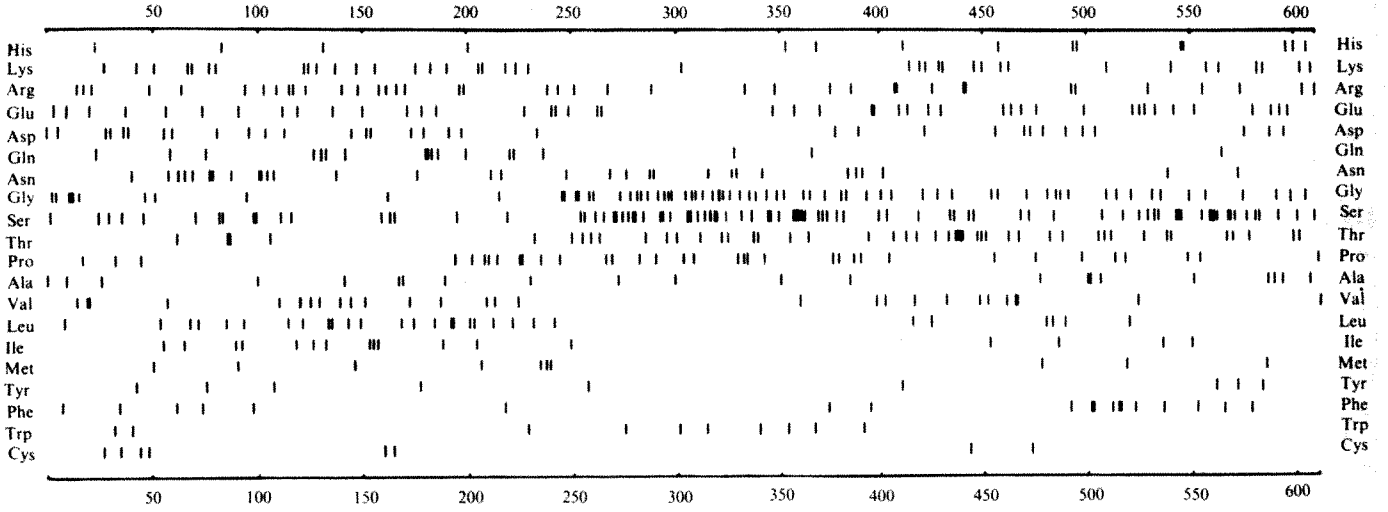


Fig. 3 Distribution of amino acids in the α-chain of human fibrinogen showing how composition changes radically from one end of the chain to the other. Zone N (amino-terminal) includes residues 1–194. The distribution of amino acids is fairly even except for the marked deficiency of proline and glycine. It is separated from the next major sector by a connecting segment (IZ = 195–245) that is rich in proline and exceedingly susceptible to proteolytic excision. Zone M (middle) extends from residues 246 to about 410. More than half the residues in this section are glycine, serine, proline or threonine, but it also contains seven tryptophan residues. The section contains only two glutaminyl side chains, both of which are cross-linking acceptor sites. The third sector (ZC) begins at about residue 411 and extends to the carboxy-terminus.

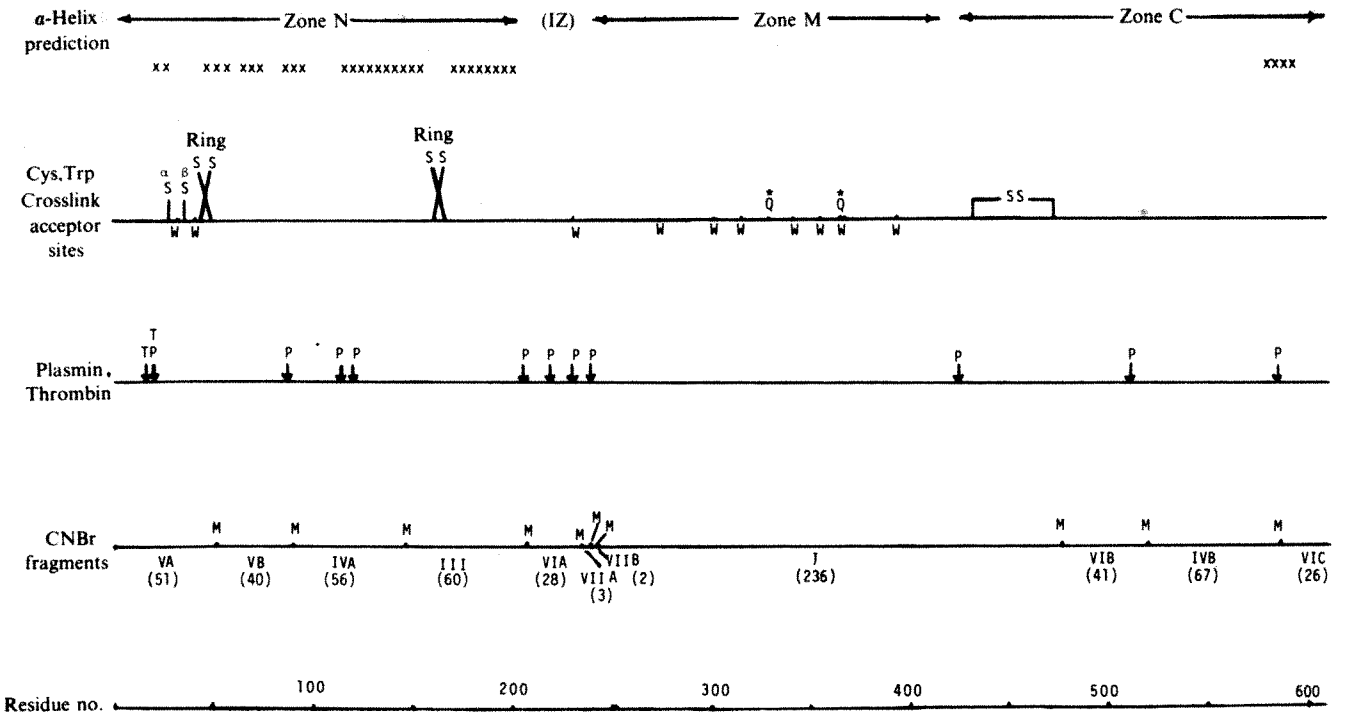


Fig. 4 Schematic representation of human fibrinogen α-chain showing thrombin attack point in releasing fibrinopeptide A(T), principal plasmin attack points (P), cross-linking sites (Q̊), 10 tryptophan residues (W) and 8 cysteine residues (C). Also shown are the 11 peptides produced by cyanogen bromide cleavage at the 10 methionine residues (M). Regions of computer-predicted α-helix are denoted by ××××.

interzonal (IZ) stretch from residues 195 to 239 includes four plasmin attack points[5]. This short segment contains eight proline residues which reflect the radical change in amino acid composition at this junction and mark the end of the long α-helical section that extends through most of ZN. Its net positive charge (Table 1) may also be a factor in its susceptibility to plasmin. The same region is apparently attacked by several proteases other than plasmin, as evidenced by the fact that digestion of fibrinogen (or fibrin) by a variety of enzymes leads to similar sets of early digestion products, at least on a gross level[14]. This kind of substrate-directed vulnerability indicates that the interzonal segment is exposed and accessible.

A second major attack region for plasmin occurs in the middle of the 'coiled-coil' region. The α-chain, in common with the β- and γ-chains (Fig. 6), is broken at several lysine and arginine residues in this region to give rise (ultimately) to the core fragments known as fragments D and E (ref. 15)

Plasmin also attacks the α-chain and/or its largest cyanogen bromide fragment, CNI (Fig. 4), at a point (Arg 424) separating ZM and ZC. Several other plasmin cleavages also occur, both in the native fibrinogen molecule and in the reduced and alkylated α-chain, especially near the ends of the chain. Finally, as is well known, thrombin triggers the polymerisation of fibrinogen to yield fibrin by cleaving the arginyl-glycine bond at α16–17, thereby releasing the fibrinopeptide A and exposing the Gly-Pro-Arg sequence at residues α17–19 which is apparently essential for polymerisation.

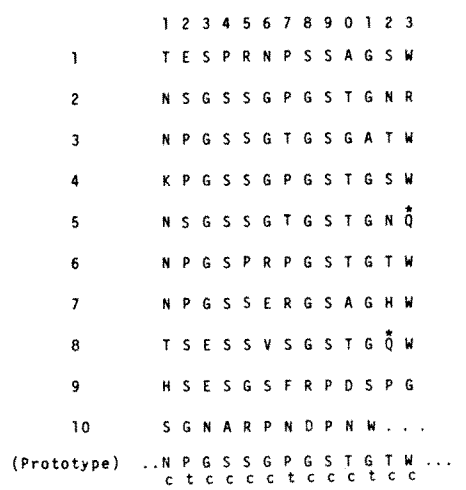


Fig. 5 Amino acid sequence of a section from zone M showing 13-residue homology repeat that occurs 10 times in tandem. A prototype sequence is shown at the bottom together with a computer-derived structural prediction for each residue (c = random coil; t = reverse turn). Asterisks denote cross-link acceptor sites.

Other functional considerations

α-Chain cross-linking: Although the first stages of fibrin polymerisation do not involve covalent bonds, the polymer is subsequently reinforced ('stabilised') by the introduction of intermolecular γ-glutamyl-ε-lysine peptide bonds. The formation of these covalent bonds between side chains is catalysed by the transglutaminase known as factor XIII. The first of these bonds formed involves γ-chains exclusively, a set of reciprocal linkages being formed intermolecularly between abutting γ-chains in the polymer, which, on reduction and dissociation of the parent fibrin(ogen) molecules, gives rise to 'γ–γ dimers' (ref.

Table 2 Distribution of charges on human fibrinogen by chain*

Chain	Lys Arg	His	Glu Asp	Sialic acid	Phospho-serine	Approximate net charge‡
α-Chain	79	15	77	0	1†	+5
β-Chain	58	7	61	2	0	−5
γ-Chain	44	10	54	2	0	−10
αβγ	181	32	192	4	1	−10
$\alpha_2\beta_2\gamma_2$	362	64	384	8	2	−20

*Adapted from ref. 13.
†Phosphoserine occurs fractionally at two or more locations.
‡Calculated for pH 7.3; histidine pK taken as 6.7.

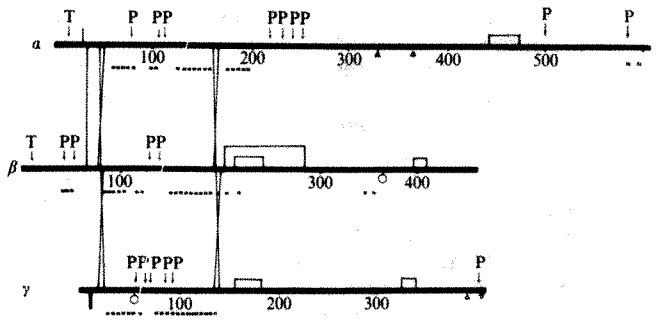


Fig. 6 Schematic depiction of three non-identical chains of human fibrinogen showing disulphide connections, carbohydrate attachment sites (◇), thrombin (T) and plasmin (P) attack points, cross-linking sites (△ and ▽) and computer-predicted helical regions (≈ ≈ ≈).

16). In a significantly slower involvement, α-chains can also be cross-linked by factor XIII, the bonding in this case leading to extended interchain involvements referred to as α-chain multimers[17]. However, the overall geometry and arrangement of these multimeric arrays has been unclear. We can now report the exact position of those α-chain glutamine residues that incorporate radioactive substitute donors (^{14}C-glycine ethyl-ester) under the influence of activated factor XIII. Thus, glutamines α328 and α366, both of which occur in ZM, are the only acceptor sites for the substitute donor. Significantly, however, they are also the only glutamine residues in ZM, thus raising the question of whether the specificity is narrow because there are only two glutamine residues or vice versa, an evolutionary exclusion of glutamine residues being required to maintain the ordered cross-linking of these randomly disposed segments. Furthermore, there is only a single glutamine residue in the carboxyl-terminal zone ZC (Gln 563), and it was devoid of radioactivity; nor were any of the 15 glutamine residues in ZN and IZ labelled. Furthermore, in neither Gln 328 nor Gln 366 does the surrounding sequence bear any resemblance to the γ-chain cross-linking acceptor site[16].

Other α-chain involvements: Fibrinogen is widely known to interact with certain other plasma proteins and particles, including associations with fibronectin ('cold-insoluble globulin')[18,19], platelets[20] and certain strains of staphylococcal bacteria[21]. These interactions are thought to involve the carboxyl two-thirds of α-chains, evidence for this including the observation that plasmic digestion of fibrinogen removes those regions concomitant with a loss of interaction[22–24].

During our determination of the α-chain structure we developed a procedure for isolating a soluble fragment corresponding to residues 241–424 ($n = 184$ residues). This fragment, which is a plasmic digestion product of the cyanogen bromide fragment CNI, includes both cross-linking acceptor sites. It also contains two tyrosine residues which can be iodinated. Thus, the fragment should prove very useful in pinpointing many of the extramolecular involvements cited above.

The α-chain protuberance comprised of ZM and ZC also affects other aspects of fibrinogen–fibrin behaviour. It is well known, for example, that fibrinogen 'sticks' to most non-biological materials, including the plastics used in most circulatory-assistance devices. It is possible that this is the result of exposing the tryptophan residues that are normally 'caged' by the repetitive Pro-Gly-Ser-Thr combinations. Also, these portions of the protein are very accessible to cross-linking agents and other types of chemical derivatisation[25]. Furthermore, it has so far not been possible to crystallise mammalian fibrinogens until portions of the α-chain have been digested away[26]. Finally, high-solubility fibrinogen preparations seem to lack these regions of the α-chain.[27]

```
α ADSGEGDFLAEGGGVRGPRVVERHQSA                                  CKDSDWPFC  SDEDWNYKCPSGCRMKGLIDEVNQDFTNRINKLKNSLFEYQKNNK DSHSLTT
β   QGVNDNEEGFFSARGHRPLDKKREEAPSLRPAPPPISGGGYRARPAKAAATQKKVERKAPDAGGCLHADPDLGVLCPTGCQLQEALLQQERPIRNSVDELNNNVEAVSQTSSSSQFYMYL
γ                                                                YVATRDNCCILDERFGSYCPTTCGIADFLSTYQTKVDKDLQSLEDILHQVENKTS EVKQLIK

α NIMEILRGDFSSANNRDNTYNRVSEDLRSRIEVLKRKVIQKVQHIQLLEKNVRAQLVDMKRLEVDIDIKIRSCRGSCSRALAREVDLKNYEDQQKQLEQVIAKDLLPSRDRQHLPLIKMK
β   LKDLWQKRQKQVKDNENVVNEYSSELEKHQLYIDETVNSNIPTNLRVLRSILENLRSKIQKLESDVSAQMEYCRTPCTVSCDIPVVSG      KECEEIIRKGGETSEMYLIQPDSSVK
γ   AIQLTYNPDESSKPNMIDAATLKSRKMLEEIMKYEASILTHDSSIRYLQEIYNSNNQKIVNLKEKVAQLEAQCQEPCKDTVQIHDITG      KDCQDIANKGAKQSGLYFIKPLKANQ

α PVPNLVPGNFKSQLQKVPPEWKALTDMPQMRM                                ELERPGGNEITRGGSTSYGTGSETESPRNPSSAGSWNSGSSGPGSTGNRNPGSSGTGSGATW
β PYRVYCDMNTENGGWTVIQNRQDGSVDFGRKWDPYKQGFGNV ATNTDGKBYCGLPGEYWLGBBKISELTRMGPTELLI  EMEDWKGDKVKAHYGGFTVQNEANKYQISVNKYRGTAGNA
γ QFLVYCEIDGSGNGWTVFQKRLDGSVDFKKNWIQYKEGFGHLSPTGTT          EFWLGNEKIHLISTQSAIPYALRVELEDWNGRTSTADYAMFKVGPEADKYRLTYAYFAGGDAGD

α KPGSSGPGSTGSWNSGSSGTGSTGNQNPGSPRPGSTGTWNPGSSERGSAGHWTSESSVSGSTGQWHSESGSFRPDSPGSGNARPNDPNWGTFEEVSGNVSPGTRREYHTEKLVTSKGDKELR
β                                                                                                                LMDGASQL MGENRTMTIHNGMFFS
γ                                                                                                                AFDGFDFGDDPSDKFFTSHNGMQFS

α TGKEKVTSGSTTTTRRSCSKTVTKTVIGPDGHKEVTKEVVTSEDGSDCPEAMDLGTLSGIGTLDGFRHRHPDEAAFFDTASTGKTFPGFFSPMLGEFVSETESRGSESGIFTNTKESSSHHP
β TYDRDNDGWLTSDPRKQCSKEDGGGWWYBRCHAANPNGRYYWGGQYTWDMAKHGTBDGVVWMNWKGSWYSMRKMSM      KIRPFFPQQ
γ TWDNDNDKFEGN      CAEQDGSGWWMNKCHAGHLNGVYYQGGTYSKASTPNGYDNGIIWAT KTRWYSMKKTTM      KIIPFNRLTIGEGQQHHLGGAKQAGDV

α GIAEFPSRGKSSSYSKQFTSSTSYNRGDSTFESKSYKMADEAGSEADHEGTHSTKRGHAKSRPV
β
γ
```

Fig. 7 Comparison of α-chain amino acid sequence with sequences of β- and γ-chains[31–37] showing significant homology among all three chains over the course of the first 210 residues, but a loss of homology in the α-chain thereafter. The 'best match' leaves a gap throughout most of zone M, some faint resemblance between the α-chain and the other two chains apparently resuming near the carboxyl-terminal segment. Other equivalent alignments are possible, however. As previously noted, the β- and γ-chains are strongly homologous over the course of their carboxy-terminal two-thirds. Matched identical residues are underlined.

Evolutionary significance

The α-, β- and γ-chains of fibrinogen are all equivalently homologous in the region spanned by ZN[28]. Thus, all three chains have two characteristic Cys-X-X-X-Cys sequences spaced appropriately for participation in the two sets of disulphide rings (Fig. 6). Also, both the α- and β-chains have thrombin-sensitive sites near their amino-termini that give rise to the fibrinopeptides A and B, respectively (Fig. 6). Shortly beyond the beginning of ZM, however, the resemblance between the α-chain and the β- and γ-chains abruptly disappears. In contrast, beyond that point the β- and the γ-chains display increasing homology[29,30]. Figure 7 shows a computer-assisted alignment of the three chains. Other reasonable alignments are possible, however, including one in which the carboxyl-terminal segments of the α- and γ-chains are directly aligned.

Given that the prototype fibrinogen molecule originated from two pairs of three identical chains, the question arises, did the β- and γ-chains lose the equivalent of zone ZM, or did it evolve in the α-chain by a more recent set of tandem duplication? In either event, the strong homology between the β- and γ-chain carboxyl-terminal segments suggests that these two chains shared a common ancestor more recently than either did with the α-chain. Moreover, the radical differences in the nature of the various α-chain zones suggests the occurrence of events other than simple gene duplication in the evolution of the α-chain. It is possible that gene splicing was involved, although we know of no other protein with a composition comparable to ZM that might be a candidate for insertion.

The International Committee on Blood Coagulation Nomenclature prefers the convention $A\alpha_2B\beta_2\gamma_2$ as a description of the six chains in the fibrinogen molecule.

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The membrane attachment protein for spectrin is associated with band 3 in human erythrocyte membranes

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Ankyrin, the membrane attachment protein for human erythrocyte spectrin, is tightly linked in a 1:1 molar ratio with band 3 in detergent extracts of spectrin-depleted membranes. Ankyrin-linked band 3, which represents 10–15% of the total band 3, spans the membrane, and is nearly identical to the major band 3 by peptide analysis. Spectrin binds to solubilised ankyrin-linked band 3, but not to free band 3. A portion of band 3 remains firmly associated with detergent-extracted cytoskeletal proteins. It is concluded that a fraction of band 3 is attached to the erythrocyte cytoskeleton through association with ankyrin, which in turn is bound to spectrin.

EXTENSIONS of the fluid mosaic model for membranes[1,2] have proposed a direct association between membrane-spanning cell-surface proteins and cytoplasmic structural proteins on the inner surface of the plasma membrane[3,4]. These protein interactions may be key events in complex cellular phenomena such as the capping of surface molecules by lymphocytes[5], redistribution and internalisation of receptors for hormones[6] and low-density lipoprotein[7], cell motility[8] and attachment of cells to surfaces[9]. Evidence has been presented for binding of actin[10,11] and intermediate filament protein[12] to cell-surface proteins. However, there is little detailed knowledge of the polypeptides involved in these linkages or of the mechanisms which regulate such interactions. The human erythrocyte provides an experimentally accessible model system for examining protein–membrane interactions at a molecular level. The shape and elasticity of red cell membranes[13,14] and the distribution of intramembrane particles[15] and glycoproteins[16] are controlled by proteins arranged in a cytoskeletal network on the inner surface of the membrane. Direct analysis of the components involved in these phenomena can be achieved, in principle, by measuring reassociation of purified cytoplasmic proteins with acceptor sites exposed on inverted membrane vesicles. This approach has been used to examine the membrane attachment site for human erythrocyte spectrin, which is the major protein in the red cell cytoskeleton.

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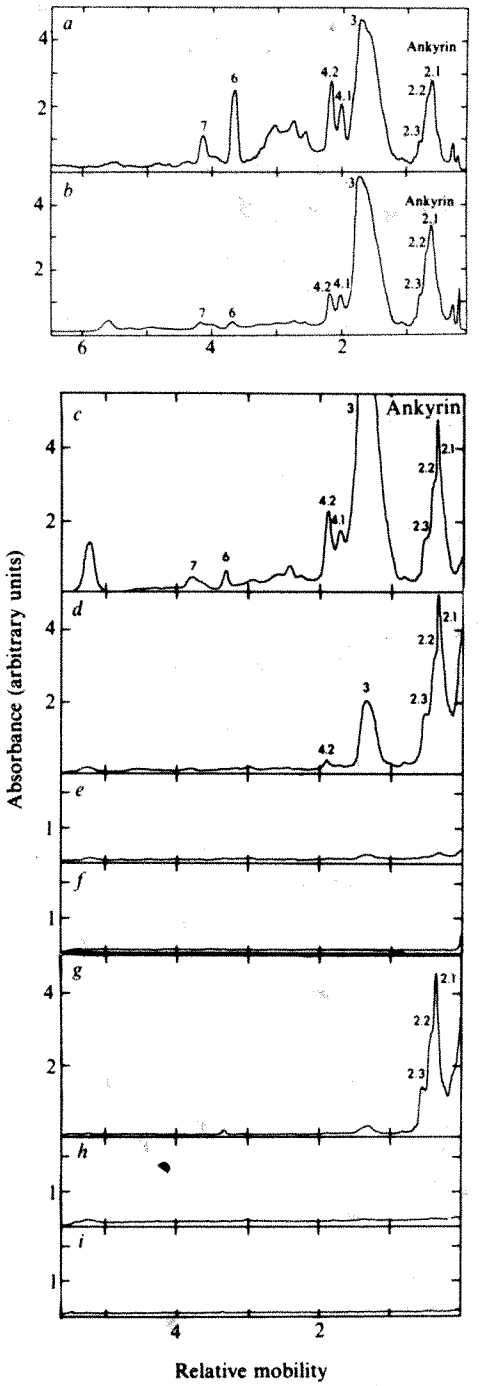


Fig. 1 Isolation of ankyrin-associated proteins by immunoadsorption with anti-ankyrin IgG of Triton X-100-solubilised spectrin-depleted vesicles labelled with [^{125}I]-Bolton Hunter reagent. Spectrin-depleted inverted vesicles[18] from 1-ml erythrocyte ghosts were reacted with 2 mCi [^{125}I]-Bolton Hunter reagent (1,500 Ci mmol^{-1}, NEN) in 0.2 ml 7.5 mM sodium phosphate, *p*H 7.5, for 60 min at 0 °C. The labelled vesicles (1.1 mCi incorporated) were washed, extracted (15 min, 24 °C) with 1.2 ml 7.5 mM sodium phosphate, *p*H 7.5, 1 mM NaEDTA, 0.2 mM dithiothreitol, 10 μg ml^{-1} phenylmethylsulphonyl fluoride and 2% (v/v) Triton X-100, and pelleted (30 min, 250,000*g* at 2 °C). The supernatant was made 0.1 M in KCl, and 10-μl aliquots were either denatured with an equal volume of 1% (w/v) SDS (30 min, 37 °C) or untreated. These samples were incubated (90 min, 0 °C) in 0.3 ml buffer containing 250 μg ml^{-1} bovine serum albumin, 2.5 μg ml^{-1} pancreatic trypsin inhibitor, 100 mM NaCl, 10 mM sodium phosphate, *p*H 7.5, 1 mM NaEDTA, 0.2% Triton X-100, 0.25 μl protein A-bearing fromalin-fixed staphylococci (Calbiochem) and either 2.5 μg affinity-purified anti-ankyrin IgG[20] or 2.5 μg preimmune IgG, and in two tubes 15 μg of the spectrin-binding fragment of ankyrin[19]. The reaction was stopped by addition of 3.5 ml 2 M urea, 1% Triton X-100 and 0.1 M glycine, and the bacteria collected by centrifugation (15 min, 4,000*g*). The bacteria with adsorbed immune complexes were resuspended with 0.1 ml 1% SDS, 40 mM dithiothreitol, 10 mM Tris HCl, *p*H 8, and 1 mM NaEDTA, and 20-μl portions analysed by SDS electrophoresis on a 1.5-mm thick 7.5% polyacrylamide slab gel[49]. The gel was stained with Coomassie blue, dried and developed by autoradiography (bottom panel). The detergent extract used for immunoadsorption was diluted to an equivalent volume to that of the precipitates for analysis. Spectrin-depleted everted vesicles used in this experiment were also electrophoresed, and the gels stained with Coomassie blue and visualised by autoradiography. *a*, Coomassie blue stained; *b* ^{125}I-labelled; *c*, Triton X-100 extract; *d*, immunoprecipitated extract + anti-ankyrin; *e*, extract + ankyrin fragment + anti-ankyrin; *f*, extract + preimmune IgG; *g*, immunoprecipitated SDS-denatured extract + anti-ankyrin; *h*, SDS-denatured extract + ankyrin fragment + anti-ankyrin; *i*, SDS-denatured extract + preimmune IgG.

Spectrin has been radiolabelled with $^{32}P_i$ in intact erythrocytes and purified as an 8S dimer which can recombine with inverted vesicles depleted of spectrin and actin[17]. ^{32}P-Spectrin binds to vesicles with a K_D of 10^{-7}–10^{-8} M, binding to a single class of protein sites tightly bound to the inner surface of the membrane, and present in about 10^5 copies per cell[18]. A water-soluble, 72,000-molecular weight (MW) fragment of the attachment protein is released by mild proteolysis of inverted vesicles, and this polypeptide has been isolated and characterised[19]. The purified attachment fragment binds to spectrin with a 1 : 1 molar ratio, and competes for reassociation of spectrin with vesicles[19]. A 200,000-MW protein localised on the cytoplasmic membrane surface has been identified as the precursor of the spectrin-binding fragment, on the basis of cross-reactivity with monospecific anti-fragment IgG (ref. 20) and by peptide mapping[21]. This protein, which has been named ankyrin, has been partially purified and found to bind to spectrin as well as competitively inhibiting reassociation of spectrin with the membrane[20].

Ankyrin dissociates from the membrane following treatment with dilute acid[20], and thus is not an integral membrane protein. It probably does interact with such proteins, as the lateral mobility of fluorescence-labelled integral proteins is increased by incubation of erythrocyte ghosts with the spectrin-binding fragment of ankyrin[22]. This report demonstrates association of ankyrin with band 3, the major membrane-spanning polypeptide of the erythrocyte membrane[23–25].

Identification of ankyrin-associated polypeptides

Ankyrin-associated polypeptides were isolated from Triton X-100-solubilised, spectrin-depleted vesicles by immunoadsorption with monospecific anti-ankyrin IgG. The antiserum was raised against the 72,000-MW spectrin-binding fragment of ankyrin which had been isolated by SDS-polyacrylamide gel electrophoresis (PAGE), and ankyrin-specific IgG was purified by affinity chromatography with immobilised fragment[20]. The immune complexes formed between anti-ankyrin and the membrane proteins were isolated with formalin-fixed protein A-bearing staphylococci, which bind with high affinity to the Fc portion of IgG[26]. The bacteria were subsequently washed with 2 M urea and 1% Triton X-100 to minimise nonspecific interactions. It should be emphasised that only proteins that are tightly associated and unaffected by Triton X-100 will be detected by this procedure.

Membrane polypeptides were analysed in nanogramme amounts without interference from immunoglobulin chains by labelling vesicles with ^{125}I-labelled Bolton Hunter reagent. This reaction is achieved in mild conditions, and radiolabels identical polypeptides as visualised by staining with Coomassie blue. Band 3 and ankyrin (bands 2.1, 2.2, 2.3) are labelled in nearly the same relative intensity, although other polypeptides are labelled to a lesser extent by the Bolton Hunter reagent (Fig. 1).

Immunoadsorption of solubilised vesicles with anti-ankyrin IgG resulted in binding of essentially all the 200,000-MW polypeptide (ankyrin) and of polypeptides of ~180,000 and 190,000 MW (bands 2.3 and 2.2) which are proteolytic fragments of ankyrin[20,27]. In addition, the immunoprecipitate

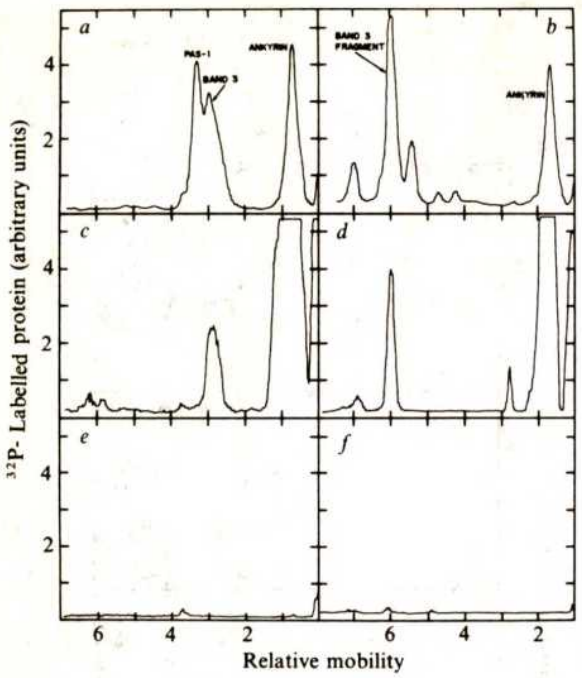
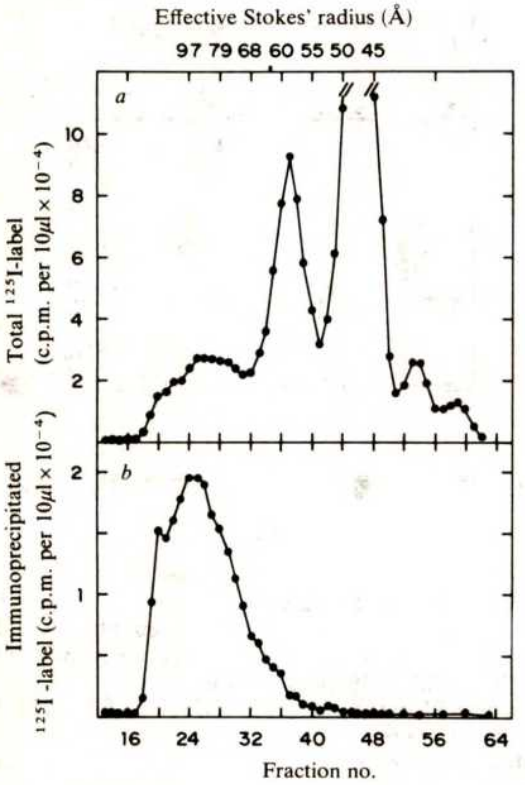


Fig. 2 Immunoadsorption with anti-ankyrin IgG of Triton X-100-solubilised spectrin-depleted vesicles from ^{32}P-labelled erythrocytes that were untreated or externally digested with α-chymotrypsin. Packed erythrocytes (3 ml) were incubated for 150 min at 37 °C in the presence or absence of 100 μg ml^{-1} α-chymotrypsin (70 U per mg) in 20 ml of Krebs–Ringer bicarbonate buffer supplemented with 200 U ml^{-1} penicillin G and 10 mM D-glucose. The cells were washed three times in 0.15 M NaCl, and then labelled with $^{32}P_i$ during a 15-h incubation at 37 °C in 5 ml of buffer containing 135 mM NaCl, 20 mM $NaHCO_3$, 4 mM KCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM D-glucose, 1 mM adenosine, 200 U ml^{-1} pencillin G, 50 μg ml^{-1} phenylmethylsulphonyl fluoride and 0.5 mM [^{32}P]$NaPO_4$ (4 Ci mmol^{-1}, NEN), *p*H 7.5. Spectrin-depleted inverted vesicles were prepared from the ^{32}P-labelled cells[18], which were solubilised, immunoadsorbed and analysed as described in Fig. 1. The data are presented as densitometer scans of the portions of autoradiograms corresponding to MWs greater than 50,000. Band 3 and band 3 fragment were identified by comparison with the Coomassie blue-stained gels. *a*, Undigested ^{32}P-labelled vesicles, Triton X-100 extract; *b*, externally digested ^{32}P-labelled vesicles, Triton X-100 extract; *c*, immunoprecipitate of undigested extract + anti-ankyrin; *d*, immunoprecipitate of externally digested extract + anti-ankyrin; *e*, immunoprecipitate of undigested extract + ankyrin fragment + anti-ankyrin; *f*, immunoprecipitate of externally digested extract + ankyrin fragment + anti-ankyrin.



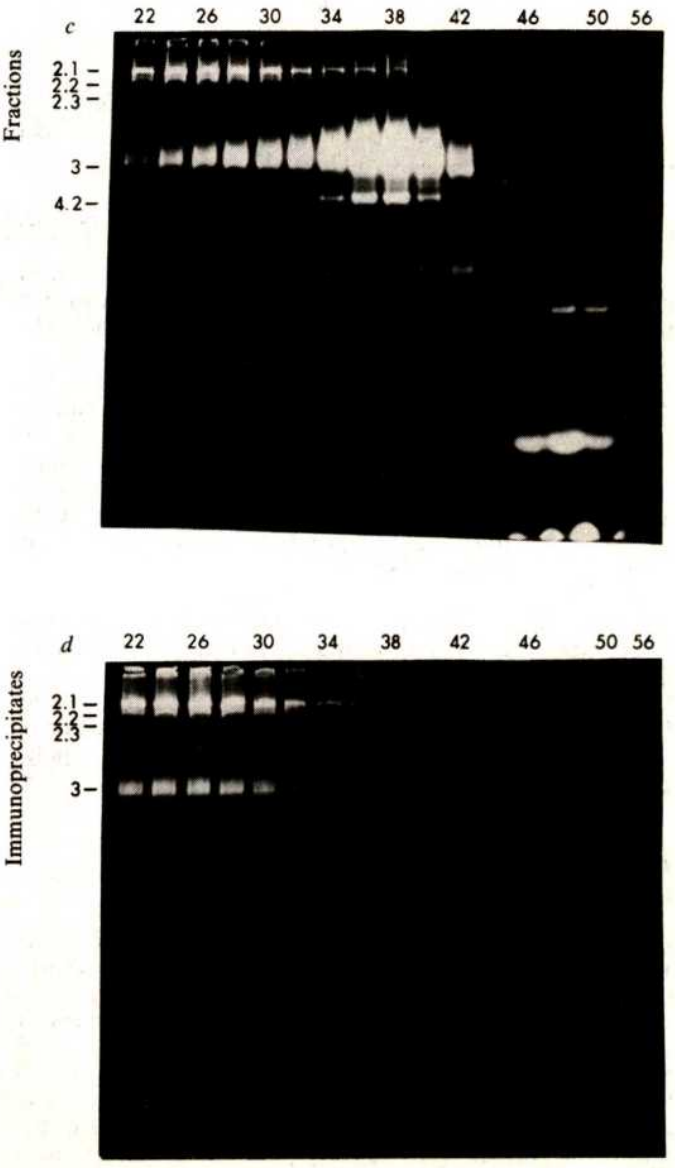


Fig. 3 Ultrogel AcA22 chromatography of Triton X-100-solubilised spectrin-depleted vesicles labelled with [^{125}I]-Bolton Hunter reagent. Vesicles were labelled and solubilised (Fig. 1) and a 0.7-ml sample chromatographed at 4 °C on a column (1.0 × 60 cm) packed with Ultrogel AcA22 (LKB) and equilibrated with 100 mM KCl, 10 mM $NaPO_4$, *p*H 7.5, 1 mM NaEDTA, 1 mM NaN_3, 0.2 mM dithiothreitol and 0.2% Triton X-100. Fractions (0.8 ml) were assayed for total radioactivity (*a*) and for immunoprecipitable radioactivity using anti-ankyrin fragment IgG as in Fig. 1 (*b*). Pooled fractions (*c*) and the corresponding immunoprecipitates (*d*) were also analysed by SDS electrophoresis. The column was calibrated with rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (44 Å) bovine liver catalase (52 Å), horse spleen ferritin (59 Å) and human IgM (120 Å).

exhibited a diffusely staining band of ~95,000 MW, which contains about 15% of the band 3 material, and a 72,000-MW polypeptide (band 4.2) (Fig. 1). Polypeptides of 28,000 MW (band 7) and 17,000 MW (band 8) were also present, but in smaller amounts which are not well visualised in these experiments. Glycophorin (PAS-1) was not clearly labelled, but was apparent in membranes from erythrocytes incubated with $^{32}P_i$ (ref. 28) (Fig. 2), and following periodate oxidation and tritiation with [^{3}H]$NaBH_4$. ^{32}P-Glycophorin was not immunoprecipitated (Fig. 2), and the same result was obtained with the tritium-labelled protein (not shown).

Several lines of evidence demonstrate that the polypeptides immunoprecipitated by anti-ankyrin IgG were associated with ankyrin, and were not nonspecifically adsorbed or bound directly to the antibody. Unlabelled ankyrin fragment blocked binding of ankyrin as well as of band 3 and the other polypeptides (Fig. 1). Immunoadsorption of solubilised vesicles previously denatured with SDS yielded only ankyrin (bands 2.1, 2.2, 2.3), with no band 3 or band 4.2, and this interaction was also blocked by the ankyrin fragment (Fig. 1). Preimmune IgG precipitated no polypeptides from either denatured or untreated vesicle extracts (Fig. 1). Finally, bands 3 and 4.2, which had been separated from ankyrin by gel filtration (see below), did not cross-react with anti-ankyrin IgG.

Ankyrin-associated polypeptides dissociated very slowly from the ankyrin–IgG complex, with less than 10% loss during a 90-min incubation at 37 °C (not shown). Conditions have not yet been developed to separate ankyrin from these proteins without using denaturing conditions. It was therefore not feasible to carry out reassociation experiments with isolated components of the complex.

Gel filtration of solubilised membranes on an Ultrogel AcA22 column separated ankyrin-linked band 3 from the major pool of band 3 in mild conditions (Fig. 4) and confirms the existence of an oligomer containing ankyrin, and bands 3 and 4.2 as its principal components. Immunoadsorption of the fractions with anti-ankyrin IgG precipitated a single broad peak which migrated with an effective Stokes' radius of about 90 Å, and preceded the major peaks of radioactivity (Fig. 3). Ankyrin chromatographed coincidentally with immunoreactive material, and was associated with bands 3 and 4.2. The relative proportions of ankyrin, band 3 and band 4.2 were about the same in the peak fractions as in the immunoprecipitates, and nearly all the radioactivity was immunoreactive. These fractions re-chromatograph at about the same position (not shown). The major peak of band 3 migrated with band 4.2 with a Stokes' radius of about 58 Å, and was not adsorbed by anti-ankyrin IgG (Fig. 3). Glycophorin labelled with ^{32}P or tritium migrated more slowly than band 3. These results agree with the previous conclusion that bands 3 and 4.2 are associated in detergent extracts, whereas band 3 and glycophorin are dissociated in these conditions[30]. Approximately 80% of band 3 migrated separately from ankyrin, in agreement with the amount of band 3 immunoadsorbed in whole extracts (Fig. 1).

Immunoadsorbed band 3 was present in a 1:1 molar ratio with ankyrin (that is, bands 2.1, 2.2, 2.3) (Fig. 1). (The stoichiometry of band 3 and ankyrin was determined by integration of the area under the respective peaks on autoradiograms, assuming average MWs of 95,000 for band 3 and 200,000 for ankyrin, and that the specific activity of the iodinated polypeptides was the same. This latter assumption is supported by fact that band 3 and ankyrin are labelled in the same relative proportions by Coomassie blue staining as by iodination (Fig. 1).) The major band 3 protein exists as a dimer in erythrocyte ghosts and in Triton X-100-solubilised extracts[30–33]. Band 3 (B3) and ankyrin (A) could thus be arranged as $((B3)_2\text{-}(A)_2)_N$ if the band 3 association remained intact during isolation, or as $((B3)_2\text{-}A)_N$ if the band 3 dimer dissociated in these conditions. Ankyrin and band 3 co-migrated on gel filtration in about a 1:1 molar ratio in mild conditions in which band 3 probably exists as a dimer[30]. It is therefore likely that the ankyrin-linked band 3 oligomer is arranged with 2 mol each of band 3 and ankyrin. This conclusion is supported by the finding that band 2.1 is cross-linked in a dimeric form[31]. In some gel filtration experiments the ratio of band 3 to ankyrin was greater than that of Fig. 3. Thus, additional associations may occur between the ankyrin–band 3–4.2 oligomer and other band 3 molecules, although experimental conditions for stabilising these complexes have not yet been developed.

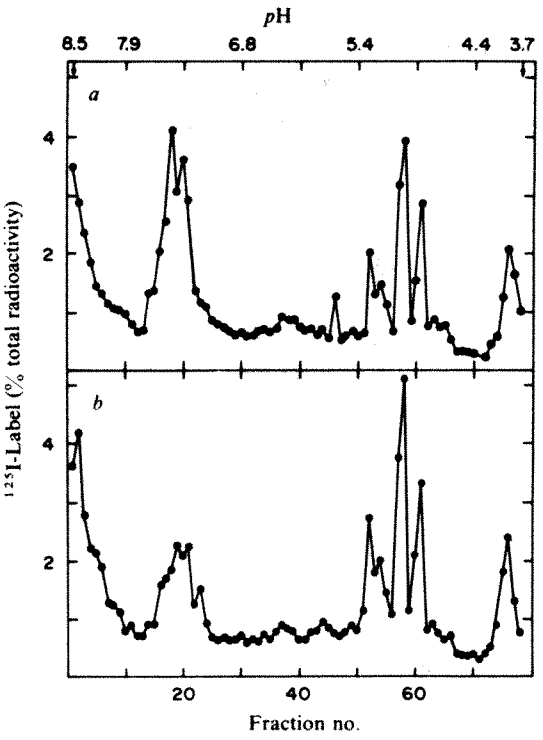


Fig. 4 Isoelectric focusing in polyacrylamide of cyanogen bromide fragments of [^{125}I]-Bolton Hunter-labelled ankyrin-linked band 3 (*a*), and free band 3 (*b*). Ankyrin-linked band 3, isolated by immunoadsorption of solubilised ^{125}I-labelled vesicles (Fig. 1), and free band 3 isolated by gel filtration (Fig. 3) were purified by SDS electrophoresis (Fig. 1). The bands (2×10^5 c.p.m.) were stained with Coomassie blue, cut out and incubated (5 h, 50 °C) in 1 ml of 70% (v/v) formic acid with 40 mg cyanogen bromide. The solutions were separated from polyacrylamide, dried and incubated with 1 M NH_4OH to convert homoserine lactone to the free acid. The samples were reconstituted with 50 μl 9 M urea, 20 mM dithiothreitol, 2% (w/v) ampholines, *p*H 3.5–10 (LKB), and 20 μg ml^{-1} bromphenol blue and analysed by isoelectric focusing (12 h, 1 W) on a 12.5% 1.5-mm polyacrylamide slab gel containing 9 M urea, 1.3% *p*H 3.5–10, 1% *p*H 5–8, 1.3% *p*H 4–6 ampholines and 0.2% Triton X-100. The gels were sliced and radioactivity determined. The data are expressed as the per cent of the total radioactivity recovered from the gel.

Ankyrin-linked band 3 is indistinguishable from free band 3

It was important to determine whether ankyrin-associated band 3 has similar properties to those attributed to the major band 3 protein. Ankyrin-linked band 3 and the total band 3 were both phosphorylated following incubation of intact cells with $^{32}P_i$ (Fig. 2). External cleavage of erythrocytes with α-chymotrpysin reduced both classes of band 3 to a characteristic 55,000-MW membrane-bound fragment[23,34,35] which retained ^{32}P-label (Fig. 2). The ^{32}P-label was cleaved entirely from these fragments by a second digestion of inverted vesicles (not shown), indicating that it is localised on the cytoplasmic surface of the membrane. Thus, ankyrin-linked band 3 spans the membrane, and exhibits a significant similarity to the major band 3 protein.

A more detailed comparison of these polypeptides was provided by isoelectric focusing of cyanogen bromide fragments of SDS-PAGE-purified ankyrin-linked band 3 from immunoprecipitates and free band 3 from gel filtration (Fig. 4). The patterns are nearly identical for the two proteins, although relative peak heights differ (Fig. 4). This difference may be explained by the

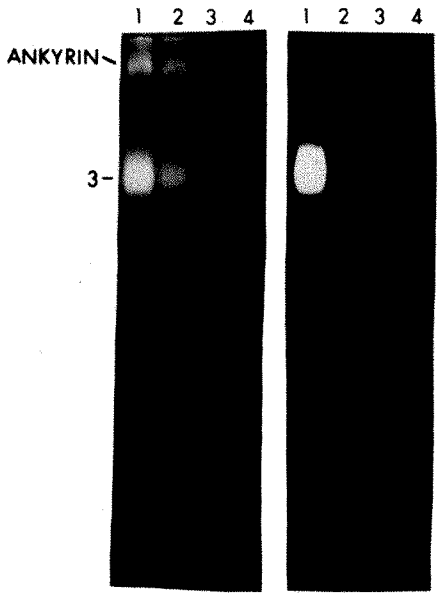


Fig. 5 Binding of purified spectrin to ankyrin-linked band 3 and to free band 3. ^{125}I-Labelled samples from gel filtration of solubilised spectrin-depleted vesicles (Fig. 3) containing ankyrin and band 3–4.2 (left panel, lane 1) or band 3–4.2 free of ankyrin (right panel, lane 1) were incubated (90 min, 4 °C) with 75 μg ml^{-1} purified unlabelled spectrin[17,18] in the absence (lane 2) and presence (lane 3) of 100 μg ml^{-1} unlabelled spectrin-binding fragment of ankyrin[19] in column buffer (Fig. 3). Some of the samples (lane 4) had been acidified to *p*H 2 with HCl and neutralised before addition of spectrin. Spectrin-[^{125}I]-protein complexes were precipitated by addition of monospecific anti-spectrin IgG (affinity purified) and protein A staphylococci, and the pellets were washed with column buffer. The immunoadsorbed proteins were analysed by SDS electrophoresis (Fig. 1) and visualised by autoradiography. The same amount of spectrin was precipitated in the presence or absence of the ankyrin fragment as determined from Coomassie blue staining (not shown).

fact that labelling was carried out with intact vesicles, and bound ankyrin could have altered the reactivity of portions of the band 3 polypeptide. Further evidence that ankyrin-linked band 3 is closely related to free band 3 is that antibodies raised against the 42,000-MW cytoplasmic domain of band 3 (ref. 35) cross-react with both polypeptides (in preparation).

Spectrin binds to ankyrin-linked band 3 but not to free band 3

Incubation of purified spectrin with ^{125}I-labelled ankyrin and band 3–4.2 isolated by gel filtration produced a spectrin–ankyrin–band 3–4.2 complex which was immunoprecipitated by monospecific anti-spectrin IgG (Fig. 5). The complex did not form if these proteins were exposed to acidic *p*H before addition of spectrin (Fig. 5), a treatment which destroys the spectrin-binding capacity of membranes[18]. The association of spectrin with these polypeptides was mediated by ankyrin, as the unlabelled spectrin-binding fragment of ankyrin reduced binding by 90% but had no effect on immunoadsorption of spectrin (Fig. 5). Furthermore, when isolated by gel filtration, band 3–4.2 (lacking ankyrin) did not bind to spectrin (Fig. 5). Other fractions from Fig. 3 also exhibited no binding to spectrin (not shown).

These results indicate that spectrin can reassociate with its membrane attachment site in the presence of Triton X-100. Consequently, it would be expected that the complex of spectrin–ankyrin–band 3–4.2 would be retained by the cytoskeletal assembly which remains after extraction of erythocytes and ghosts with Triton X-100[36,37]. Nearly all of spectrin and 10–15% of band 3 are recovered in Triton X-100-extracted ghosts along with bands 4.1, 4.2 and 7, and erythrocyte actin (Fig. 6). This residual band 3 is tightly adsorbed, and persists at a constant level during repeated washes in detergent (Fig. 6). Ankyrin is poorly visualised due to interference by spectrin, but is detected by radioimmunoassay in the same amounts as in whole ghosts (not shown). Furthermore, ankyrin together with band 3, band 4.2 and band 7 can be extracted without solubilising these Triton residues[38] (Fig. 6). Cytoskeleton-linked band 3 is cleaved to a 55,000-MW fragment by external cleavage of erythrocytes with α-chymotrypsin before detergent extraction (Fig. 6). A second limited digestion of the cytoskeletons with α-chymotrypsin cleaves band 3 and the 55,000-MW fragment (not shown). It can thus be concluded that the cytoskeleton-bound band 3 spans the membrane and is the same polypeptide as the ankyrin-linked band 3.

Discussion

These data indicate that about 15% of band 3 is tightly linked to the erythrocyte cytoskeleton through association with ankyrin, with a stoichiometry of 1 mol band 3 per mol of ankyrin. The ankyrin– band 3 oligomer also contains a 72,000-MW polypeptide (band 4.2) which has been demonstrated to bind to band 3 in solution[30]. Band 3 is present in about 10^6 copies per cell arranged in a dimeric form visualised as intramembrane particles[32]. Ankyrin, which is present in about 10^5 copies per cell, is thus associated with 10^5 band 3 molecules which are presumably also dimers and form from 5×10^4 to 10^5 intramembrane particles, depending on the ankyrin–particle stoichiometry. These ankyrin-linked structures are also the points of attachment of spectrin to the membrane, and may be nucleation sites for assembly of the cytoskeleton. The average nearest neighbour distance between ankyrin–band 3–4.2 oligomers would be 350–500 Å, which is comparable to the dimensions of tetrameric spectrin[39], the form of this protein in erythrocyte ghosts[39,40]. Scanning electron microscopy of erythrocyte ghosts and cytoskeletons reveals a net-like appearance with globular structures 200–1,000 Å apart interconnected by filaments[41]. These dimensions are consistent with the possibility that the interstices of the network contain ankyrin-linked oligomers.

The finding that only a fraction of band-3 proteins are linked to spectrin helps reconcile two conflicting sets of data regarding association of integral proteins with the cytoskeleton in red cell membranes. Spectrin, and possibly actin, are thought to restrict lateral translation of intramembrane particles[15] and to be associated with sialoglycoproteins[16]. Spectrin has been localised in

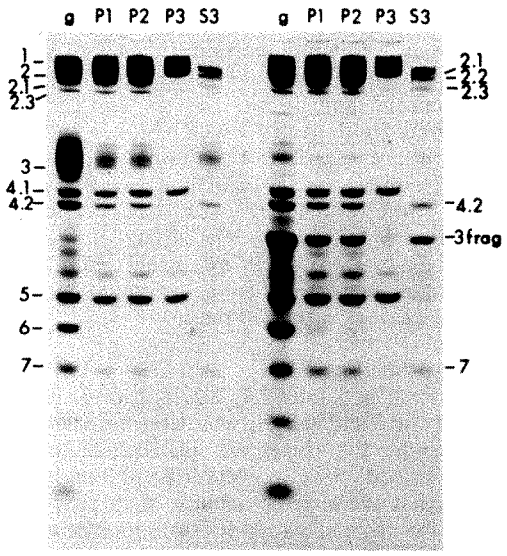


Fig. 6 Retention of band 3 in Triton X-100-extracted cytoskeletons of erythrocyte ghosts. Ghosts were prepared from erythrocytes that were untreated (left) or had been digested externally with α-chymotrypsin (right) as in Fig. 2. Cytoskeletons were prepared by incubating (15 min, 4 °C) the ghosts with 20 vol of 100 mM KCl, 10 mM $NaPO_4$, *p*H 7.5, 1 mM NaEDTA, 10 μg ml^{-1} phenylmethylsulphonyl fluoride, 0.2 mM dithiothreitol and 0.2% (v/v) Triton X-100. Cytoskeletons were pelleted (15 min, 40,000*g*) (P1), washed once in extraction buffer (P2) and incubated (15 min, 0 °C) in extraction buffer plus 1 M KCl. The suspensions were pelleted, the supernatants (S3) removed and dialysed, and the pellets (P3) resuspended in the original volume. The samples were analysed by SDS electrophoresis (Fig. 1) and proteins stained with Coomassie blue.

patterns coincident with intramembrane particles[42]. The lateral mobility of integral membrane proteins is restricted, with a diffusion constant of 5×10^{-12} cm^2 s^{-1} at 22 °C (refs 43, 44), and capping of lectins does not occur in erythrocytes[4]. These experiments have led to the view that intramembrane particles and their component proteins are attached to the cytoskeleton. However, no association could be demonstrated between spectrin and Triton X-100-solubilised band 3 or glycophorin[30]. A direct role for band 3 and the sialoglycoproteins in the attachment of spectrin to the membrane has been ruled out by several experiments[18–20]. The rotational diffusion of band 3 occurs at a rate which indicates that the membrane viscosity should support lateral diffusion at 10^{-9}–10^{-10} cm^2 s^{-1} (ref. 45). Moreover, rotational diffusion of band 3 was unchanged following extraction of spectrin[45]. Further evidence that integral proteins are not all immobilised is the formation of membrane vesicles depleted of spectrin and actin, but containing a full complement of integral proteins[46,47].

The data indicating that band 3 is unrestricted in the membrane are consistent with the present results that only ankyrin-associated band 3 is linked to spectrin. The fact that immobilisation of only a portion of band 3 proteins apparently leads to restriction of all these proteins suggests the involvement of an additional process. It is conceivable that the free proteins are simply entangled in the underlying cytoskeletal meshwork. Another possibility is that the integral proteins associate with each other with low affinity. It is pertinent to this point that intramembrane particles are not dispersed randomly, but rather exist in groups of two to eight. The effect of such aggregation would be to decrease the diffusion constant due to increased size, and to immobilise particles associated with an ankyrin-linked particle.

These studies may have direct relevance to cells other than erythrocytes, as ankyrin has been detected by radioimmunoassay in many tissues and cell types[48]. Future areas of interest include elucidation of the nature of the linkage between band 3 and ankyrin, the role of band 4.2 in this complex, and examination of possible association between ankyrin, band 3 and other membrane proteins such as hormone receptors.

We thank Dr Michael Sheetz for sharing data before publication which demonstrated that cytoskeleton-linked band 3 can be released by high salt extraction.

Note added in proof: Further evidence for association of a portion of the band 3 proteins with the cytoskeleton is provided by the finding that a small amount of band 3 can be chemically cross-linked to band 1 of spectrin in erythrocyte membranes[50].

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letters

A precessing ring model for SS433

THE wavelengths of the shifted optical lines in SS433 seem to show a periodic variability at a period of 160 ± 3 d (or a multiple integer thereof)[1,2]. We have suggested that such periodicity could arise if the lines were emitted from a precessing ring of matter orbiting around a massive black hole (BH) of mass M (ref. 3). We also suggested that SS433 may be located inside an old globular cluster[3]. We describe here, in principle, how such precession model may come about and what some of its observational constraints might be.

We suggest that a normal, solar type, star has been captured[4] by a $\sim 10^6\, M_\odot$ BH. Its orbit then circularises at the tidal radius[5], which is located at $R \sim 45\, R_G (R_G \equiv GM/c^2)$, as is indicated by the wavelengths of the shifted lines[3,2]. Note that the tidal radius for this captured star, of average density ρ, is given by[6]

$$R_T \sim 45 \left(\frac{\rho}{1\ \mathrm{g\ cm^{-3}}}\right)^{-1/3} \left(\frac{M}{3\times 10^6\, M_\odot}\right)^{-2/3} R_G$$

and that the lifetime of that orbit against gravitational radiation is[5]

$$(R/\dot{R})_T \equiv \tau \sim 10^6 \left(\frac{M}{3\times 10^6\, M_\odot}\right)^{-2/3} \ \mathrm{yr}$$

(The lifetime may be shorter when appreciable gas dissipation occurs).

We now assume that this star experiences steady Roche-lobe overflow due to either self expansion or steady drift inwards. Once overflow commences, the particle jet through L1 follows an orbit which is almost identical to that of the star, because of the relative smallness of the stellar Roche-lobe radius (this is unlike some 'normal' X-ray or dwarf novae binaries, where the jet goes right into the other lobe). The jet thus forms a cool circular keplerian ring (or torus) around the BH, of small width

$$\sim 0.04\, R \left(\frac{r}{10^{11}\ \mathrm{cm}}\right)\left(\frac{3\times 10^6\, M_\odot}{M}\right)$$

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where r is the stellar radius. This ring is steadily controlled by the L1 overflow.

We assume the BH to be rotating, not necessarily at extreme Kerr frequencies, because such massive BHs tend to reduce their a/M when 'swallowing' stars[7]. If the star is orbiting in an inclined orbit, a precession of its orbital plane around the rotation axis will occur due to the spin–orbit coupling, at a period given by[8]

$$T_p \sim 48.6\,\mathrm{d}\left(\frac{a}{M}\right)^{-1}\left(\frac{M}{3\times10^6\,M_\odot}\right)\left(\frac{R}{45\,R_G}\right)^3$$

This period will be consistent with the value of 160 ± 3 d for $a/M\sim1/3(M/3\times10^6\,M_\odot)$. (Note that a mass $M>10^7\,M_\odot$ will not be consistent with this model if the period is, indeed, 160 d).

A similar precession motion is also performed by the ring particles, at slightly different frequencies; however, as the ring is always additionally controlled by the L1 overflow, with a time scale of ~100 orbital revolutions ($\gtrsim10$ d), it moves essentially with the stellar precession, having a 'thickness' comparable to its width. This time scale is, essentially, the time it takes for a matter blob leaving L1 to separate from L1 by about an orbital length, and the 'thickness' results from the precession frequency gradient across the ring.

Frictional processes in the ring will bring about a slow drift inwards of 'old' ring material and, eventually, because of dephasing of its precessional motion, its settling down to the outer ring of an equatorial disk[9], which is where all of these out-of-phase inclined inner rings intersect. The disk may have pre-existed around the BH[3]. It provides continuum UV and optical flux to excite the 'new', cold, ring (jet) material into line emission. As the 'old' material drifts inwards, its density first decreases in the diffusion process and then it heats up on approaching the disk. Both these effects should secure the uniqueness of that ring, relative to all material inside it, as an optical line emitter. Note that since the inside material tends more and more to lie in the BH equator[9], the ring is likely to 'see' the inside UV source without much interruption from intervening opacity. The volume of the ring is $\sim10^{37}$ cm^3, requiring a density of $\gtrsim5\times10^{11}$ cm^{-3} (ref. 10) to account for the observed line luminosity. A cool ring of such dimensions and density and with solar type composition will indeed be optically thick to UV radiation but marginally optically thin in the visible when viewed pole-on.

To analyse observational constraints on our model, we write the centre of mass (COM) of the lines[3] as

$$\frac{1}{2}\frac{v^2}{c^2}+\frac{\phi}{c^2}\equiv\frac{3}{2\eta^2}\frac{v^2}{c^2}\simeq0.033 \qquad \text{(ref. 2)}$$

with ϕ the gravitational potential and v the velocity of the emitting region. For ring geometry $\eta^2=1$, while $\eta^2\sim3$ for $v^2\gg\phi$.

Recent data of Abell and Margon[11] suggest that the ring radiation geometry should be such that the ring does not radiate homogeneously but rather radiates predominantly from the regions which lie close to the BH equator, possibly due to the hot outer ring of the disk[15].

This geometry is equivalent to the double-water-hose kinematic geometry of Milgrom[2] and Abell and Margon[11], except that for the ring, $\eta^2=1$ while Abell and Margon take $\eta^2=3$, (that is, they use $v/c=0.27$ while we have $v/c\simeq0.15$). On repeating the Abell and Margon[11] analysis for $\eta^2=1$, we find that

$$(i,\theta)\cong(66°,32°)\text{ or }(32°,66°)$$

where θ is the angle between the plane of the ring and the BH rotation axis, and i is the angle between the line of sight and the BH rotation axis.

The width of the shifted lines emitted from these nodal regions depends on the length of these regions and on the thickness of the ring. Note that since the intrinsic intensity distribution per unit Doppler-shifted frequency along such ring segment maximises, almost always, at one end[3], and since the illumination should maximise at about the middle of the segment, one may sometimes expect double-peaked lines to be observed, depending on the relative heights of these maxima. Furthermore, as the optical depth through which the segment is viewed in the lines increases when the line of sight becomes more parallel to it, one also expects intensity variations with precessional phase.

Finally, we mention briefly a possible origin for the unshifted lines in the spectrum of SS433, which show a variability over time scale of a day[12], self-absorption and widths of $\gtrsim1{,}000$ km s^{-1}. Such variability limits the emission region to $\leq2\times10^{15\text{–}16}$ cm. For a BH of $\sim10^6\,M_\odot$, velocities of a few thousand km s^{-1} are compatible with such distances. If SS433 is accreting clouds, possible in the SNR W50 (ref. 3), moving at $\gtrsim100$ km s^{-1}, or else is accreting interstellar gas[3] moving at $\gtrsim10$ km s^{-1},

$$\left[\left(\frac{n}{1\ \mathrm{cm}^{-3}}\right)\left(\frac{v}{10\ \mathrm{km\ s}^{-1}}\right)^{-3}\sim0.01-1\right],$$

then cooling in the accreted matter will be efficient down to $\sim10^{15\text{–}16}$ cm[13]. In that region, density and temperature may be adequate to emit most of the unshifted emission lines, and its illumination and velocity distribution, modified by angular momentum of infalling matter[13], may cause the line width and shape (see for example ref. 14). Note that the interior part of the accreting matter may become sufficiently hot to radiate at X-ray wavelength, and that its influence on the kinematics and structure of the ring will be small due to the high, supersonic, velocities. It may, however, deposit by electrons up to $\sim3\times10^{36}$ erg s^{-1} in the ring, to add to its excitation.

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On the nature of SS433

THE report of large wavelength variations associated with SS433[1] illustrates the large amount of information to be gleaned from preliminary data when effects large enough to involve the theory of relativity are present. It seems most plausible to interpret the large periodic wavelength variations in the Balmer lines of this object as arising from periodic motion, requiring, as a result of its magnitude, the special relativistic Doppler formula for its interpretation. Here we comment on some recent observations that have made it possible to make quantitative estimates of some aspects of the model.

If the motion may be characterised by a constant speed, then the classical Doppler effect contains information about the radial velocity, while the special relativistic form also involves

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the actual speed. The relativistic expressions for these parameters are

$$\frac{v \sin i}{c} = (z_2 - z_1)/(2 + z_1 + z_2)$$
$$\text{and} \quad \frac{v}{c} = [1 - 4/(2 + z_1 + z_2)^2]^{1/2} \quad (1)$$

where z_1 and z_2 are the minimum and maximum values respectively for the observed redshift relative to the centre of mass. Using the values given for the object in ref. 1, one finds that it may be characterised by a velocity of 80,000 km s^{-1}, inclined 30° to the plane of the sky.

Since the object exhibits both X rays[2] and spectral lines of hydrogen and neutral helium[1], it seems reasonable to suggest that the latter are formed far from the ionising effects of the former. Thus any attempt to achieve the observed velocities by dynamical means incorporating the observed period of 160 d[1] requires a mass in excess of 10^8 solar masses. If the distance of 3.5 kpc (ref. 3) is correct then the presence of such a massive object is incompatible with stellar dynamics in that region of the Galaxy. Thus it is plausible to suggest that some form of hydromagnetic acceleration is responsible for the extremely large velocity. It has also almost become commonplace to suggest accretion by a collapsed object as the source of X rays in other systems exhibiting periodic variations.

These two suggestions strongly imply that a stellar binary system exists, one component being a rather massive star possessing a strong magnetic field (not unlike VV Cephei[4]), and the other a collapsed object, which is indeed the source of the X-ray spectrum. The rapid mass loss from the star would force matter not only through the inner lagrangian point but also through the outer lagrangian points, into the magnetic field. Except for the immediate X-ray shadow cast by the star, this material would be fully ionised by the X-ray source, and thus be frozen to the co-revolving magnetic field of the star. This ionised material would be accelerated outwards until it exceeds the Alfvén velocity or passes the Strömgren radius dictated by the X-ray source. The outward motion could be expected by means of cyclotron radiation to produce the observed non-thermal radio radiation[5]. At the Strömgren radius, the material will radiate and rapidly cool, giving rise to the observed high-velocity cool gas spectrum.

Additional observational data have recently become available which enable us to make quantitative estimates of several aspects of this model. The observed Hα flux of Liebert *et al.*[6], the 3.5 kpc distance, and the reddening of Margon *et al.*[3] imply an emitted energy at Hα of about 3×10^{34} erg s^{-1} or 5×10^{45} Hα photons per s per stream. If β is the number of Hα photons emitted for each outflowing proton, then the implied mass outflow would be 10^{-4} per β solar masses per yr. The divergence of the field would allow the material streaming outwards to spread, so that radiation is produced in a large volume. If the emitting region subtends about 15° as seen from the binary, then the time required for this region to cross the location of maximum Doppler shift would be about 4% of the 160 d period, and would produce the flat tops of about one week duration of the radial velocity curves[1]. The rotationally outflowing material will compress the field in the rotational plane over that of a static dipole geometry, in a manner perhaps similar to that of the Sun or Jupiter. As the distance from the binary system increases, the weakening field will form a spiral, which when combined with the above compression, yields a field decreasing in strength much more slowly than a static dipole field. This phenomenon is well observed in both the Sun and Jupiter, and implies a field strength that varies with distance as $r^{-\alpha}$, with $\alpha \approx 1.3$ (ref. 7). The spiraling of the magnetic field lines implies that the mass motion at time of radiation will have both tangential and radial components. If we take the magnetic and kinetic energies to be equal in the radiating volume, and equating the radial and tangential velocity components, then assuming co-rotation, the distance to the emitting regions is fixed. In addition, the previously calculated mass flux will specify the density, and hence the magnetic field strength, in the emitting region. If we extrapolate the field to the stellar surface using the above power law dependence, we may place a lower limit on the mass of the star such that it is stable against magnetic distortion[4]. The expression that relates these parameters is

$$\frac{M_*}{M_\odot} \geq \frac{10^{-8} v^{\alpha+1} P^\alpha \sin^\alpha \xi}{R_\odot^\alpha (2\pi)^{\alpha-1/2}} \left(\frac{R_*}{R_\odot}\right)^{2-\alpha} \rho^{1/2} \quad (2)$$

where ξ is the radial angle of the recombining gas, which we have taken to be 45°. With the previously determined values of α, period P and velocity v, this becomes

$$\frac{M_*}{M_\odot} \geq 1.4 \times 10^9 (R_*/R_\odot)^{0.7} \rho^{1/2} \quad \text{cgs} \quad (3)$$

The geometrical arguments previously mentioned and the outflowing particle flux yield a density in the beam of

$$\rho = 4\pi N m_H (\beta v^3 P^2 \theta^2 \cos \xi \sin^2 \xi)^{-1} \approx 1.6 \times 10^{-19}/\beta \text{ g cm}^{-3} \quad (4)$$

where N is the number of Hα photons emitted per s and θ is the half-angle subtended by the emitting regions as seen from the binary system. We may take the keplerian separation implied by the large mass indicated in equation (3), and the period P, to suggest an upper limit to the stellar radius of 2 AU. Equation (3) implies a lower limit to the mass of the primary of 40 $M_\odot/\beta^{1/2}$.

The absence of forbidden lines in the spectrum of SS433 and the recombination time for the hydrogen spectrum require a high-density medium for the emitting region. In order to keep the kinetic energy loss through the beam within reasonable bounds, one would expect the beam density to be low. These two requirements suggest a high speed particle beam interacting with a high density medium at the boundary of the Strömgren sphere. Although the details of such an interaction are complicated, mechanisms such as charge exchange imply that a value of β very much larger than that obtained from classical recombination theory would result. A large value of β implies that typical stellar masses would be gravitationally stable against the required stellar magnetic field. The evidence that the object contains a massive blue star[8] indicates that the field, although large, can be well below the disruption strength. Also a large value of β reduces the required mass loss rate to values typical for massive stars.

Thus we see that many aspects of this object are consistent with a model containing a massive blue star of the order of 50 solar masses in orbit with a collapsed object, separated by about 3 AU. The strong magnetic field of this blue star declines slowly with distance to a value of about 1G at 1,000 AU, where the outflowing matter interacts with the surrounding medium. Note that this distance, which is largely set by the geometry of the model, is consistent with the Strömgren radius expected for the X-ray source[2] and densities we have used.

Recently Abell and Margon[9] have shown that the shape of the observed segment of the radial velocity curve implies that the expanding streams may be inclined to the orbital plane at the location of emission. If confirmed, this interpretation would imply that the stellar field be inclined to the orbital plane, introducing only minor modifications to our model.

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Cool gaseous nebulae

THE electron temperatures of diffuse gaseous nebulae have long been thought to be close to 10^4 K. Much lower temperatures were derived from some of the early radio continuum and recombination line work, but these were generally considered to be wrong for a variety of reasons (cf. the review by Seaton[1]). However, most of these considerations applied only to the easily measurable bright nebulae like Orion, and these are expected to be at high temperatures because of their high densities and the effects of collisional de-excitation on cooling agents such as oxygen and neon ions[2]. While there is little doubt that the bright nebulae do indeed have temperatures of ~8,000–9,000 K (refs 3–5), there are strong indications that some nebulae of lower densities have much lower temperatures, ≲4,000–5,000 K (ref. 5). We have made radio recombination line observations of several low-density nebulae, in order to determine electron temperatures in the absence of such effects as collisional de-excitation, stimulated emission, and pressure broadening. Several of these nebulae have been found to have temperatures below 5,000 K, and for two of them which are discussed here (RCW94 and G339.1–0.2) absolute upper limits of ~4,700 K are imposed by the line widths alone.

The observations were made at 5 GHz with the Parkes 64-m radio telescope in March and April 1979. The system temperature was about 50 K, and the half-power beamwidth 4.4 ± 0.1 arc min. The temperature scale was based on observations of Hydra A, assumed to have a flux density of 13.1 Jy; the ratio of point source flux density to main beam brightness temperature was 1.43. The digital correlation spectrometer provided two simultaneous 512-channel spectra of a 10-MHz band containing the H 109α, H 137β, and He 109α lines; the spectral resolution was 2.3 km s^{-1} after Hanning smoothing. The total power observing mode was used; after each on-source integration, lasting 20 min, a reference integration of the same duration was made over the same range of hour angle. Special care was taken to assure compatibility of the continuum and line observations; the continuum measurements were interspersed with the line measurements of a given source, and were made with long scans through the peak of the source in order to separate the source from the galactic background accurately. The final spectra of the two sources RCW94 and G339.1−0.2 are shown in Fig. 1, compared with a spectrum of the Orion Nebula; measured and derived parameters are given in Table 1.

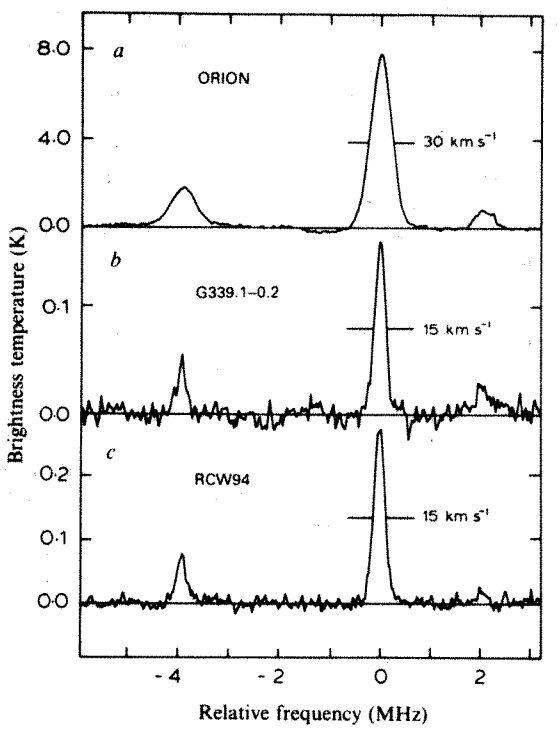


Fig. 1 5-GHz recombination line spectra of the Orion Nebula (*a*), G339.1–0.2 (*b*) and RCW94 (*c*). Hanning smoothing has been applied. A quadratic baseline was removed from the spectrum of the Orion Nebula and linear baselines from those of G339.1 − 0.2 and RCW94. The frequency scale is relative to the H 109α line; the H 137β and He 109α lines appear at −3.890 and +2.041 MHz respectively.

Table 1 Observed and derived parameters

	RCW94	G339.1 − 0.2
α (1950)	15 h 38 min 13 s	16 h 40 min 48 s
δ (1950)	−54°06.7′	−45°58.5′
T_C(K)	1.69 ± 0.09	0.85 ± 0.05
H 109α:		
ΔT_L	0.273 ± 0.005	0.160 ± 0.005
ΔV_L (km s^{-1})	14.9 ± 0.2	14.9 ± 0.3
V_L (km s^{-1})	−43.1 ± 0.2	−119.7 ± 0.2
I(H 137β)/I(H 109α)	0.27 ± 0.02	0.28 ± 0.02
I(He 109α)/I(H 109α)	0.06 ± 0.02	0.10 ± 0.02
T_D(K)	4,730 ± 130	4,730 ± 190
T_e^*(K)	4,660 ± 280	3,960 ± 280
T_e(K)	4,490 ± 280	3,800 ± 280

Quoted errors are 1 s.d. and allow for baseline uncertainties. Continuum (T_C) and peak line (ΔT_L) temperatures are in units of brightness temperature averaged over the main beam. The linewidth (ΔV_L) is the FWHM value, and the line velocity (V_L) is with respect to the local standard of rest. The integrated line intensities (I) were obtained by integrating under the observed line profiles. The Doppler temperature (T_D) allows for the instrumental resolution of 2.3 km s^{-1}. T_e^* and T_e are electron temperatures computed from the line-to-continuum ratio; LTE was assumed in determining T_e^*, and T_e takes into account all non-LTE effects assuming a local electron density of 100 cm^{-3}.

The most striking features of these lines are their widths, both 14.9 km s^{-1}. This is extremely small compared with the widths of recombination lines from other gaseous nebulae; the average of 128 lines observed in one survey[6] was 34.5 km s^{-1}, and the Orion Nebula with a linewidth of 30 km s^{-1} is typical of bright nebulae. These narrow lines almost certainly originate within the nebulae themselves, and not from cold partially ionised regions which may surround the nebulae; supporting evidence includes the absence of any significant carbon recombination line emission, approximately normal helium-to-hydrogen line intensity ratios, and β/α ratios close to the local thermodynamic equilibrium (LTE) value of 0.276. In addition the observed H 109α profiles are well represented by single gaussians; if some fraction of the line emission were supposed to originate outside the nebulae, the central velocities of the two line components would have to agree within ~0.5 km s^{-1}, and the widths and intensities would have to be interrelated to an improbable degree to satisfy the tight condition that the overall line shape is nearly gaussian. The linewidths thus imply an absolute upper limit of 4,730 K (T_D in Table 1) for the electron temperatures of the nebulae. If there are any turbulent mass motions, the electron temperatures must be smaller still.

The electron temperatures determined from the line-to-continuum ratios are consistent with these upper limits. Assuming LTE, they are 4,660 and 3,960 K for RCW94 and G339.1 − 0.2 respectively. Corrections for non-LTE effects reduce these temperatures still further, to 4,490 and 3,800 K respectively assuming a local electron density of 100 cm^{-3} (the corresponding β/α ratio is 0.29 in both cases); these corrections include stimulated emission due to the continuum from the nebulae, the galactic background, and the 2.7 K microwave background (about 5% of the total line emission). The r.m.s. electron density is ~20–30 cm^{-3} for both nebulae, so the adopted local value corresponds to filling factors of ~0.05–0.10, typical values; use of the r.m.s. densities gives the lowest possible values for the electron temperatures, 4,130 and 3,530 K for RCW94 and

0028–0836/79/330476–02$01.00

G339.1−0.2 respectively, but the temperatures obtained using a density of 100 cm^{-3} are probably more realistic. The corresponding r.m.s. turbulent velocities are small, ~3–5 km s^{-1}, as might be expected for well evolved, low-density nebulae such as these.

The low electron temperatures probably require relatively high metal abundances[7,8]. The normal helium-to-hydrogen line intensity ratios suggest that the nebulae are fully ionised. The excitation parameters (90 and 60 pc cm^{-2} for RCW94 and G339.1−0.2 respectively) correspond to early O-stars; the exciting star of RCW94 has been identified as LS3386 (ref. 9), and recent near-IR measurements of it (to be published) are consistent with an O5 star at the distance of the nebula (2.7 kpc), with $A_V \sim 5$. And recent work indicates that dust which may be present in the nebulae could not produce the necessary cooling[10]. The most likely explanation for the low temperatures is therefore a high metal abundance; indeed, recent radio and optical observations of another low-temperature nebula, G339.2−0.4, reveal metal abundances higher than the solar values by a factor of 4–5 (ref. 11).

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Note added in proof: New optical observations of RCW94 (in preparation) reveal metal abundances higher than the solar values by a factor of 6–7.

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Measurements of the trigonometric parallax of pulsars

THE distances of pulsars from the Earth have been determined using the dispersion measure and an assumed constant value of the mean electron density through the Galaxy of about 0.03 cm^{-3}. Independent distance estimates are available for a number of distant pulsars from the measurement of the H I absorption in the pulsar radiation and these results are in general accord with the dispersion measure estimates over large distances through the Galaxy. There is some indirect evidence[1,2] that the mean electron density in the solar neighbourhood is typical of the rest of the Galaxy but all estimates of pulsar distances within 1 kpc of the Sun rely on this assumption. We report here measurements of trigonometric parallax of pulsars and from the distance estimates we obtain a value for the mean electron density within about 500 pc of the Sun.

The observations formed part of the programme at Jodrell Bank for the measurement of the proper motions of pulsars[3]. Observations were made at 408 MHz using a radio-link interferometer between the 76-m MkIA telescope at Jodrell Bank and a 25-m telescope at Defford, a baseline of 127 km length. The technique involved a search for apparent motion between the pulsar and one or two nearby small diameter radio sources, assumed to be extragalactic and much more distant than the pulsar. Observations were made over a period of between 2 and 4 yr, each pulsar being observed once or twice a year.

Table 1 Parallax determinations with r.m.s. error $<10^{-2}$ arc s

Pulsar	Parallax (p) (10^{-3} arc s)	Dispersion measure (D) (pc cm^{-3})	Electron density (n_e) (cm^{-3})
0329+54	1.3±1.7	26.8	0.035±0.046
0809+74	1.8±7.1	5.8	0.010±0.041
0950+08	8.3±8.8	3.0	0.025±0.026
1237+25	−6.2±5.2	9.3	−0.058±0.048
1818−04	3.1±6.1	84.4	0.262±0.515
1929+10	21.5±8.0	3.2	0.069±0.026
2016+28	0.9±3.6	14.2	0.013±0.051
2020+28	−1.3±4.6	24.6	−0.032±0.113
2021+51	1.8±4.9	22.6	0.041±0.111

A least squares fitting process was used to determine linear proper motions in right ascension and declination and the parallax. For many of the pulsars most of the observations were made when the Earth was in approximately the same part of its orbit around the Sun so that the parallactic motions were small and consequently the errors in the measured parallaxes large. However, for nine pulsars out of the 26 studied, data were collected sufficiently often to allow determinations of parallax with r.m.s. errors of <0.01 arc s and these are listed in Table 1. The measured proper motions are not included and the complete results for all 26 sources will be published elsewhere. The major errors in the parallax determinations, apart from the random errors caused by poor signal-to-noise ratios and limited sampling through the year, are due to secular variations in the properties of the ionosphere. Systematic errors are to be expected because of the solar cycle and annual ionospheric variations above the observatories. There are not sufficient ionospheric data to make accurate corrections to the observations but a computer analysis using plausible data indicates that these errors amount at most to a few milliarcseconds per year in proper motion and a few milliarcseconds in parallax. This is confirmed by the small parallaxes measured on some of the pulsars which have large dispersion measures and are therefore expected to be very distant. The dispersion measures are shown in Table 1. The errors in these values are all <1% (ref. 4).

Taken individually, only one pulsar, PSR1929+10, shows a significant parallax of 21.5±8.0 marc s. This pulsar has the second smallest dispersion measure of all known pulsars and the parallax places the pulsar at a distance of about 50 pc from the Sun. The mean electron density (n_e) along the line of sight to the pulsar can be obtained from the product of the parallax (p) and the dispersion measure (D). That is

$$n_e = p.D$$

In this case, $n_e = 0.069 \pm 0.026$ cm^{-3}

This is somewhat larger than the mean electron densities measured along the lines of sight to pulsars over large distances through the Galaxy determined from observations of H I absorption. However, this pulsar lies at a low galactic latitude ($b = -4°$) and the line of sight to the pulsar is always within the disk of the Galaxy where the mean electron density is probably enhanced somewhat by material and ionisation associated with the population I stars and H I and H II regions. For pulsars, the galactic height distribution is several times wider than this enhancement, so that most of the lines of sight lie within this layer for only a small fraction of their lengths.

The errors in the parallax determinations for the other pulsars in Table 1 are too large to allow meaningful distances to be estimated. However, because the derived value of n_e is proportional to parallax, it is possible to obtain an unbiassed estimate of the mean electron density for each pulsar and this is shown in

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Table 1. Combining these results we can obtain a weighted mean of 0.029 ± 0.014 cm^{-3}.

There is no strong evidence to suggest any inhomogeneity in the electron distribution, as the individual estimates of the electron density are all substantially in accord with the mean value of about 0.03 cm^{-3} when their errors are taken into consideration.

This value for the mean electron density is derived essentially from those pulsars within about 500 pc of the Sun (that is, dispersion measures <15 pc cm^{-3}) since the estimates of n_e for the more distant objects all have large errors and therefore carry little weight in the formation of the mean. This value of the mean electron density is consistent with estimates over much larger distances in the Galaxy and indicates that the Sun does not lie in a region which is particularly rich or poor in interstellar plasma. The estimates of the local space density of pulsars made by Taylor and Manchester[5] and by Davies, Lyne and Seiradakis[2] were based on assumed mean electron densities of 0.03 and 0.025 cm^{-3} respectively. As the most numerous pulsars in the Galaxy have a very low luminosity, we observe them only within a few hundred parsecs of the Sun. To estimate the local space density of pulsars reliably, it is important to have a good estimate of the local mean electron density near the Sun, or better still a direct measure of distance. It seems therefore that their estimates of the pulsar space density will not be seriously in error due to this assumption and that the rather high pulsar birth rates of between 1 every 4 and 1 every 15 yr required to sustain the derived galactic pulsar population are accordingly still justified. More precise measurements are in progress and will help to determine whether supernovae occur often enough to be the sole birth places of pulsars.

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On the Pioneer 11 observation of the ring of Jupiter

SHORTLY before closest approach to Jupiter, the imaging experiment on board Voyager 1 detected a ring of particulate matter around the planet as the spacecraft crossed the equatorial plane of Jupiter. The radial position of the ring is at approximately $1.7R_J$ (jovian radii), the total ring thickness is reported to be ≤ 30 km and the radial extent $\leq 10^4$ km (refs 1, 2). Although this discovery was rather unexpected, the possible existence of a dust belt within the orbit of the innermost satellite, Amalthea, actually had been suspected as a result of the Pioneer 11 measurement of the jovian trapped radiation belt[3,4]. In this note we briefly outline this interesting observation and evaluate some of the basic properties of this newly discovered ring.

During the Pioneer 11 flyby of Jupiter in December 1974, the minimum distance of the spacecraft to the centre of the planet was as small as $1.6R_J$ and hence the intensity variations of the trapped radiation belt charged particles between the outer magnetosphere and the region around $1.6R_J$ were sampled by energetic particle detectors. As in the earlier Pioneer 10 encounter, absorption structures near the orbits of Ganymede ($L = 15.0$), Europa ($L = 9.4$) and Io ($L = 5.9$) were found in the particle fluxes in certain energy channels[3,5-7]. Sometimes the intensity dropped by a factor of 10 in the vicinity of the satellite sweeping corridors. Because of its closer encounter distance, Pioneer 11 enabled a survey to be made of the jovian radiation belt near Amalthea. As well as two absorption features apparently related to Amalthea there are two minima at $L \approx 1.6$ and 1.7 not accounted for by any known satellite[3]. On one hand, such minima may be simply caused by the anamolous precipitation of the mirroring charged particles into the planetary upper atmosphere at this radial distance as a result of the large quadrupole moment of the jovian magnetic field[4,8]. On the other hand, the absorption effect of a small satellite or a ring of dust particles can also produce just such structure in the intensity variations of the charged particles[3,4]. It has been noted, in any case, that even with the anomalous precipitation effect operating in reducing the particle fluxes near the minima in question some particulate matter may still exist and help to sweep up the charged particles in this region[9]. In the light of the Voyager 1 discovery, it seems appropriate to reconsider the Pioneer 11 data in terms of the dust belt interpretation. Because the distances of closest approach to Jupiter for both Voyager spacecraft are more than $4.9R_J$, no information can be obtained for the trapped radiation belt in the vicinity of the ring. This certainly makes the measurements of Pioneer 11 even more interesting. In this letter, we make some estimates (in a manner essentially similar to the treatment discussed by Van Allen[10] for the saturnian rings) of the optical thickness of the ring, number density, as well as the average size of the ring particles. Only proton ($E \geq 80$ MeV) data from the UCSD Trapped Radiation Detector instrument described by Fillius[3] will be used in the present discussion. This calculation, together with the Voyager 1 imaging result, should provide preliminary information on the new ring of Jupiter.

The satellite absorption effect is determined by two basic factors[11-13]: (1) the effective absorption cross-section of the satellite, and (2) the time scale for the charged particles to diffuse across the sweeping corridor of the satellite. Furthermore, the absorption process will be affected by the drift frequency and pitch angle of the particle motion in the dipole field of the planet. If, for a certain kinetic energy, the drift frequency is comparable to the frequency of the relative motion between the satellite and the co-rotating magnetosphere and the two motions are in opposite directions, the resulting 'resonance' would tend to suppress the satellite absorption effect. At the same time, because of the 10° tilt of the magnetic dipole moment with respect to the rotation axis of the planet, mirroring charged particles with equatorial pitch angle larger than 70° would be able to diffuse past the satellite more easily without being absorbed[11]. In the case of absorption of charged particles by a ring of particulate matter, the pitch angle effect might still be important in determining the degree of absorption; but the drift frequency should not influence the particle absorption since the ring particles are presumably distributed uniformly along the whole orbit. The only energy-dependent factor then would be the stopping range of the charged particles, that is the integrated mass intercepted by the charged particles as they diffuse across the ring plane. For 100-MeV protons, the stopping range is typically a few grammes for matter of various composition (ice, aluminium and so on). Hence, if most of the ring particles have sizes of >1 cm, just one pass would be enough to absorb the impacting proton; and the survival probability of the energetic protons as they diffuse inward past the ring can be written as

$$P = \exp(-2N_b\tau_\perp) \quad (1)$$

where N_b is the total number of bounces executed by the mirroring protons and $\tau_\perp$ is the optical thickness in the direction perpendicular to the ring plane. The value of $\tau_\perp$ is related to the number density and average diameter (d) of the ring particles, and the thickness of the ring place ($l_\perp$) by the following expression,

$$\tau_\perp = \pi n d^2 l_\perp / 4 \quad (2)$$

The value of N_b can be approximated by taking the ratio of the pertinent diffusion time scale t_d to the bounce period t_b of the

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100-MeV protons. In the Pioneer measurements, the radial diffusion coefficient (D) for 100-MeV proton is estimated to be $\approx 10^{-6}\ s^{-1}$ at the orbit of Io ($L = 5.9$) and $10^{-8}\ s^{-1}$ at the orbit of Amalthea ($L = 2.6$) (see ref. 12). If D is proportional to L^3 or L^4 the corresponding value at $L \approx 1.7$ is $\approx 7 \times 10^{-9}\ s^{-1}$. Now, according to the preliminary report[1,2], the total radial extent of the ring is $\geq 9 \times 10^3$ km ($\Delta L \geq 0.1$). At the same time, an upper limit for ΔL of about 0.16 can be set from the Pioneer data[3]. The diffusion time scale can then be approximated as[14]:

$$t_d = \Delta L^2 / 4D \tag{3}$$

with $\Delta L \approx 0.1$.

Therefore $t_d \approx 3.6 \times 10^5$ s. The bounce period of mirroring charged particles is given by[15]

$$t_b \approx 5.2 r_0 / V \tag{4}$$

where r_0 is the equatorial radial position of the mirroring charged particle and V its velocity. For $r_0 = 1.7 R_J$ and $V = 1.2 \times 10^{10}\ cm\ s^{-1}$ we have $t_b \approx 5.2$ s; and hence $N_b \approx 7 \times 10^4$. Judging from the depth of the minima in the proton intensity as measured by the UCSD experiment we can put $P = 0.1$ (that is, the flux is reduced by a factor of 10 across the absorption structure). From this we can immediately obtain $N_b \tau_\perp \approx 2.3$ and therefore $\tau_\perp = 6.2 \times 10^{-6}$ for the absorbing particles. Such a small optical thickness may be compared with $\tau_\perp \approx 1$ for the B ring of Saturn[16] and a value between 10^{-7} and 10^{-2} for the D' ring suspected to exist beyond the outer edge of the A ring[17,18]. [The result that the $\tau_\perp$ value of the ring of Jupiter should be a factor of 6×10^{-6} smaller than that of the saturnian B ring is in good agreement with the most recent IR detection of Jupiter's ring[19]. In this observation it is concluded that the number of particles in Jupiter's ring should be of the order of 10^5 less than that in Saturn's if both ring systems have similar kinds of particles and the viewing geometries are about the same.]

If the ring plane thickness $l_\perp$ is known we can derive the product nd^2 using equation (2). No exact value of $l_\perp$ is yet known—only that it is less than 30 km (refs 1, 2). Considering a thickness of ~1 km estimated for the saturnian rings[20] it may be reasonable to adopt $l_\perp \approx 1$ km for the ring of Jupiter as a working number. The actual thickness is not likely to be larger or smaller by a factor of 10. Substitution of this value into equation (2) gives $nd^2 \approx 8 \times 10^{-11}\ cm^{-1}$; that is

$$n \approx 8 \times 10^{-11} / d^2\ cm^3 \tag{5}$$

With the total volume of the ring given as $U = 2\pi r_0 \Delta r l_\perp$ (thus $U \approx 6 \times 10^{24}\ cm^3$ if $r_0 = 1.7 R_J$, $\Delta r = 0.1 R_J$ and $l_\perp = 1$ km) and the density of the ring particles is 2 g cm^{-3}, the total mass of the ring can be expressed as

$$M \approx 5 \times 10^{14} d \text{ grammes} \tag{6}$$

Thus $n \approx 8 \times 10^{-11}\ cm^{-3}$ and $M \approx 5 \times 10^{14}$ g if $d \approx 1$ cm, and $n \approx 8 \times 10^{-21}\ cm^{-3}$ and $M \approx 5 \times 10^{19}$ g if $d \approx 1$ km.

From simple geometrical considerations we find the maximum optical path ($l_\parallel$) along the ring plane as it is observed edge-on to be about $1.1 R_J$, therefore the corresponding optical thickness ($\tau_\parallel$) can be approximated as $\tau_\perp \times (l_\parallel / l_\perp)$, and this gives $\tau_\parallel \approx 0.5$. If the ring particles have a certain size distribution, the bodies with diameter much less than 1 cm may contribute significantly to a total light reflecting area ($A \approx 6 \times 10^{14}\ cm^2$ if $\tau_\perp \approx 6 \times 10^{-6}$) even though their absorption power is rather limited (since most of the mass is likely to be concentrated in the large particles). In this manner, the effective optical thickness may be increased by one or two orders of magnitude—but probably not by a factor much larger—considering the absence of the ring from ground-based observations in the past. If we argue that the ring particles are as old as the Solar System, their size must be large enough to resist orbit decay caused by the Poynting–Robertson effect. Following the similar treatment for the saturnian ring particles[21], the minimum diameter can be estimated to be ≈1 m, at least, for particles carrying most of the mass. A determination of the size distribution could possibly be achieved by analysing the absorption effect on electrons and protons of different energies (since they have different stopping ranges). Before this is attempted, little can be said.

In this connection, we would like to mention that detailed examination of the Pioneer 11 data with a small time resolution may also provide vital information on the radial structure of the ring. For example, the radial distance of the 2:1 two-body resonance with Amalthea is $1.61 R_J$. It would be interesting to see if there is energetic particle signature along the magnetic flux tube in this vicinity indicating the existence of a gap reminiscent of the Cassini's division in the saturnin rings. Or, the orbital resonance may actually define the mass concentration of the ring as previously suggested for the narrow rings of Uranus (but for a different type of orbital resonances) (see refs 22–24). The Pioneer 11 energetic charged particle data might then provide information on the dynamical models of ring formation as well.

Thus, based on the Pioneer 11 UCSD proton ($E \geq 80$ MeV) measurement of the absorption structures in the vicinity of the ring of Jupiter, the optical thickness of the absorbing ring particles in the direction perpendicular to the ring plane is estimated to be about 6×10^{-6}, and the corresponding value as the ring is seen edge-on is approximately 0.5. If the ring is as old as the Solar System, most of the mass should be concentrated in particles with diameter larger than 1 m and the total mass of the ring would be larger than 5×10^{16} g. Comparison with the Voyager imaging data may narrow down the uncertainty. But only further analysis of the energetic charged particle data can give us more insight about the size distribution and radial structure of the ring.

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Compositional differences between Arctic aerosol and snow

UNIQUE information on trace elements in polar atmospheres is available through records of deposition in snow and ice. Proper interpretation of these data requires a knowledge of the nontrivial chemical relationship between the deposition and its parent aerosol. Because several complex processes determine the trace-element content of precipitation, polar ice and snow cannot be considered *a priori* to have the same composition as polar aerosol[1]. In most of the current literature, such an assumption is usually made. Within the past year or so, the first tests of the long-term relationship between aerosol and deposition have been (inadvertently) produced for the Antarctic and

Table 1 Elemental concentrations and crustal enrichment factors in Barrow aerosol and snow

Element	Aerosol* Concentration (ng m^{-3})	EF_{crust}‡	Snow† Concentration (μg l^{-1})	EF_{crust}‡
Na	770	74	144	15.9
Mg	160	21	41	6.1
Al	30	1.0	26	1.0
Ca	55§	4.1	100	8.6
V	0.65	13.0	0.086	2.0
Mn	1.12	3.2	0.79	2.6
Zn	14.8§	580	1.16	52
Cd	0.37§	5,000	0.047	730
Hg	<0.4§	$<14,000$	0.011§	430

*Mean of December 1976–April 1977 and December 1977–April 1978 at Barrow, Alaska.
†From ref. 5, 28–224 km south of Barrow, Alaska, 15 February 1974. Na, Ca from 28- and 84-km points; Cd from all except 28-km point; other elements from all except 56-km point.
‡For element X, $EF_{crust} = (X/Al)_{aerosol\ or\ snow}/(X/Al)_{rock}$, where the rock composition is from ref. 10.
§Estimated from incomplete series of measurements; uncertainties a factor of 2.

the Arctic. The results are different for each region, and illustrate the great caution that must be exercised in this entire field. At the South Pole, snow from 1973–74[2] compares very well with aerosol from 1974–75[3]—both media give the same impression of the environment[2,4]. In the Arctic, on the other hand, opposite conclusions about the atmosphere have been drawn from snow and aerosol, and this problem is discussed here.

Weiss *et al.*[5] have used crustal enrichment factors of trace elements in recent Barrow, Alaska, snow and pre-1900 Greenland ice to conclude that (1) the 'lithospheric' elements Al, V, and Mn are not enriched at Barrow and Milcent (Greenland), and hence are derived from a (natural) 'windborne lithospheric component'; (2) the enrichments of Na, Zn and Cd are similar at Barrow, Milcent, and other Greenland sites (which implies that these elements are natural in origin at Barrow); (3) other enriched elements such as S, Se, Hg, Cd, As and Sb are naturally enriched in pre-1900 Greenland deposits. The overall impression given by Weiss *et al.*[5] is of the natural character of polar deposition in both Greenland before 1900 and Barrow today. Weiss *et al.* refer interchangeably to 'atmosphere' and 'aerosol' 'snow', 'ice', and 'deposit', implying that this natural character also holds for the polar atmosphere.

In contrast, we have recently concluded from chemical analysis of two years' samples of the Barrow aerosol that it is strongly pollution-derived, particularly during winter, to the extent that abundances of even 'lithospheric' elements can be affected[6]. This deduction is based on actual V/Al and Mn/Al ratios, which are more characteristic of mid-latitude pollution than of crust. It is supported by ancillary evidence such as abundant grey, sooty carbon and high concentrations of sulphate (1–5 μg m^{-3}) in the aerosol[6] (both of which imply pollution), and high concentrations of ^{210}Pb in the aerosol[6,7] (which indicate that it is continental in origin). Tests at the surface (spring 1979) and aloft[8] have shown that this pollution does not originate on the North Slope; it is associated with air coming to Alaska from farther north. In analogy to the similarity of snows from various parts of the Arctic reported by Weiss *et al.*, we find a similar (pollution) aerosol at the widely dispersed Arctic sites of Spitsbergen, northern Norway, and northern Greenland during winter[6].

We therefore have two lines of evidence (aerosol and snow) which have arisen independently, are both solid and defensible, but offer divergent pictures of the Arctic environment. How can they be reconciled? Part of the difference for Barrow can be explained by recalculating snow–crust enrichment factors with Al rather than Mn as crustal reference element. Weiss *et al.* used Mn because it was common to all their snow samples. In general, however, Mn is enriched by factors of 3–10 in urban and rural atmospheres[9], presumably by pollution sources, and therefore should not be used as a crustal reference element wherever it can be avoided. (Of course, without knowing how much the Barrow aerosol is affected by pollution, it would seem reasonable to use Mn.) Concentrations and revised enrichment factors for the Barrow winter aerosol and the near-Barrow snows are shown in Table 1. These calculations, the basis for which can be justified from many aerosol samples[9], show that both V and Mn have modest but definite enrichments in the near-Barrow snows. But this recalculation removes only part of the discrepancy between Barrow aerosol and snow—V and Mn are still several times more enriched in the aerosol than in the snow, and all other elements except Ca have greater enrichments in aerosol than in snow. For Na, Mg and Ca, this difference may be marine-related, because the aerosol-sampling site was closer to the sea than were the snow-sampling sites. The other elements, however, are not marine in origin; their different enrichments must have another explanation.

We believe that the broad discrepancy between Arctic aerosol and Arctic snow is a genuine feature of the present Arctic environment. Possible explanations for it include: (1) formation of snow from a parent aerosol aloft whose composition differs from the surface aerosol; and (2) aerosol–snow fractionation during or after snowfall. We favour the second explanation for two reasons: first, we see little reason that the composition of the aerosol in such a remote, source-free area should change markedly with altitude. Second, formation of snow crystals by nucleation on clay minerals, such as are found at the centre of many or most polar snow crystals,[11,12], could fractionate snow chemically relative to aerosol. A central clay nucleus which contained 90% of the foreign matter if the ice crystal (estimated by M. Kumai, personal communication) would decrease enrichments in snow by the factor of 10 that is observed. We stress, however, that aerosol–snow fractionation in the Arctic is only a hypothesis.

We emphasise that whatever the eventual explanation, aerosol-snow differences in the Arctic imply, care is needed in interpreting ice and snow data atmospherically. Modern snows may not be automatically equated with modern aerosols; historical trends of deposition may not be immediately equated with historical trends of parent aerosol. Until thorough studies of the factors controlling the elemental composition of polar snow and ice are undertaken, these uncertainties will remain.

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The 20-yr cycle in Greenland ice core records

CLIMATIC oscillations with a period of ~20 yr have been reported in North American winter temperature records[1,2], midwestern US drought indicators (ref. 3 and J. M. Mitchell, Jr personal communication), dendrochronological data[4], and sea-surface temperature anomalies[5]. These oscillations have mostly been attributed to double sunspot cycles, which seem to occur at about 22-yr intervals. Other possible causes include an 18.6-yr modulation of the diurnal and semidiurnal lunar tidal forces[5]. Critics[6,7] of these observations argue that such oscillations may only be an artefact of a red noise record, and are not uniformly present in a wide range of climatic data. It has also been argued that even if the oscillations are significant, their small amplitude renders them relatively unsuitable for climatic prediction[1]. However, if such oscillations prove to be real, identifying their nature and possible causes, even though their amplitude is small, can enhance our understanding of climatic change. To gain more insight into such 20-yr oscillations, we have examined characteristic oscillations in Greenland ice core oxygen isotope records. The results show that the strongest spectral peak occurs at a period of 20 ± 0.5 yr with a statistically significant amplitude. This period is essentially the same as that found in eastern North American winter temperatures[1]. Moreover this 20-yr oscillation is found to be coherent in phase with the dominant variation in the Sun's motion about the centre of mass of the Solar System. This motion has been hypothesised[8] to be closely linked with sunspots. On the basis of these observations we speculate here that a 20-yr oscillation may be a more fundamental solar and/or climatic oscillation than the nominal 22-yr cycle often mentioned.

Because dating procedures[9] are highly accurate, climatic time series obtained from Greenland ice core records are especially suited for detailed spectral analysis. From recent cores several well-dated coincident oxygen isotope records spanning 728 yr (1244–1971) have been obtained. These data supply an adequate-length time series for resolving frequencies accurately, while still yielding a typical sampling interval of <0.2 yr. These records do, however, contain significant amounts of spatially incoherent non-climatic noise due to depositional irregularities. In an analysis of several shallow cores separated by <60 km, Reeh *et al.*[10] found this non-climatic component accounted for about 50% of the variance of the isotopic records and generally had a 'red' spectral content. This 50% figure has also been verified using deeper (100 m) cores (N. Reeh, personal communication). Because of this depositional noise, the significance of spatially coherent oscillations in the records can be substantially enhanced by averaging several records at different locations.

Although not critical for this study, it should be noted that the precise meteorological significance of the isotopic records is not determined. In Greenland, correlations suggest that the isotopic records indicate atmospheric temperature variations[11]. Comparison of one of these records[12] with temporally smoothed Icelandic and central England temperatures supports this interpretation. However, the isotopic values are possibly indicative of shifts of storm tracks, which in turn tend to modify the source of the precipitation.

For analysis here, coincident portions of three cores were used: Crete [71.12° N, 37.32° W], Milcent [70.31° N, 44.58° W] and Dye 3 [65.18°N, 43.82° W]. All three of these cores were obtained as part of the International Greenland Ice Sheet Program (GISP). To reduce the depositional noise, oxygen isotopic records from these three cores were averaged to form a combined time series for portions of the analysis.

Of the core sites Milcent is on the western side of the ice divide, Dye 3 on the eastern side and Crete effectively on the divide. Primarily because of this, Dye 3 and Milcent tend to receive precipitation from different directions, whereas Crete probably receives precipitation from either direction[13]. Also, Dye 3 is further south, and hence the core contains more melt features which yield some measure of summer temperature[14].

To analyse these isotopic records spectrally both conventional lagged product[15] and maximum entropy[16] (ME) spectral analysis methods were used. Conventional techniques allow statistical confidence levels for the spectral peaks to be estimated, while the ME techniques aid in precise determination of frequencies and power. For the maximum entropy analysis, the Burg algorithm as formulated by Andersen[17] was used to estimate a minimum delay autocorrelation function from which the ME spectrum was calculated. In all cases 2 yr non-overlapping averages were taken before analysis in order to display the low frequencies better and to reduce the noise level. The use of such averages was supported by analyses of the whole 1 yr averaged combined record which yielded low frequency

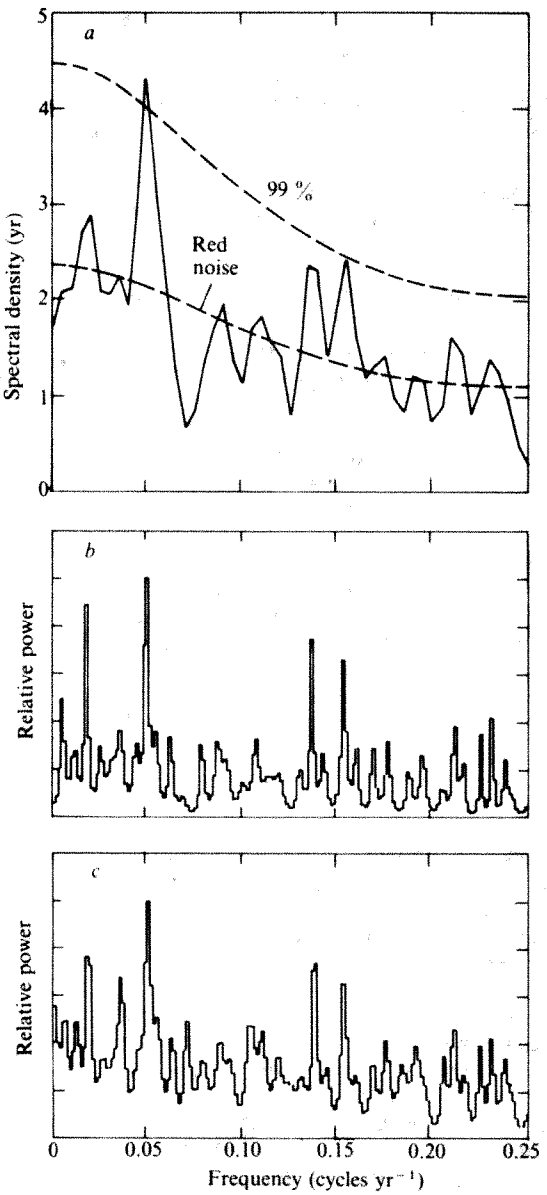


Fig. 1 Spectral densities of ice core oxygen isotope records. Two-year averaged data were used for the analysis. *a*, *b*, The conventional and ME spectra of the combined isotopic record formed by combining the three core records from 1244 to 1971. *c*, The average spectrum of the three records taken separately. In *a* 50 lags were used to analyse a 364-point time series of 2-yr averages, together with a Hamming Spectral Window. Twenty degrees of freedom were used in estimating confidence limits. This estimate was based on the expression $2.7\ T/M$ (ref. 15) where T is the record length and M is the auto covariance length.

Table 1 Periods and relative power of ME spectra of combined Crete, Milcent, Dye 3 oxygen isotopic ratio record

Whole record (1244–1971) (80 filter wts)		Bottom half of record (1244–1607) (40 filter wts)		Top half of record (1608–1971) (40 filter wts)	
Period	Relative power	Period	Relative power	Period	Relative power
20.00	8.17	6.47	16.97	20.36	17.28
54.41	5.88	28.35	12.73	7.35	12.51
6.53	5.59	56.67	10.00	51.27	11.09
7.31	5.00	19.73	9.75	144.20	7.52
183.70	4.27	5.28	6.36	9.32	6.87

2-yr averages sampled every 2 yr were used in the analysis.

(≤ 0.25 cycles yr^{-1}) results almost identical to those obtained from 2-yr averaged data.

Figure 1*a* shows the lagged product spectrum of the combined isotopic record. Figure 1*b* is the ME spectrum of the combined isotopic record, and Fig. 1*c* is an average ME spectrum obtained by finding a common unit prediction filter for all three records—a straightforward extension of the Burg algorithm. (This average spectrum is essentially the sum of the individual time series spectra.) The primary difference between the 'combined' spectrum and the 'average' spectrum is that in the 'combined' spectrum cycles with phase differences will cancel, whereas in the 'average' spectrum phase differences from record to record do not matter. Note that both ME spectra have similar dominant peaks with the 'combined' spectrum having less noise. This indicates a phase coherence between the dominant oscillations in the different records. This phase coherence was also verified directly for the 20-yr cycle by direct correlation of bandpassed individual records.

The dominant feature in these spectra is a pronounced peak with about a 20-yr wavelength. As demonstrated in Fig. 1*a*, the 20-yr peak is statistically significant at the 99% level when compared with red noise. Based on this spectrum it seems unlikely that this peak is simply an artefact of simple 'persistence' in the climate (which in the simplest case is a first order Markov process with a red noise spectrum[18]).

To determine more precisely the frequency of this peak, the analytical integration procedure of Johnsen and Andersen[19] was used. In this method, the analytical expression for the maximum entropy spectrum is integrated using residue theory. This procedure gives a precise value for both the power and frequency of a given peak. Table 1 lists the first five peaks (ordered by their power) for the average isotopic record. Also listed are the first five peaks in the bottom and top halves of the record. While there is some scatter in these results we feel they conservatively place the period of the 20 yr oscillation at 20.0 ± 0.5 yr. This result was also verified by higher resolution conventional lagged product spectra with a frequency resolution of 0.0025 cycles yr^{-1}.

This period agrees with a 20-yr period found in eastern North American January temperatures by Mock and Hibler[1]. It is, however, in contrast to a 23-yr cycle found by Bain[20] in central England temperatures and a 22-yr spectral peak in Bristlecone pine data found by Epstein and Yapp[4]. (Epstein and Yapp[4], however, used 10-yr averaged data, which could effectively filter out a 20-yr cycle. For example, taking 10-yr averages of the combined isotopic record reduced the 20-yr cycle amplitude and shifted it to 21 yr.) This period also contrasts somewhat with a 20–22 yr drought cycle in the Midwest suggested by Roberts and Olsen[3] and found in tree-ring drought data by Mitchell, Stockton and Meko (personal communication).

One of the main reasons that researchers favour a 22-yr cycle in climatic records has been the belief in possible weather links to a hypothesised 22-yr double solar cycle. In principle, this cycle arises because of different magnetic polarities[21] of sunspot cycles which, in observations over the past 90 yr, have been found to reverse in alternate cycles nominally occurring at 11 yr intervals. It also appears that magnetic activity is consistently different in adjacent cycles[21]. Pittock[7] has argued that no link between solar cycles and climatic change has been established, and a 20-yr rather than a 22-yr cycle could support his scepticism. By this view the 20-yr cycle in the ice core data could be simply a resonance in the climate system. However, examination of available sunspot data indicates that it is difficult to ascertain the precise period of the 'double solar cycle'. Consequently, an alternative hypothesis which, in our judgement, cannot be ruled out is that a double solar cycle is occurring at 20 yr rather than 22 yr. Under this hypothesis the relatively irregular sunspots could be viewed as a stochastic manifestation of some more regular 20-yr cycle. Certainly such a coincidence would not prove a link between solar variations and the ice core records, but it would at least provide a possible forcing at the correct frequency.

Although he performed no spectral analyses, Jose[8] indirectly advanced the hypothesis of a 20-yr solar oscillation in a detailed calculation of the Sun's position relative to the Solar System centre of mass. In particular, Jose compared various solar orbital parameters (which typically vary with a 20-yr period) with sunspot data. He found that if, in early sunspot records without polarity observations, the polarities of adjacent sunspot cycles were in special cases not reversed, then excellent correlation with the Sun's motion could be obtained. Note that such a deterministically forced theory of sunspots, while not necessarily in agreement in period, is consistent with a recent suggestion by Dicke[22] that the sunspots are irregular manifestations of a more regular phenomenon.

To compare the period and phase of the solar oscillation at 20 yr with the ice core oscillation, we have made a simple (but less precise than Jose) calculation of the Sun's orbital parameters. We assume, as a first approximation, that the planetary orbits are circular, and in the same plane, then by conservation of momentum (that is $\sum_i m_i \dot{\mathbf{r}}_i = 0$) the position of the Sun relative to the centre of mass of the Solar System may be easily estimated. Using planetary data from Allen[23], we calculated various orbital parameters over the same 728-yr period spanned by the ice core data. Figure 2 shows a ME spectrum of the rotation rate $[(\dot{x}\ddot{y} - \dot{y}\ddot{x})/(\dot{x}^2 + \dot{y}^2)]$ of the centre of the Sun about its instantaneous centre of curvature. This parameter is chosen because it would be a portion of the effective 'coriolis' parameter seen by any rotating turbulence in the convective zone of the Sun. (Parameters such as the Sun's velocity do, however, yield similar spectra.) The spectrum is strongly dominated by a 19.8-yr peak. This peak physically arises due to the synodic period of Jupiter and Saturn, being ~19.85 yr. There is also a secondary weaker peak at ~11.9 yr which corresponds closely to the 11.86 yr orbital period of Jupiter.

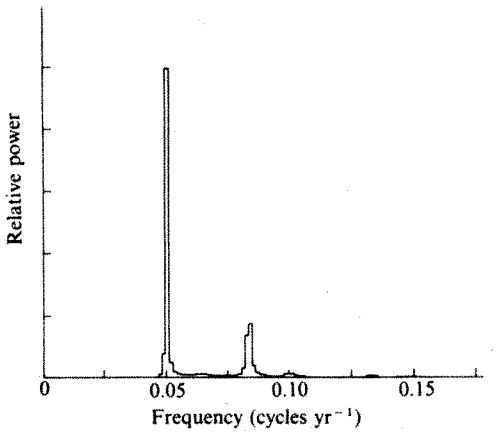


Fig. 2 Spectrum of rotation rate of the centre of the Sun about its instantaneous centre of curvature (relative to a fixed coordinate system at the centre of mass of the Solar System).

Table 2 Periods and relative power of the sunspot record (1699–1971) using various polarities

Single cycle (polarity ignored)		Double cycle (polarity reversed in adjacent cycles)		Double cycle using Jose's polarities	
Period	Relative power	Period	Relative power	Period	Relative power
10.93	33.12	21.99	70.22	20.12	55.82
9.99	20.22	18.37	12.21	24.24	18.76
93.74	11.68	26.39	2.89	30.73	6.75
12.15	7.23	15.87	2.29	13.94	3.81
50.37	6.61	13.71	1.96	16.11	3.76

Annual mean values together with 100 filter wts were used in the analysis.

To establish the phase drift of the isotopic cycle relative to the Sun's 'coriolis' variations, we correlated a narrow frequency band of the isotopic record with the solar motion. To construct this bandpassed record the procedure of Ulrych *et al.*[24] was used. In particular the record was first extended forwards and backwards using ME, and then bandpass filtered, using a filter[25] with pass band from a period of 21.05 yr to 19.05 yr. Since the ratio of this band width to the Nyquist frequency is about 0.02, we estimate the degrees of freedom to be about 1 per 100 yr. In both the bottom and top halves of the record the best correlation occurred at a 180° phase shift (within 2 yr), indicating a good phase coherence. While this phase coherence could be a statistical fluke, it suggests a deterministic forcing rather than an internal stochastic oscillation.

To demonstrate the effect of Jose's hypothesised polarity on the sunspot spectra, we used the Johnsen–Andersen[19] spectral integration technique to determine the first five peaks in the various sunspot records (see Table 2). As can be seen, using Jose's polarities the inverted cycle yields a sharp 20-yr peak. Note also that in addition to an 11-yr cycle there is a strong 10-yr cycle in the normal (polarity ignored) sunspot record[21].

Studies by Eddy[26] have suggested that the occurrence of sunspots may be quite irregular, with the sunspots even disappearing for several decades. This fact in conjunction with the uncertainty in periods exhibited by Table 2 suggests that it is probably not possible to establish whether a 'double solar cycle' is occurring at 20 or 22 yr from available sunspot records alone.

In conclusion, the results presented here demonstrate a dominant oscillation in Greenland ice core isotopic records at a period of about 20 yr. While we know of no causal link, such a period also coincides with the Sun's motion relative to the centre of mass of the Solar System. This motion has been suggested to correlate with sunspot variations. On the basis of these observations we speculate that a 20-yr oscillation may be a more fundamental climatic variation than the nominal 22-yr 'solar cycle'.

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Combined structural and chemical analysis of 3,800-Myr-old microfossils

CELL-like inclusions detected in the cherty layers of a quartzite, which is part of the Isua series in South-west Greenland, consist of biological materials, according to analyses by Raman laser molecular microprobe. The available radiometric data place the age of the sequence at around 3,800 Myr[1]. Thin sections of our specimens were taken in their primary positions within the rock matrix. The material used was compact and unweathered. No maceration, etching, impregnation or other methods were applied which might have produced artefacts.

About 100 well preserved specimens noted in the material were sealed within the quartz grains or in the silica cement in a three-dimensional condition (Fig. 1*d*, *f*). This sealing apparently results from a synsedimentary permineralisation caused by colloidal silica, which subsequently crystallised to form chalcedony. Our observations suggest that these specimens are primary constituents of the sediment, and are not contaminants from a later time.

The fossils occur as individual unicells (Fig. 1*c*), filaments (Fig. 1*a*, *b*) or cell colonies (Fig. 1*e*). Cells and cell families are usually surrounded by multilaminate sheaths which show a characteristic laminar structure. All specimens observed apparently belong to the same kind of organism, named *Isuasphaera*[2].

The individual cells are more or less ellipsoid in shape. The mature cells range between 20 and 40 μm in diameter. The cell encloses a more or less globular hollow which is partly filled with organic matter (p in Fig. 1*e*). Apparently, this filling is a remnant of the former protoplasm which has been degraded during fossilisation. A vacuole is often contained in the cell lumen (v in Fig. 1*c*).

The mode of reproduction indicated resembles the kind of budding known in recent yeasts. Figure 2 shows the characteristic stages of reproduction frequently observed in the material.

The best way of confirming the biogenicity of a fossil residue is by relating morphology and chemistry. In practice, the chemical proof becomes a problem: most of the conventional analytical techniques require powdering and leaching of the rock samples. Consequently the results obtained are representative of the whole-rock bitumen and not specifically the enclosed microfossils.

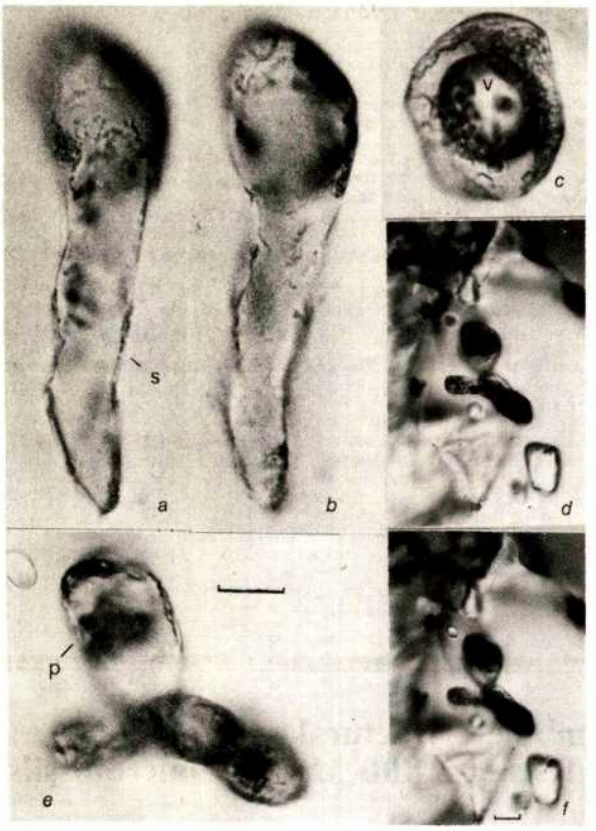


Fig. 1 *Isuasphaera isua* Pflug, 1978 Isua quartzite Greenland 3,800 Myr *a*, *b*, Filament: s, sheath; *c*, single cell: v, vacuole; *d–f*, ramified colony: p, organic cell filling; *d*, *f* are two foci, showing the specimen in its primary position within a quartz grain (bright). Magnifications: *a–c*, *e* ×900; *d–f*, ×290. Scale bar in *e* and *f*, 10 μm.

Individual and non-destructive analysis of microfossils in rocks has proved possible with the Raman microprobe analyser Mole[3]. This is a new physical method of microanalysis, based on the vibration spectra characteristic of polyatomic structures. A focused laser beam excites the sample. The light diffused by the Raman effect is used to identify and locate the molecular constituents present in the sample[4].

For the present work only one of the possible modes of the Mole was used: the monochannel spectrometer Raman mode. A light-optical microscope investigates the interesting structures in the sample and where the laser beam should be directed. The measuring spot can be restricted to ~1 μm in diameter. The diffused light is collected and analysed by a double monochromator equipped with holographic gratings. It is then received by a photomultiplier, amplified and recorded on paper as a function of wavenumber. The light used for excitation was the green line (5,145 Å) of an ionised argon laser.

Unmounted thin sections, ~40–100 μm in thickness, were taken from the rock samples. Polishing powder and distilled water only were applied to the slides during preparation. Any contact with organic substances was carefully avoided.

Thirteen well preserved specimens were selected for the analysis. They were all located below the surface cut and were completely enveloped by the mineral matrix. Separated analyses were made of different portions of the individuals—the cell interior, the cell wall and the enveloping sheath. Control measurements were extended to the mineral vicinity of the specimens and to the surface of the slide.

Two different types of spectra were obtained. One (Fig. 4) is typical of the dark brownish substance composing sheath, cell wall and cell filling (s, p in Fig. 1*a*, *e*), the other spectrum is typical (Fig. 5) of the cell vacuole (v in Fig. 1*c*).

Hydrocarbons of high aromatisation and condensation similar to those present in asphaltites are indicated by most of the spectra obtained from cell walls and fillings (Fig. 4*a–c*). Some spectra differ from this type by developing a stronger intensity at 1,360 cm^{-1} (Fig. 4*e*, *f*). Such patterns are characteristic of

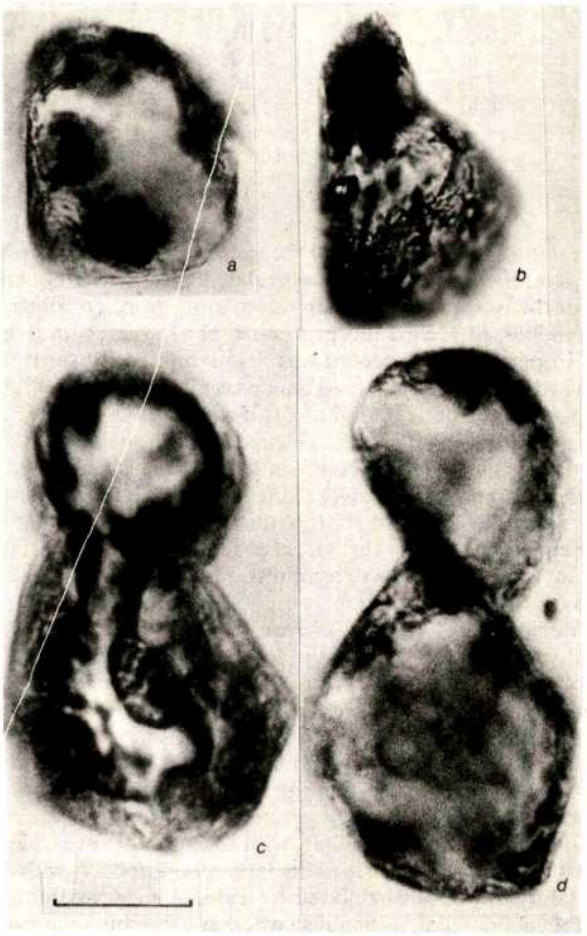


Fig. 2 *Isuasphaera* in different stages of budding (compare Fig. 3). Scale bar, 10 μm.

amorphous carbon produced by charring of organic material. It is therefore possible that some graphite may also be present in the *Isuasphaera* matter. The Raman spectrum of graphite[5] is characterised by a strong, sharp line at 1,580 cm^{-1}, and clearly differs from the larger band formed in the *Isuasphaera* spectra at about 1,595 cm^{-1}. Another difference is the band at 1,360 cm^{-1} which seems to be narrower in the graphite spectrum than in the *Isuasphaera* spectrum. Thus, graphite can account for, at most, only part of the fossil matter. It can be concluded from the analyses that *Isuasphaera* consists of organic material which is partly present in a carbonised condition, partly in a high rank of coalification very similar to a final stage of graphitisation. This is in accordance with the metamorphic condition of the enclosing rock which seems to be in the range of an upper greenschist facies[6].

Characteristic Raman spectra were obtained from two cell vacuoles of *Isuasphaera* (Fig. 5). In these, we find the typical lines of organic functional groups. A stretching vibration of the carbonyl group C=O appears at 1,745 cm^{-1}. The high wavenumber corresponds to that of an ester carbonyl. Indications of the presence of an ester are also found with the line at

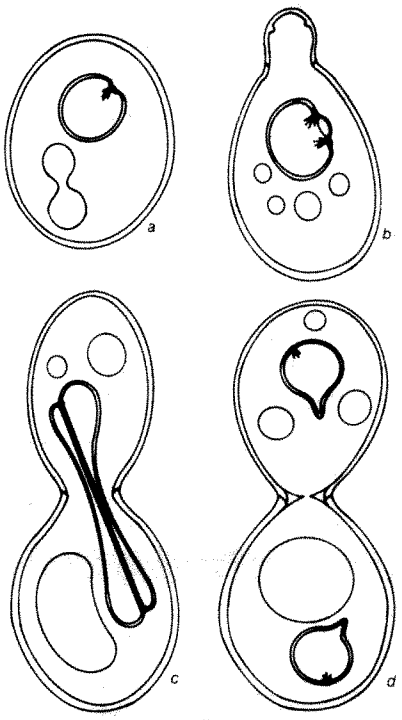


Fig. 3 Stages of a budding yeast cell: n, nucleus, v, gas vacuole (simplified after Matile *et al.*[10]).

850 cm^{-1}, which is the region of stretching vibration νC—C adjacent to a carbonyl carbon bond. Another indication is the high frequencies of νC—H vibrations near 3,000 cm^{-1}. An intense line at 1,460 cm^{-1} shows that a large amount of aliphatic hydrocarbons is present because the line corresponds to the deformation vibrations of CH_3 and CH_2 groups. The same groups are responsible for the broad signal at about 3,000 cm^{-1} which is a region in which the C—H stretching vibrations appear. The highest peak is a doublet at 2,950 and 2,967 cm^{-1}; a position which is typical for C—H vibrations in an electronegative environment corresponding to that of an ester function.

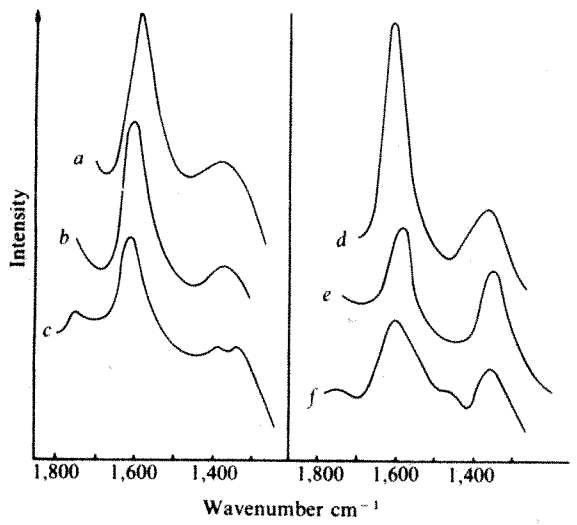


Fig. 4 Raman spectra obtained from cell wall and cell filling of *Isuasphaera* (*b–f*) compared with that of resinous asphaltite from Boscan (*a*).

Between 1,460 and 1,745 cm^{-1}, no significant lines for C=C double bonds occur.

We believe that the preservation of the vacuoles is due to the special conditions in the cherts. Normally, the volatile compounds set free from the mother bitumen during metamorphosis escape through pores and cracks of the rock. However, degassing cannot take place from material which is hermetically sealed in a quartz grain. Here the volatile fractions produced remain trapped in the matrix and after cooling, condense at the place of their origin, that is, at the cell lumen or nearby.

Structural comparisons have shown[2] that *Isuasphaera* conforms with recent asporogenous yeasts in all its preserved morphological properties, such as size, shape, structure of the cell wall and sheath, mode of growth and reproduction (Figs 2 and 3). But in the present context budding is not an exclusive

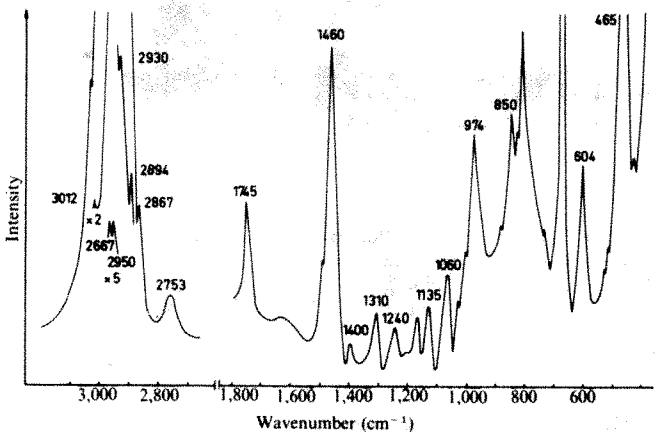


Fig. 5 Raman spectrum obtained from the cell vacuole of *Isuasphaera* (v in Fig. 1*c*).

characteristic of living cells. Multilateral budding, which is not dissimilar to that of *Isuasphaera* is, for example, known from Fox's microspheres—the artificial protein aggregates produced by certain laboratory experiments (Fig. 6). Because of their interesting budding abilities, the microspheres are often considered as a possible model for the hypothetical protocell[8].

However, fossil *Isuasphaera* differs from the microspheres in a series of specific features showing that it was not an abiotic product but a true organism. The exterior multilaminate sheath enveloping the *Isuasphaera* cell can only be understood as a product of biological activity.

Metabolic production is also indicated by the esters filling the cell vacuole of *Isuasphaera* (Fig. 4). Similar fat globules are found in recent yeast cells. They commonly make up 30–40% of the dry weight of the organism and reach 50–60% in some genera. Compounds of the lipid fraction are known to be resistant to decomposition and can, in the proper environment, last for long periods of time. They are also known to be a main source of the petroleum hydrocarbons.

There is little doubt that *Isuasphaera* is an organism. This is indicated by the morphological and chemical details. A problem results from the similarities between *Isuasphaera* and recent yeasts. If we conclude that yeasts existed during Isua times, life must be much older than 3,800 Myr. According to our present knowledge, the Earth's sphere formed about 4,450 Myr ago[9]. Consequently biophile conditions, implying a solid crust, cool temperatures and liquid water cannot have been realised on Earth earlier than about 4,400 Myr ago. The time span of roughly half a billion years, however, appears too short for the evolution from a simple organic compound to a eukaryotic organism to have occurred.



Fig. 6 *Isuasphaera* (*b*) compared with Fox's microsphere (*a*), an artificial budding structure consisting of protein, which has often been considered as a model of the hypothetical protocell (*a* from 8). Scale bars in *a*, 0·5 μm; *b*, 10 μm.

However, the classification of *Isuasphaera* to yeasts or other eukaryotes is uncertain. The question of whether an organism is an eukaryote or not can hardly be decided without knowledge of the protoplasm which no longer shows structural details in its fossil condition. The example of the microspheres (Fig. 6) indicates that cellular budding does not have to be a very complicated process nor does it have to depend on a complicated endocellular apparatus.

Thus, it seems that *Isuasphaera* in spite of its yeast-like appearance may occupy an evolutionary level far below that of eukaryotes. Consequently, *Isuasphaera* may represent a half-way line between a microsphere-like protobiont and subsequent evolution.

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An early Cretaceous terrestrial crocodilian and the opening of the South Atlantic

THE discovery in the lower Cretaceous of Niger of *Araripesuchus*, a small terrestrial crocodilian previously known only from Brazil, corroborates the existence of an African–South American faunal community until the Aptian. We show here that this provides additional evidence that an open seaway connecting the northern and southern parts of the Atlantic was not established until well into the early Cretaceous.

Among the abundant remains of terrestrial and freshwater vertebrates found (by P.T.) in the vast early Cretaceous fossil locality of Gadoufaoua (northeastern part of the Republic of Niger)[1] are those of a small short-snouted crocodilian. They were first discovered in 1966, and have been mentioned[1] as those of a 'brevirostrine crocodilian'. These specimens include incomplete skulls and lower jaws, vertebrae, dermal scutes, and limb bones. After acid preparation (by E.B.) of the very hard sandstone matrix, they were first found to belong to the genus *Araripesuchus*, a notosuchian crocodilian previously recorded by Price[2] from the early Cretaceous of Brazil.

The most significant specimen is a partial skull with the lower jaw still attached (Fig. 1); the part of the head located behind the orbits has disappeared. This skull is small: its complete length must have been about 12 cm, which indicates a crocodilian about 90 cm long. The snout is remarkably short and comparatively broad. The external nostrils are located in a very anterior position. The maxillae are somewhat 'swollen' behind their junction with the premaxillae. There is a large well defined antorbital fenestra, bordered anteriorly by the maxilla, posteriorly by the lacrymal. The orbits were large. A palpebral bone is present. In the upper jaw, there are 4 teeth in the premaxilla and 11 in the maxilla. The third premaxillary tooth and the third maxillary tooth are enlarged. The back teeth of the maxilla are small, laterally compressed, and have a rounded apex. The internal nares are large and located between the palatines and the pterygoids in a typically mesosuchian position. The lower jaw has a moderately long symphysis, in which the splenials take part. Postcranial remains indicate that the vertebrae are amphicoelous and that a dorsal and a ventral armour of dermal scutes were present.

These characters are consistent with the inclusion of this form in the mesosuchian infraorder Notosuchia and the family Uruguaysuchidae, as defined by Gasparini[3]. The Notosuchia are a group of small, short-snouted mesosuchian crocodilians, represented in South America by the families Uruguaysuchidae (early Cretaceous and beginning of the late Cretaceous) and Notosuchidae (late Cretaceous)[3]. The Libycosuchidae[4], from the Cretaceous of Africa[5], could also be referred to this infraorder. Closer comparison shows that the fossils from Gadoufaoua are very similar to the uruguaysuchid *Araripesuchus gomesii* Price, 1959, known from a skull and lower jaw found in the Santana Formation of northeastern Brazil[2]. The resemblances (Fig. 2) are such that it seems safe to refer the African form to the genus *Araripesuchus*. However, some minor differences in the shape of the teeth (which in the African form are reminiscent of *Uruguaysuchus* from the Cenomanian of Uruguay[6]) and in their relative sizes, as well as in the shape of the antorbital fenestra, will probably justify the suggestion of a new species, closely related to *A. gomesii*. This is thus the first record of an uruguaysuchid in Africa, and it has significant palaeobiogeographical implications.

The Elrhaz Formation of Niger, which has yielded the African *Araripesuchus*, and the Santana Formation of Brazil, from which comes the only known specimen of *A. gomesii*, are approximately of the same age. According to stratigraphic and palaeontological evidence[1], the Elrhaz Formation is probably of late Aptian age. The age of the Santana Formation has been disputed, but recent research both on the invertebrate[7] and verte-

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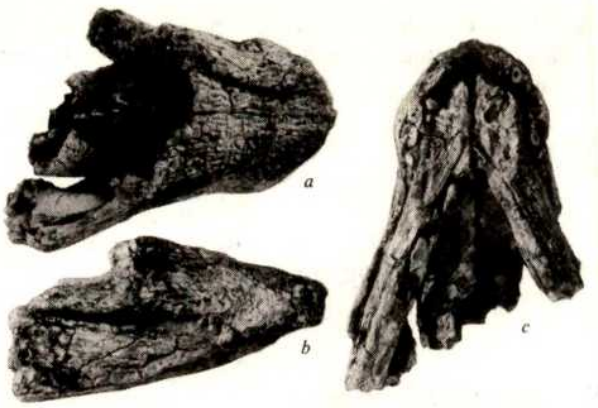


Fig. 1 *Araripesuchus* sp. from the Aptian Elrhaz Formation of Gadoufaoua, Republic of Niger. Incomplete skull and lower jaw collected by P.T. Muséum National d'Histoire Naturelle, Paris, No. GDF 700. *a*, Dorsal view; *b*, lateral view, showing the antorbital fenestra; *c*, ventral view, showing the internal nares. Total length of specimen: 90 mm. Photographs by D. Serrette.

brate[8] faunas tends to refer it to the late Aptian[9]. The discovery of *Araripesuchus* in the Aptian of Niger provides further evidence that the Santana Formation is not of late Cretaceous age, as was formerly believed.

The occurrence of very closely related species of *Araripesuchus* in the Aptian of Niger and Brazil also provides additional evidence of the existence of a terrestrial and freshwater vertebrate fauna little if at all divergent between Africa and South America until late in the early Cretaceous. This was already indicated by the presence of several very closely related forms (including freshwater fishes and the giant crocodilian *Sarcosuchus*) at Gadoufaoua and in the freshwater deposits, of probably Aptian age, of the Bahia/Reconcavo basin, on the eastern coast of Brazil[10]. We interpreted this fact as evidence that South America and Africa had not yet completely separated in the Aptian[10]—that there was no complete marine connection between the North Atlantic and the South Atlantic at the time—a hypothesis supported by studies on the ostracod faunas of the early Cretaceous coastal basins of Gabon and Bahia[11]. Note that the anatomy of the Notosuchia indicates that these

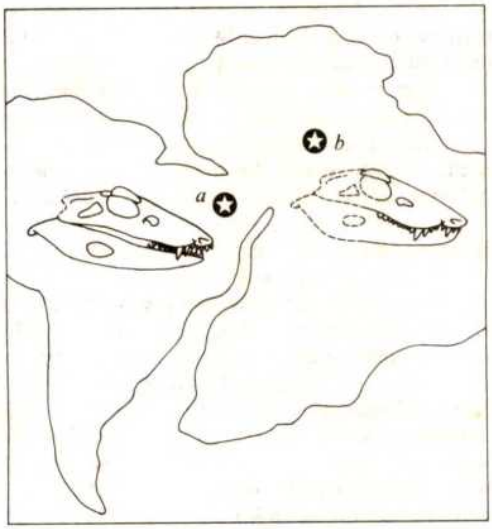


Fig. 2 Geographical distribution of the genus *Araripesuchus* in the Aptian. *a*, *Araripesuchus gomesii* Price, 1959, from the Santana Formation, Chapada de Araripe, northeastern Brazil (after Price[2]). *b*, *Araripesuchus* sp., from the Elrhaz Formation of Gadoufaoua, Tegama basin, Niger. Length of the lower jaw in both about 130 mm. Position of South America and Africa in the late Aptian after Förster[14].

small crocodilians were adapted to a terrestrial, somewhat 'mammal-like', way of life; *Araripesuchus* certainly could not cross any large area of saltwater.

Evidence relating to the date of the opening of a continuous seaway between Africa and South America has been based largely on the distribution of ammonites. On the basis of these kind of data, Reyment[12] concluded that a complete marine connection allowing a faunal interchange became established in the middle Turonian. Kennedy and Cooper[13] have then claimed that such a connection existed as early as the late Albian, a conclusion also supported by Förster[14] and Förster and Scholz[15]. Palaeobathymetric[16] and sedimentological[17] studies support the hypothesis of the establishment of a marine connection at the end of the early Cretaceous. These views, which suggest that a seaway separating Africa from South America came into being in the Albian, about 110 Myr ago, are consistent with our conclusions based on the Aptian terrestrial faunas of these continents. However, in a recent collection of palaeocontinental maps[18], Smith and Briden favour an earlier date—the beginning of the Cretaceous—for the opening of the South Atlantic and the separation of Africa from South America. We believe that palaeontological evidence based on the distribution of terrestrial and freshwater vertebrates, reinforced by the discovery of the crocodilian *Araripesuchus* in Niger, shows that a land connection between South America and Africa may still have existed as late as Aptian times. This obviously favours a comparatively late opening of the South Atlantic, at the end of the early Cretaceous.

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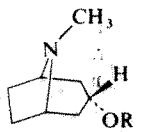
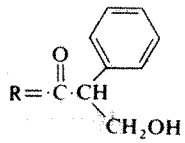
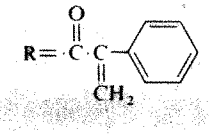
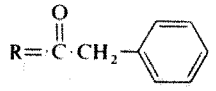
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Toxicity induced in the tobacco horn-worm (*Manduca sexta* L.) (Sphingidae, Lepidoptera)

INSECTS feeding on toxic plants demonstrate three fairly distinct types of life-style[1–3]. There are cryptic species, which metabolise or rapidly excrete the toxic substances present or avoid their ingestion by selective feeding; aposematic or warningly coloured species which store plant toxins in their tissues unchanged or slightly modified[4]; and aposematic species which superficially resemble or mimic toxic species—without actually storing poisonous plant products—or those warningly-coloured

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Table 1 Alkaloids from insect and plant

Compound		Pupae	Larval frass	*Atropa belladonna* L.
				
I		+++	+++	+++
II		–	++	–
III		–	(Trace)	–

Symbols represent relative amounts determined by gas liquid chromatography[10].

non-storers which secrete their own toxins. It is generally agreed that the cryptic life-style is more 'successful' than either of the other two, which are relatively rare, and it is difficult to envisage the evolutionary steps necessary to enable a species to change to the more hazardous warning life-style. If, however, circumstances favour a switch to certain toxic host plants, a cryptic insect is frequently destined to become warningly coloured[1,5]. On the basis of experiments with *Manduca sexta*, the tobacco horn-worm, we suggest that the evolution of an aposematic poisonous insect, from the more common, harmless, cryptic type, may simply involve a change to a related food plant containing different toxic properties from those of its usual host. Storage and the acquisition of toxicity and warning colour could follow this crucial switch.

In the Sphingidae there are several examples of aposematic larvae which feed on toxic plants, such as *Plumeria* (ref. 6 and unpublished results of M.R. and N.M.), but which give rise to cryptic, non-poisonous adults. This suggested that storage, even if temporary, sometimes occurs in this group. It seemed more likely that a cryptic insect such as *Manduca*, which excretes toxins rapidly[7,8], rather than those which metabolise them, would be able to switch more readily to tissue storage.

We reared *M. sexta* (from hatching to the pupal stage) on the abnormal food plant belladonna (*Atropa belladonna* L.), and controls on leaves of tobacco (*Nicotiana tabacum* L.) or standard Yamamoto[9] artificial diet lacking leaf powder. Between one and three of the pupae resulting from belladonna-reared larvae were then fed to each of six hens, all of which died within 1–7 d. Two of the tobacco-reared specimens, and 14 of those fed on artificial diet (controls) were eaten by two hens, neither of which suffered any visible ill effects and continued egg-laying throughout the 7 d of the experiment.

Extracts of four belladonna-reared pupae (3.354–4.207 g each) were injected intraperitoneally into laboratory mice, of which three out of four died within 20–25 h[6]. Extracts of pupae reared on tobacco and artificial diet failed to elicit any visible response in the mice.

Analysis of the belladonna-reared pupal stage revealed the presence of atropine in the body tissues (Table 1) and both atropine (I) and two metabolites (II and III) were found in larval frass. The pupae of *M. sexta*, when reared on belladonna, therefore contain lethal toxins.

We thank Carroll Williams for *M. sexta* eggs and Brian Gardiner and Catherine Godfrey for assistance with rearing caterpillars.

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Retinal site of transient tritanopia

WHEN the eye is adapted to a bright yellow light, a marked loss of sensitivity for short-wavelength stimuli occurs in the first few seconds after extinction of this light. This phenomenon was first reported by Stiles[1] and has been termed transient tritanopia by Mollon and Polden[2]. The latter authors suggest that this apparent suppression of the short-wave sensitive cones (hereafter called blue cones) during rapid dark adaptation is effected through inhibition by the recovering long- and middle-wave sensitive cone mechanisms[2] (hereafter called red and green cones, respectively). In this study we attempted to localise the site of blue cone inhibition through intra-retinal recordings from the intact rhesus monkey eye. We found transient tritanopia to be absent at the receptor level but present at the b-wave level, and we conclude that transient tritanopia originates at a site between the receptors and the bipolar cells, probably at the horizontal cells, as was earlier hypothesised by Augenstein and Pugh[3].

The signal that is recorded with a microelectrode positioned in the central fovea of a rhesus monkey eye, the local electroretinogram (LERG), consists mainly of the positive-going cone late receptor potential (LRP), also termed PIII, with a superimposed negative-going b-wave[4], representing activity at the bipolar cell level[5,6]. Figure 1 shows typical waveforms of responses to 447-nm and 551-nm flashes in two conditions. In the first condition, 'during yellow', a 10^5-troland yellow/orange background was present. The second condition, 'after yellow' (the transient tritanopia condition), is in the dark, shortly after extinction of the yellow/orange background. In the lower panels (Fig. 1), the 551-nm flash response 'after yellow' shows a large increase of both receptor potential and b-wave, compared with the 'during yellow' condition (note the difference in vertical scale). This increase reflects the removal of a desensitising field, that is, switching off the background. The situation is different for the 447-nm flash responses (Fig. 1, upper panels), where the removal of the background field leaves the receptor potential unaltered, but virtually abolishes the b-wave.

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To assess which cone systems are involved in evoking these responses, the spectral sensitivities of both LERG components in the two conditions were determined for three monkeys (Fig. 2*a*, *b*). Spectral sensitivity is defined as the inverse of the flash intensity necessary to evoke a criterion response. Figure 2*a* gives the receptor potential data. The data points were fitted with the blue cone spectral sensitivity function[7] in the short-wavelength region and the CIE standard photopic spectral sensitivity function for a 10° stimulus[8] ($V_{10,\lambda}$) in the rest of the spectrum. The low sensitivity of the long- and middle-wavelength-sensitive cones relative to the blue cones is apparent

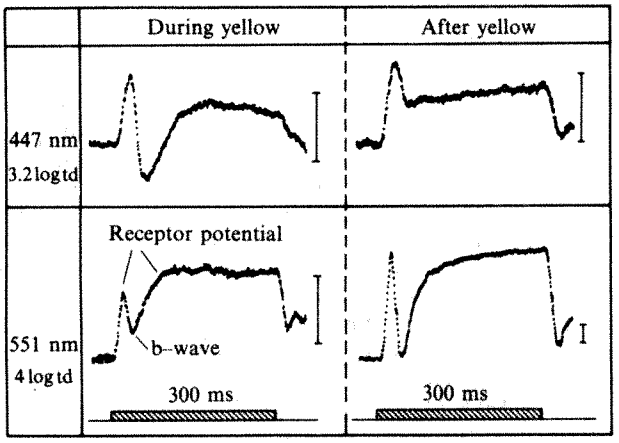


Fig. 1 Responses obtained from the central fovea of the intact rhesus monkey retina with a technique adapted from Baron and Boynton[15]. A microelectrode, protected by a guide needle, was driven through the sclera and directed to the macular region under direct observation. The tip of the electrode (~1 μm) was positioned in the outer segment layer of the central fovea and a reference electrode was placed in the vitreous at 1 mm from the retina. The responses are averages of 90 flashes, each lasting 300 ms; repetition time was 1,200 ms. Vertical calibration bars represent 50 μV. The left-hand panels show responses to 447-nm and 551-nm flashes presented on a 10^5 troland yellow/orange background (Schott OG 570 cut-off filter). Stimulus intensities were 3.2 log td and 4 log td, respectively. Both waveforms have a distinct b-wave. The right-hand panels show responses to exactly the same stimuli, 1 s after extinction of the background. At 447 nm, top right, the b-wave has disappeared, whereas at 551 nm, bottom right, the whole response has increased in amplitude (note the smaller calibration bar). For the purpose of response averaging in the 'after yellow' condition, the background was repeatedly turned on and off, the on-phase lasting 10 s, the off-phase 1.6 s. In each dark interval one 300-ms flash was given 1 s after extinction of the background. This cycle was repeated until 90 responses were averaged. The extinction of a 10^5 troland yellow background field produces a large off-response that contaminates the recordings in the 'after yellow' condition. Therefore, this off-response was recorded separately, averaged, stored and subtracted from each averaged response in the 'after yellow' condition. Both stimulus and background field subtended 10°, centred around the fovea. The receptive field of our microelectrode is about 8°. Responses from stray light beyond the 10° stimulus field therefore do not contribute to the signal.

in the 'during yellow' condition and this is usually called chromatic adaptation. Turning off the yellow/orange background leaves the blue cone sensitivity unaltered (see the 'after yellow' data points), but the long-wave sensitivity increases by about 1.6 log units which is as expected after removal of a yellow/orange chromatic adapting field. Figure 2*b* shows the b-wave spectral sensitivity data. The 'during yellow' data at short wavelengths are again fitted with the macaque blue cone spectral sensitivity function[7], but the long- and middle-wavelength points are now fitted with the green cone spectral sensitivity function[9] (this will be discussed below). In the 'after yellow' condition the sensitivity at short wavelengths is about 1.5 log units lower than in the 'during yellow' condition, which is indeed transient tritanopia: shortly after extinction of a yellow/orange background there is a marked decrease in sensitivity for blue stimuli[10]. The middle- and long-wave sensitivity increases by about 1 log unit when the background is removed.

To determine which cone mechanisms mediate the b-wave spectral sensitivity 'after yellow', we tried to fit the $V_{10,\lambda}$ function and the blue cone spectral sensitivity function to the data. However, a good fit could not be obtained. Apparently, the blue cone system does not contribute to the response. The green cone spectral sensitivity function[9], however, gives a very satisfactory fit between 400 and 620 nm. The sensitivity at 660 nm is clearly higher than predicted by the green cone sensitivity curve but this is probably caused by a contribution of the red cone system to the response. These findings corroborate the psychophysical results of Mollon and Polden[2], who describe the short-wavelength spectral sensitivity (<500 nm) in the 'after yellow' condition using Stiles' π_4 (green sensitive) mechanism. It is indeed surprising that the green cone spectral sensitivity function, which certainly does not fit the receptor potential data, fits the b-wave data points 'during yellow' and 'after yellow' in the above mentioned spectral regions, and that the $V_{10,\lambda}$ function does not. This might be explained by previously reported colour-opponent features of the b-wave[11,12].

Pugh and Mollon[13] recently proposed a two-site adaptation model for Stiles' π_1 (blue sensitive) mechanism that includes an explanation of transient tritanopia. The first site is in the receptors themselves and the second site is believed to be in the retina proximal to the receptors. This second site is thought to have blue–yellow colour-opponent properties. It is supposed that sensitivity at the second site depends on the balance of the blue and yellow signals, being least when the site is polarised. To account for transient tritanopia it is suggested that a restoring force, which acts to reduce the polarisation of the site, is built up slowly during adaptation to the yellow field and continues to act for some time after extinction of the field, so producing a polarisation in the opposite sense. The present results fit this view rather well: the operational inhibition of the blue cone signal does not take place at the receptor level, but at some more proximal site. As the suppression is manifest at the level of the b-wave, it must take place at the bipolar cells[6] or earlier. The horizontal cells, which are distal to the bipolar cells, are the most likely candidates for blue cone inhibition: first, because of their anatomical suitability (they make cross-connections between various receptor cells) and second, because, at least in lower vertebrates, they are chromatically coded[14].

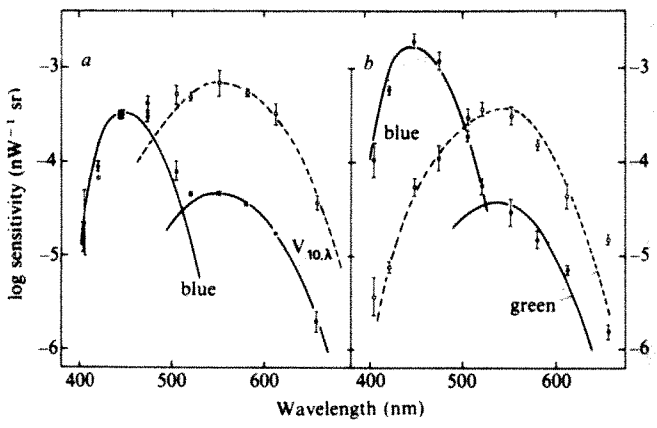


Fig. 2 *a*, Spectral sensitivity of the receptor activity, measured on a yellow/orange background (●) and 1 s after extinction of this background (○). Data are averages of three experiments. Receptor activity, the LRP or proximal PIII, was estimated by measuring the slope of the leading edge of the LERG response. Taking the initial slope allows consideration mainly of distal PIII without contamination of subsequent proximal PIII, onset of the b-wave potential or feedback from higher order cells[16]. Criterion for sensitivity was a response of 2 μV ms^{-1}. Error bars represent ±1 s.e.m. *b*, Spectral sensitivity of the b-wave 'during yellow' (●) and 'after yellow' (○). The b-wave amplitude was defined as the distance between initial positive peak and first negative deflection. Criterion was 45 μV and error bars represent ±1 s.e.m. This figure shows transient tritanopia: the sensitivity for blue (short wave) stimuli is much reduced when the yellow/orange background is removed ('after yellow' data points), compared with when the background is present. This is in contrast to *a*, where the blue sensitivity 'during yellow' and 'after yellow' is the same.

In conclusion, our electrophysiological data confirm the hypotheses based on psychophysical studies[2,3] in two ways: short-wavelength flashes, after switching off a yellow adaptation light, are 'seen' by the green cones rather than by the blue cones; and the inhibition of the blue cones can probably be located at the horizontal cell level.

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Rapid effect of change in cerebrospinal fluid sodium concentration on salt appetite

THIRST can be evoked within 1–2 min by intracarotid infusion of hypertonic NaCl. In contrast, the onset of a specific appetite for sodium salts as a result of rapid loss of body sodium is slow. A parotid fistula in naive sheep can produce a significant Na deficit (200–700 mmol) within 1–3 d but a Na appetite usually develops after 2–5 d (ref. 1). With large urinary water and Na loss induced by frusemide, water intake occurs within an hour but Na appetite is delayed until 24 h (ref. 2). Moreover, in the Na-depleted sheep or goats, increase in the Na concentration of cerebral arterial blood by 10–30 mmol l^{-1} by slow intracarotid infusion of 4 M NaCl for 7 min before or during presentation of Na solution does not reduce Na intake[3,4]. However, rapid intravenous infusion of significant amounts of isotonic and 2–4 M NaCl solutions in Na-deficient sheep substantially reduces Na appetite after 40–120 min[5,6]. In the rat, tissue fluid sequestration produced by subcutaneous polyethylene glycol injection causes water drinking within an hour, but onset of salt appetite is delayed by 6–10 h.[7] This delay also occurs in salt appetite produced by peritoneal dialysis[8], tourniquet release[9] and formalin injection[10,11]. New data indicate the powerful effects of the hormones involved in the reproductive process in inducing salt appetite. With pregnancy and lactation in Na-replete rabbits, avid salt appetite develops with a daily turnover of 50% or more of extracellular fluid (ECF) Na (ref. 12). This is caused by the combined action of oestradiol, cortisol, ACTH, prolactin and oxytocin[13]. ACTH seems to act directly on the brain[14]. Recent experimental data provide new information on the central nervous system control mechanism. Weisinger *et al.*[15] reported that in Na-depleted sheep, the systemic influx of hypertonic NaCl significantly reduced motivation for salt within 10–20 min. In the light of these data, we decided to evaluate the effect on Na appetite of increasing the cerebrospinal fluid (CSF) Na concentration or osmolality in Na-deficient sheep. We now report the first experiments to show that it is feasible to induce salt appetite within an hour or less by a physiological procedure.

Sheep with a parotid fistula were trained to bar press for 600 mM $NaHCO_3$ (15 ml per delivery = 9 mmol Na). They were allowed to bar press for 2 h daily (1200–1400 h), and thus had been deprived of Na for 22 h (deficit of 350–450 mmol) when the bar press was made available. On the day of the experiment, the infusion (1 ml h^{-1}) into a lateral brain ventricle was begun 1 h before and continued until the end of the 2-h bar press period. The composition of artificial CSF has been described previously[16,17]. CSF was sampled immediately after the end of infusion and analysed for [Na], [K] and osmolality. Data were analysed by a two-way analysis of variance with subsequent *t*-tests.

The results (Fig. 1, $n = 6$) show that within 10 min, intraventricular infusion of CSF [Na, 500 mM] caused a significant decrease in Na intake relative to infusion of CSF [Na, 150 mM]. When an infusion of 0.7 M mannitol–CSF[Na, 150 mM] was used to test the effect of increasing CSF osmolality without increasing CSF [Na], Na intake was almost doubled (Fig. 1). The increase relative to the CSF [Na, 150 mM] infusion was significant within 60 min and thereafter. In experiments where no i.v.t. infusion was given, the intake was similar to that with CSF [Na, 150 mM]. In five animals tested Na appetite was significantly increased within 60 min whether the mannitol infusion was begun 60, 15 or 0 min before the bar press period. No significant increase in water intake occurred. The effect of mannitol infusion on Na intake was the same whether water was concomitantly available to the animal or not.

Relative to infusion of CSF[Na, 150 mM], CSF (Na, 500 mM) increased CSF [Na] and therefore osmolality and decreased CSF [K]. Infusion of 0.7 M mannitol–CSF[Na, 150 mM] decreased CSF [Na] and [K], and increased CSF osmolality (Table 1).

The question of whether hypertonic mannitol would induce a Na appetite in Na-replete sheep was then examined. Naive Na-replete sheep with no known history of Na deficiency were given continuous access to water. Two-hour access to a bin containing 600 mM $NaHCO_3$ was given daily for a control period of 2–4 weeks. After two or three control infusions of artificial CSF[Na, 150 mM], which had no significant influence on $NaHCO_3$ intake (Fig. 2), the animals ($n = 5$) were infused on three consecutive occasions with 0.7 M mannitol–CSF[Na, 150 mM] (Fig. 2). This was usually done on three consecutive weeks. A 3-h infusion (1 ml h^{-1}) into a lateral brain ventricle was begun 1 h before access to Na.

Na intake increased in all animals on each of the three mannitol–CSF infusion days relative to the mean Na intake on the two days preceding the infusion ($P < 0.01$) in all experiments (Fig. 2). The following day intake was basal or below. A small increase in water intake occurred during the first hour of infusion on trial 3 ($P < 0.02$) but was never increased during the 2-h period when $NaHCO_3$ was available. In 18 other experiments with a cafeteria of Na, K and Mg salts the specificity of choice of Na salts was clearly shown (300–500 mmol increase,

Table 1 Effect of a 3-h i.v.t. infusion of various solutions on CSF composition

Condition	Na (mM)	K (mM)	Osmolality (Mosmol)
CSF[Na, 150 mM]	151.2 ± 0.4 (25)	2.84 ± 0.03 (25)	297 ± 1.3 (15)
0.7 M Mannitol CSF[Na, 150 mM]	129.9 ± 1.4‡ (28)	2.52 ± 0.02‡ (28)	379 ± 7.8‡ (17)
0.3 M Mannitol CSF[Na, 0 mM]	127.0 ± 2.8‡ (5)	2.72 ± 0.11 (5)	319 ± 7.2‡ (5)
CSF[Na, 500 mM]	177.2 ± 13.7‡ (4)	2.47 ± 0.08‡ (4)	—

CSF was sampled from the ipsilateral ventricle immediately after the end of infusion. Statistical comparisons with CSF[Na, 150 mM] value by Student *t*-test (*$P < 0.05$, ‡$P < 0.01$; ‡$P < 0.001$). Numbers in parentheses indicate the number of animals tested.

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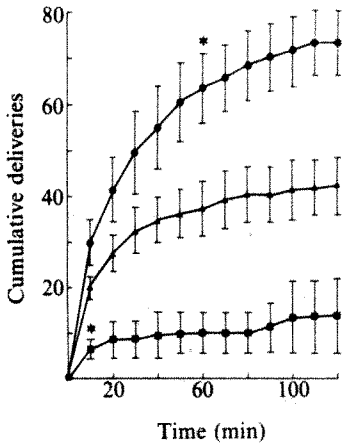


Fig. 1 Effect of i.v.t. (lateral ventricular) infusions on bar pressing for $NaHCO_3$ solution. The sheep were Na depleted as a result of parotid salivary loss for 22 h. Cumulative mean (±s.e.m.) number of deliveries (15 ml 0.6 M $NaHCO_3$ = 9 mmol) over the 120-min period in the various conditions: ■, 3 h i.v.t. infusion (1 ml hr^{-1}) of artificial CSF [Na, 500 mM]; ▲, artificial CSF[Na, 150 mM]; ●, 0.7 M mannitol–artificial CSF[Na, 150 mM]. Infusion was started 1 h before the bar press period. $n = 6$ Na-deficient sheep. *Significantly different from CSF[Na, 150 mM] values, $P < 0.05$.

$P < 0.01$), although a small increase in $KHCO_3$ sometimes occurred.

With i.v.t. hypertonic mannitol the decrease in CSF [Na] and [K] was presumably due primarily to the osmotic withdrawal of water from the choroid plexus and surrounding brain tissue capillaries. A decrease of CSF [Na] with less water movement was obtained by infusion of 0.3 M mannitol–CSF[Na, 0 mM] ($n = 7$) (Table 1). The Na appetite induced was similar to that with 0.7 M mannitol–CSF[Na, 150 mM] (Fig. 2). No increase in water intake occurred.

The results of these experiments indicate that a change in the composition of CSF involving an increase or decrease in [Na] may rapidly alter salt appetite drive. A rapid induction, within 60 min or less, of a large Na appetite has been shown.

The induction of a Na appetite in the naive Na-replete sheep, augmentation in the moderately deplete animal, the demonstration by both voluntary intake and operant behaviour and the inhibition of appetite as a result of increase of CSF [Na] in the Na-deficient animal strengthen the inference that altered CSF [Na] affected the physiological control system. In all cases, the

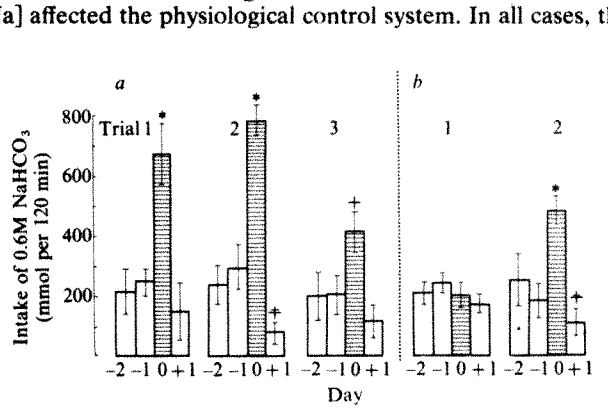


Fig. 2 *a*, Effect of 3 h i.v.t. infusion (1 ml h^{-1}) of 0.7 M mannitol–artificial CSF[Na, 150 mM] on voluntary intake of 0.6 M $NaHCO_3$ by 5 Na-replete sheep. Infusion was started 1 h before the 2 h access period on day 0. No infusion on days −2, −1 or +1. Water was continuously available. The effect of infusion on Na intake on three trials on consecutive weeks is shown. *b*, Effect in the same conditions of infusion of artificial CSF[Na, 150 mM] (1), 23 experiments in 13 sheep, and 0.3 M mannitol–artificial CSF[Na, 0] (2), 7 sheep. *$P < 0.001$; †< 0.01; ‡$P < 0.02$.

experimentally induced changes in CSF [Na] were in the physiological range. The absence of significant water drinking despite an increased osmotic pressure may be due to concurrent decrease of CSF [Na][17,18]. With i.v.t. CSF [Na, 500 mM] infusions, if water was available during the whole infusion, or in the period 0–120 min when Na HCO_3 was available, drinking of 1–2 l occurred.

The results are consonant with the suggestion by D.A.D. that reduction in intracellular [Na] of the hypothalamic nerve cells subserving Na appetite is the basic mechanism for the genesis of this specific drive[19]; however, further experiments are required to confirm this. Whether the time interval for CSF changes to induce behaviour is too short for a transcription mechanism is also a matter for further investigation. Intracellular ionic change could act at the level of translation[20]. A change in brain ECF [Na] may also have a direct effect on the membrane. The fact that the large salt appetite in the Na-replete animal during reproduction is generated by a sequence of steroid and peptide hormone actions[21], presumably by the same neural mechanisms which respond to body Na deficit, is consistent with the idea that a complex neurochemical mechanism underlies the genesis of salt appetite.

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Immunisation of guinea pigs and cattle against ticks

IXODID TICKS attach to the skin of their host and remain there for several days, engorging on blood and intermittently passing salivary secretions into the host[1]. Several species of Ixodid ticks transmit pathogenic microorganisms along with their saliva into the host, and tick-borne diseases are of great economic importance, particularly in cattle in subtropical and tropical areas[2]. The control of cattle ticks by chemical acaricides has become increasingly difficult due to the emergence of acaricide-resistant strains of ticks[3]. An apparently novel immunological method of tick control in guinea pigs and cattle is reported here. Hosts were immunised with antigens extracted from the gut and

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Table 1 Results from *D. andersoni* infestations* of immunised and control guinea pigs

Guinea pigs		Mean weights (±s.e.) of fed female ticks (mg)	Mean weights (±s.e.) of egg mass/female (mg)	Mean numbers of larvae (±s.e.) per female
Controls	1	297.26 (±63.6)	232.22 (±24.3)	3,575 (±806)
	2	401.62 (±56.7)	194.85 (±36.1)	2,709 (±478)
	3	396.27 (±72.3)	184.95 (±28.7)	2,973 (±288)
	4	318.91 (±96.1)	89.72 (±42.1)	1,366 (±209)
	5	31.12 (±2.3)	13.28 (±12.8)	102 (±73)
	6	38.66 (±2.8)	18.32 (±9.8)	0
Immunised with antigen I†	7	29.56 (±1.3)	0	0
	8	22.73 (±1.8)	0	0
	9	279.48 (±82.3)	47.70 (±36.2)	0
	10	76.71 (±31.8)	14.82 (±8.2)	0
	11	74.42 (±24.6)	12.08 (±10.9)	0
	12	146.61 (±76.3)	55.35 (±26.7)	0
Immunised with antigen II†	13	29.43 (±1.1)	0	0
	14	23.76 (±1.0)	0	0
	15	17.81 (±1.2)	0	0
	16	35.72 (±1.8)	0	0
	17	28.16 (±0.9)	0	0
	18	30.32 (±1.6)	0	0

* Each guinea pig was infested with four male and four female ticks.
† Antigen preparations: midgut, reproductive and other organs were dissected from female ticks which had fed for 5 d on guinea pigs. Dissected organs were held in ice-cold phosphate buffered saline (*p*H 7.2, 0.01 M in 0.15 M NaCl). Antigen I was extracted from midgut and reproductive organs, antigen II from all internal organs. Tissues were homogenised, centrifuged at 10,000*g* for 30 min, and the supernatants held at 4 °C. Protein concentrations were estimated by the Lowry method[12].

other internal organs of partially fed ticks. Ticks which subsequently parasitised these hosts showed significantly reduced feeding and reproductive performance. These experimental results raise the theoretical possibility of controlling some species of cattle ticks by appropriate immunisation of their hosts.

It has been known for some time that guinea pigs acquire a resistance to tick feeding[4]. For example, they allow approximately 80% of *Dermacentor andersoni* larvae to engorge during a primary 5-d infestation of one ear, but <10% of the larvae engorge during a subsequent similar infestation of the opposite ear. Such acquired tick resistance is an immunological phenomenon, abrogated by the use of immunosuppressants, and transferable between syngeneic guinea pigs with viable lymph node cells. Tick resistance is accompanied by a cutaneous hypersensitivity to antigens derived from the salivary glands of the tick, and a degree of resistance has been induced artificially in guinea pigs using such antigens[5–7].

Cattle may also acquire a similar resistance to the tropical cattle tick *Boophilus microplus*, following a series of infestations. However, the ability to acquire high level resistance to the tick is confined to certain strains of cattle. This ability is more often evident in *Bos indicus* than in *Bos taurus*, but the ability varies markedly from animal to animal within and between breeds[8–11]. At present, the causes of this variability are not known, and artificial induction of high level tick resistance has not been achieved in cattle by use of tick salivary gland antigens.

Immunisation trials reported here used antigens from the ticks' midgut and reproductive organs, and were based on the crude concept that ticks feeding on appropriately immunised hosts might ingest antibodies specific for antigens within the alimentary tract and reproductive organs of the tick, producing deleterious effects on the feeding and reproductive behaviour of the tick.

Antigenic extracts were prepared from female *D. andersoni* ticks which had been allowed to feed for 5 d on outbred guinea pigs. Extracts from tissues of unfed ticks had previously been found to be ineffective. Midgut, reproductive and other organs were dissected from the ticks. Two antigenic extracts were prepared; antigen I from the midgut and reproductive organs, and antigen II from all internal organs of the tick.

Eighteen outbred guinea pigs, weighing 400–450 g, were randomly placed in three groups. Animals in group I received antigen I 1.2 mg per kg body weight in Freund's complete adjuvant (FCA) on days 0 and 14. Animals in group II were immunised with similar doses of antigen II in FCA. Control animals (group III) received similar injections, lacking tick antigens, at the same time. All injections were given intradermally into footpads. On day 28, each guinea pig was infested with four male and four female *D. andersoni*. Ticks were removed from the guinea pigs 2 weeks later. The ticks were weighed, and females were placed individually in vials held at 24 °C in a relative humidity of 85%. The egg mass produced by each female was weighed, and the numbers of larvae hatching within 35 d of removing the ticks from their hosts were recorded.

The results of this experiment are shown in Table 1. Ticks engorged on four of the six control guinea pigs and produced egg masses from which large numbers of larvae hatched. Ticks feeding on animals immunised with antigen I produced relatively few eggs from which no larvae hatched. Those placed on guinea pigs immunised with antigen II neither engorged nor laid

Table 2 Results from *D. andersoni* infestations* of immunised and control calves

		Total ticks recovered	Total weight live ticks (g)	No. of dead part-fed females	No. of females laying eggs	Total egg production ($\times 10^3$)	Total larvae produced ($\times 10^3$)
Immunised†	1	79	4.31	42	12	26.84	25.89
	2	101	2.52	40	10	10.57	0
	7	101	3.79	56	11	20.20	19.03
	8	105	6.79	38	26	52.91	47.99
Mean ± s.e.		96.5 (±5.2)	4.35 (±0.8)	44 (±3.5)	14.75 (±3.3)	27.63 (±7.8)	23.23 (±8.6)
Controls	5	115	26.55	5	57	171.66	92.98
	6	111	24.01	20	47	165.53	128.87
	10	122	31.35	15	58	224.51	183.27
	12	80	15.83	8	38	93.45	56.97
Mean±s.e.		107 (±8.0)	24.44 (±2.9)	12 (±2.9)	50 (±4.3)	163.79 (±23.3)	115.52 (±23.3)
Significance (*P* value)‡		—	+(<0.001)	+(<0.001)	+(<0.005)	+(<0.005)	+(<0.01)

* Each calf was infested with 30 male and 100 female *D. andersoni*, confined to the ears within double cotton bags.
† Antigen I was prepared as before, from female ticks which had fed on cattle. Protein concentrations of the extracts were estimated by the Lowry method[12]. Antigen preparations were held at 4 °C until used.
‡ Differences in the performances of ticks on immunised and control calves were analysed by Student's *t* test.

eggs. The success in using antigenic extracts of partially fed ticks, in contrast to previous failure with extracts of unfed ticks, may reflect the developmental and presumably antigenic changes which occur in the midgut epithelium and other tissues of the feeding female Ixodid tick[1]. These results were considered to be sufficiently encouraging to undertake a pilot experiment with *D. andersoni* infestations of immunised and control cattle.

Antigen I was prepared, as before, from female *D. andersoni* which had fed for 5 d on the ears of Holstein steers. Eight Hereford-cross calves, which had not previously met ticks, and which weighed between 180 and 280 lb, were randomly divided into test and control groups. Each calf to be immunised received 67 mg antigen in Freund's incomplete adjuvant on days 0 and 16. A similar dose without adjuvant was administered on day 25. All injections were given by the intramuscular route. Control calves received similar injections, lacking the tick antigens, on the same days. All calves received 10-d tick infestations starting on day 28. Measurements of the ticks' feeding and reproductive performances were recorded as shown in Table 2. Serum samples were obtained from all calves on days 0, 16, 25, 28 and 38. Immunodiffusion tests were run with these samples and the immunising antigen.

From the results shown in Table 2, it is evident that there was no significant difference in the total numbers of ticks recovered from immunised and control calves. Significantly lower total weights of live ticks, lower egg production and fewer larvae were found in infestations of immunised compared with control calves. No larvae were produced by ticks which infested immunised calf number 2.

Immunodiffusion studies revealed single precipitation bands between the immunising antigen and sera collected on days 16, 25 and 28 from all immunised calves. Strong multiple bands were obtained with sera collected from calves 1, 2 and 7 on day 38, but single bands only were obtained from the serum collected from calf 8 on that day. This calf was the youngest of the group and produced the least impressive results on tick challenge.

It seems that the feeding and reproductive performances of ticks infesting immunised calves were significantly reduced. It seems reasonable to speculate that more impressive results may be obtained in cattle immunised with antigen II. Such immunisations are now being investigated. Theoretically, such an immunological method of tick control might be expected to be most effective against such species as *Boophilus microplus*, a relatively host-specific cattle tick, the larvae, nymphs and adults of which must feed in sequence from the same host. There would be potential problems in purification and large scale production of antigens but, if all cattle in an area could be successfully immunised, one might expect significant reduction in both numbers of ticks and in the tick-borne diseases of cattle.

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Involvement of a gene on chromosome 9 in human fibroblast interferon production

HUMAN fibroblast interferon is a single monomeric glycoprotein[1], and presumably a single structural gene codes for the human interferon polypeptide. It was originally reported that chromosomes 2 and 5 were necessary together for human interferon production[2]. More recent evidence suggests that both chromosomes 2 and 5 carry separate human interferon genes[3,4]. However, we have found, in two separate series of human–mouse somatic cell hybrid cell lines and their diphtheria toxin-resistant subclones[5,6], that human interferon production segregated from chromosome 5 and, furthermore, did not correlate with the presence of chromosomes 2 and 5 together[7]. Furthermore, using 29 hybrid clones (13 independent primary hybrids and 16 subclones) we could exclude chromosomes 1–8, 10–22 and X as candidates for the localisation of a single gene coding for human interferon on the basis of discordance of more than three clones for the segregation of human interferon production and the presence of a particular chromosome. However, there was a good correlation between the production of human interferon and the presence of chromosome 9. Here we demonstrate this directly.

The human fibroblast cell line, GM705 (Human Genetic Mutant Cell Repository, Camden, New Jersey) contains one normal inactive X chromosome and an X; 9 translocation (q12; p24) in which the long arm of X carrying the genes coding for hypoxanthine–quanine phosphoribosyl transferase (HPRT) and glucose-6-phosphate dehydrogenase (G6PD) is translocated onto chromosome 9 (refs 8, 9). When these cells are fused with mouse cells lacking HPRT, the X; 9 translocated chromosome bearing the gene for human HPRT can be selected for using the standard hypoxanthine–aminopterin–thymidine (HAT) selection procedure and selected against by 8-azaguanine. Thus, we used hybrid cells containing the X; 9 translocated chromosome to test directly the involvement of chromosome 9 in human interferon production.

Human GM705 cells, grown in Ham's F12 medium plus 20% fetal bovine serum (FBS) were fused with the $HPRT^-$ mouse cell lines L-A9 (ref. 10) and PG19 (ref. 11) using polyethylene glycol (molecular weight 6,000 (ref. 12)). Hybrid cells were selected in Ham's F12 plus 10% FBS containing HAT (hypoxanthine 13.6 $\mu g\,ml^{-1}$, aminopterin 0.19 $\mu g\,ml^{-1}$, and thymidine 3.9 $\mu g\,ml^{-1}$) and ouabain (10^{-5} M)[13], which kill mouse and human parental cells, respectively. Hybrid cell lines were isolated as previously described[7], grown to confluency in 100-mm plastic dishes ($1.5–4.0 \times 10^6$ cells per dish) and induced for interferon production with Newcastle disease virus (NDV) strain F (1×10^{-4} haemagglutinating units per cell). Interferon containing fluids were collected at 20–24 h post infection, dialysed for 5 d at 4 °C against *p*H 2 buffer to inactivate residual NDV and returned to *p*H 7.4 by dialysis against culture medium. They were then assayed for human and mouse interferon in human embryo or foreskin fibroblasts and mouse L-A9 or L-929, respectively, using the method of 50% inhibition of viral ribonucleic acid synthesis ($INAS_{50}$)[14]. Neither human nor mouse interferon gave a detectable antiviral response in the respective heterologous cell system. Use of specific antisera showed that the hybrids produced human fibroblast interferon and not human leukocyte interferon.

Eight independently selected hybrid cell lines formed from the fusion of human GM705 and mouse PG19 all produced human and mouse interferons, although titres of human interferon produced were substantially less than those of mouse interferon (Table 1). GM705 fibroblasts produced 10^2 U per ml per 10^6 cells when virally induced in the same conditions. Isozyme analyses of GP hybrids indicates that all eight contain enzyme markers for chromosomes X and 9 (that is, G6PD, AK1, AK3 and $ACON_s$, see Table 1 legend for abbreviations) and in

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Table 1 Interferon production and human chromosomes in human–mouse cell lines

Hybrid cell line	Interferon yields* Human	Mouse	Methods of analysis	1			2		3	4	5	6	7	8	9			10	11	12	13	14	15	16	17	18	19	20	21	22	X	X; 9
				ENO_1	PEPC	MDH_S	ACP_1	ICD_S	—	—	HEX_β	ME_S	—	—	AK_1	AK_3	$ACON_S$	GOT_S	LDH_A	PEPB	ESD	NP	MPI	APRT	GALK	PEPA	GPI	ADA	SOD_A	$ACON_M$		G6PD
GP-1	620	8,890	I	+	+	+	+	ND			−	+			+	+	+	ND	+	+	ND	+	ND	+	+	+	+	+	ND	ND		+
GP-2	890	178,000	I	+	+	−	−	±			−	+			+	+	+	±	±	+	+	+	−	+	+	+	+	+	−			+
GP-3	445	31,500	I	+	+	+	+	ND			−	+			+	+	+	ND	+	+	ND	+	ND	+	+	+	+	+	ND	ND		+
GP-4	890	22,400	I	+	+	+	+	+			−	+			+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−		+
GP-5	125	11,200	I	+	+	+	+	ND			±	+			+	+	+	ND	+	+	ND	+	ND	+	+	+	+	+	ND	ND		+
GP-6	790	88,900	I	+	+	+	+	ND			±	+			+	+	+	ND	ND	+	ND	+	ND	+	+	+	+	+	ND	ND		+
GP-9	500	31,600	I	ND	+	+	+	ND			−	+			+	+	+	ND	+	+	ND	+	ND	ND	+	+	+	+	ND	ND		+
GP-15	620	112,000	I	+	+	+	+	+			±	+			+	+	+	+	+	+	+	+	+		+	+	+	+	+	+		+
GL-A	125	1,000	C(%)	0			36		44	0	52	60	20	60	4			0	60	44	0	28	32	48	0	60	56	52	64	0	60	64
			I	−	−	+	ND	ND			+	ND			see X; 9			−	+	±	−	+	ND	+	ND	+	+	+	ND	ND		+
GL-B	125	2,810	C(%)	0			16		40	0	0	64	36	52	0			0	56	48	0	28	32	52	0	60	52	52	60	0	64	64
(Derived by diphtheria toxin selection of GL-A)			I	−	−	±	ND	ND			−	ND			see X; 9			−	+	+	−	+	ND	+	ND	+	+	+	ND	ND		+
GL-C	0	6,700	C(%)	0			8		40	0	0	68	28	60	0			0	68	0	0	28	20	72	0	64	52	52	72	0	0†	0
(Derived by 8-azaguanine selection of GL-B)				−	−	±	±	ND			−	+			see X; 9			ND	+	−	ND	+	ND	+	ND	+	+	+	ND	ND		−

Metaphase chromosomes were banded and stained using the method of Bishun *et al.*[15]. Twenty-five metaphase figures were analysed for each hybrid cell line and numbers of human chromosomes expressed as percent. Human isozymes used as markers for individual chromosomes were, with the exception of *N*-acetyl hexosaminidase-β, tested by routine electrophoretic methods (Harris and Hopkinson[16,17]). *N*-acetyl hexosaminidase-β was assessed by immunodiffusion (Swallow *et al.*[6]). Abbreviations: ENO_1, enolase-1; PEPC, peptidase-C; MDH_S, soluble malate dehydrogenase, ACP_1, acid phosphatase-1; ICD_S, soluble NADP-dependent isocitrate dehydrogenase; HEX_β, *N*-acetyl hexosaminidase-β; ME_S, soluble malic enzyme; AK_1, adenylate kinase-1; AK_3, adenylate kinase-3; $ACON_S$, soluble aconitase; GOT_S, soluble glutamate-oxaloacetate transaminase; LDH_A, lactate dehydrogenase-A; PEPB, peptidase-B; ESD, esterase-D; NP, purine nucleoside phosphorylase; MPI, mannose phosphate isomerase; APRT, adenine phosphoribosyl transferase; GALK, galactokinase; PEPA, peptidase-A; GPI, glucose phosphate isomerase; ADA, adenosine deaminase; SOD_A, superoxide dismutase; $ACON_M$, mitochondrial aconitase; G6PD, glucose-6-phosphate dehydrogenase. I, isozyme analysis; C, chromosome analysis; ND, not done.

* Titres expressed as reference research units per ml per 10^6 cells.

† The normal inactive X chromosome was lost in GL-C, presumably due to the 'random' segregation of this chromosome.

addition have several other human chromosomes in common. The chromosome 5 marker HEX_β was absent or present only in trace amounts (Table 1). The three chromosome 2 enzyme markers tested (MDH_S, ACP_1, ICD_S) were also absent in one clone (GP-2). This particular clone (GP-2) also responds to polyriboinosinic acid: polyribocytidylic acid and superinduction and has retained the capacity to produce human interferon when virally induced after 6 months in continuous serial culture in medium containing HAT.

Further hybrids were prepared from the fusion of GM705 and mouse L-A9 cells. One of these, GL-A, contained chromosome 5, was selected with diphtheria toxin at 10^{-1} L_f ml^{-1} (see ref. 5) in the presence of HAT, and the resulting resistant (segregant) cells grown and tested for interferon production. These derived cells, GL-B, maintained the ability to produce human interferon at the same level as the parental hybrid, GL-A (Table 1). However, when GL-B cells were exposed to 8-azaguanine (10 μg ml^{-1}) to select against the X; 9 chromosome, and resistant hybrid cells (GL-C) grown and tested for interferon production, the capacity to produce human interferon was lost (Table 1). Recently, similar results have been obtained after growth of GP-2 in 8-azaguanine. Karyotype and isozyme analyses of GL-A, B and C (Table 1) showed that chromosome 5 was eliminated by diphtheria toxin (GL-B) and X; 9T by 8-azaguanine (GL-C).

The production of high levels of human interferon by all the clones selected to contain the X; 9 chromosome, and the loss of human interferon production in the 8-azaguanine-resistant clones strongly suggest that a gene on the X; 9 chromosome is involved in human interferon production; this is presumably on 9 rather than on X, as previously we have found 11 clones discordant for the presence of X and human interferon production[7]. The only other chromosomes lost in GL-C that were present in GL-B were chromosome 12, which has been excluded in our previous study[7], and the inactive X chromosome.

Thus, our data point to the assignment of the human fibroblast interferon gene to chromosome 9 rather than 2 or 5, as these are absent in one or more of our hybrid clones which are inducible for human interferon. As fibroblast interferon is a glycoprotein, the products of genes other than the structural gene must be required for the secondary modification of the human interferon polypeptide. This modification may be completed by products of the rodent genome. However, we cannot exclude the possibility that genes on the other human chromosomes in addition to chromosome 9 are required for human interferon production in our hybrids, but neither 2 nor 5 seem to be involved. The basis for the discrepancy of our results from those of Tan[3] and Slate and Ruddle[4] is not clear. These authors did not observe chromosome 9 in any of their reported hybrids[2,4]. However, Slate and Ruddle (persbnal communication) have now also shown that chromosome 9 does contain a structural gene for human interferon by using a human–mouse hybrid containing chromosome X; 9 as the only human chromosome. C. Chany (personal communication) also has evidence for the involvement of chromosomes 2, 5 and 9. Thus, it is possible that there is more than one structural gene for human interferon, and that they are on different chromosomes, although our evidence so far fails to show this.

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Sheep fetal haematopoietic cells produce adult haemoglobin when transplanted in the adult animal

DURING the fetal stage of development, the human erythroid cells synthesise mainly fetal haemoglobin ($\alpha_2\gamma_2$) but switch to almost exclusive formation of adult haemoglobin ($\alpha_2\beta_2$) soon after birth. Similar ontogenetic changes in the activity of the closely linked γ- and β-genes are seen in most primates and few other mammals. In goats and sheep over 95% of the haemoglobin synthesised in the fetus is of the fetal type; the switch from fetal to adult haemoglobin formation starts at about 130 gestational days and is completed within a few weeks[1]. Several hypotheses have been formulated to account for these striking changes in haemoglobin gene activity during ontogeny, but the factors responsible for haemoglobin switching *in vivo* remain unknown. We report here an experiment which shows that the fetal haematopoietic cells readily switch to adult haemoglobin formation once transplanted to an adult animal. Our findings provide evidence that the switch from fetal to adult haemoglobin formation is not restricted to the perinatal developmental stage, and raise the possibility that the haematopoietic microenvironment determines the programme of haemoglobin formation in the haematopoietic progenitor cells of the fetus or the adult animal.

The experiment involved transplantation of haematopoietic cells from sheep fetuses aged 100 gestational days to adult female sheep. To have definitive markers of a successful graft of the transplanted tissue and to distinguish between the globin produced by the donor and the recipient red cells, we used the polymorphism at the β-haemoglobin locus of the sheep[2]. There are two common alleles of this locus, β^A and β^B, directing the synthesis of the two sheep haemoglobins A($\alpha_2\beta_2^A$) and B($\alpha_2\beta_2^B$). The two haemoglobins can be clearly separated and quantitated by isoelectric focusing[3]. We therefore transplanted fetal haematopoietic cells known to be homozygous for the Hb *A* gene to recipient adult animals who were homozygous for the Hb *B* gene.

The fetal haematopoietic cells were obtained from the livers and bone marrow of twin sheep fetuses. No matching between donor and recipient cells was done. The cells (10^7 cells per kg body weight) were infused in the donor animal, which had been lethally irradiated (950 rads per total body) 2 h before the cell infusion. One of the irradiated animals died 1 d after transplantation; two other animals died at post-transplant days 11 and 15. The fourth Hb *BB* animal was transplanted with cells from a closely related Hb *AA* fetus (coefficiency of kinship 0.725), received post-transplant support with leukocyte transfusions (the leukocytes were obtained from a donor sheep that was homozygous for Hb *B*) and survived for 45 d; cause of death was an infection due to defective cannulation.

In this fourth animal, sequential haemoglobin analyses were carried out and findings are shown in Fig. 1. It is apparent that (1) there was a successful graft of the donor pluripotent stem cells, as Hb *A* appeared in the circulation of the Hb *BB* recipient; (2) the haematopoietic cells of the 100-d-old fetal donor switched to almost exclusive adult haemoglobin formation once they started supporting the erythropoiesis of the adult animal. There was no detectable production of Hb *C* by the transplanted *AA* cells. The small amounts of the fetal haemoglobin synthesised by the transplanted animal (Fig. 1*a*) were not unexpected as experiments in humans have shown that a transient production Hb *F* characteristically appears in post-transplantation erythropoiesis[4]. A control experiment of homotransplantation in a *BB* homozygous sheep showed that in the adult sheep, as in humans, post-transplantation erythropoiesis is associated with a transient increase in fetal haemoglobin production (Fig. 1*b*).

In vivo studies in man have clarified certain aspects of the cellular regulation of fetal haemoglobin production. Studies in patients with clonal haematopoietic stem cell disorders suggest that a single pluripotent stem cell can provide erythroid progeny which produce fetal haemoglobin as well as progeny which produce adult haemoglobin[5]. The interpretation of this is that the regulatory events that determine Hb *F* or Hb *A* production in the adult take place in haematopoietic cells that have left the

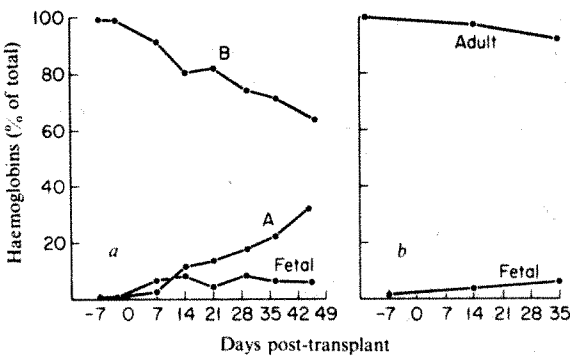


Fig. 1 Time course of haemoglobins A, B and F formation in sheep transplantation experiments. *a* Findings in a *BB* sheep transplanted with *AA* fetal sheep cells. *b*, The results of a homotransplantation experiment in a *BB* sheep; in *b* bone marrow cells were removed by multiple aspirations, the animal received 970 rads of whole body irradiation and was transfused with its own bone marrow cells. Note the appearance of a small amount of Hb *F* in the homotransplanted animal. The amount of Hb *F* is equivalent to that observed in experiment *a*.

pluripotent compartment of haematopoiesis. Evidence against the possibility that the pluripotent stem cells are irreversibly programmed for either fetal or adult haemoglobin expression is provided by the transplantation experiment described here. The transplanted stem cells were from a fetus which synthesised exclusively Hb *F*. If the Hb *F* phenotype of the red cell was determined at the pluripotent stem cell level, large amounts of fetal haemoglobin should have appeared in the circulation of the transplant recipient; instead, adult haemoglobin of the donor cell genotype (Hb *A*) appeared in the circulation as soon as there was evidence for engraftment of the transplant. Considering the size of the blood volume of the recipient and the volume of the transplanted cells, the best interpretation of the finding is that the erythroid progeny of the transplanted haematopoietic stem cells readily switched to adult haemoglobin formation.

This experiment is also informative with regard to the factors which are involved in the regulation of fetal to adult haemoglobin switching. Thus, the findings show that it is unlikely that the

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temporary presence of a regulatory substance during the perinatal period of ontogeny mediates the switching from fetal to adult haemoglobin formation. It is more likely that the fetal cells were switched to adult haemoglobin formation either because they were pre-programmed to switch at a given developmental time (130–140 d from conception) or because they were re-programmed to form adult haemoglobin when they were found in the adult environment. Of the two possibilities, we consider the first one less probable. Between 100 and 135 gestational days, the cells in the sheep fetus produce predominantly Hb *F* (refs 1, 6); if the transplanted stem cells continued to express a pre-determined developmental programme, predominantly Hb *F* would have been formed during the first month of repopulation of the haematopoietic tissue of the recipient animal, but, as shown in Fig. 1*a* and *b*, the amount of Hb *F* in the transplanted animal was no more than that produced by the adult cells of the homotransplant control. This finding suggests that the fetal cells readily switched to an adult haemoglobin formation programme when they were moved to an adult haematopoietic environment, and raises the possibility that the change in haematopoietic environment underlays the Hb *F* to Hb *A* switching in the transplanted cells. We therefore proposed that it is the continuous presence of certain microenvironmental influences in the fetal and their absence in the adult haematopoietic tissue (or the reverse) which makes the erythroid progenitors express the fetal or the adult developmental programmes.

The phenomenon of haemoglobin switching has a twofold interest: (1) as a prototype for understanding the regulation of gene activity in development and differentiation; (2) as a therapeutic model for the treatment of sickle cell anaemia and homozygous β-thalassaemia. Patients with both these disorders could become virtually asymptomatic if it was possible to reverse the switch from Hb *F* to Hb *A* formation. If environmental factors mediate this switch, identification of these factors and their mode of the interaction with the progenitor cells may provide clues to the treatment of the β-chain haemoglobinopathies.

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Evidence of continuous evolutionary change in structures mediating adherence of lymphocytes to specialised venules

MANY cell–cell interactions are similar in a wide variety of species; for instance, penetration of ova by spermatozoa, morphogenetic cell movements during embryogenesis, and specific cellular cooperation during the immune response in vertebrates. The operation of apparently identical functional interactions in diverse species, however, does not exclude the possibility that the cell surface receptors and ligands involved may have changed considerably during evolution. In fact, studies of the molecular evolution of certain enzymes and proteins present in numerous species have demonstrated that structural evolutionary change (that is, amino acid substitutions) may proceed even in the absence of observed functional modification[1]. For this reason, we suggest that the investigation of the degree of evolutionary change in structures mediating conserved cell–cell interactions requires determination of the degree to which the interaction is possible between cells from different species. The present study was designed to examine quantitatively the effect of progressive evolutionary divergence on interspecies cellular cooperation in a simple, highly conserved cell–cell interaction: the specific adherence of lymphocytes to specialised lymphocyte-binding high endothelial venules (HEV) in lymph nodes. Based on results of quantitative *in vivo* and *in vitro* assays, we report that the ability of lymphocytes from different vertebrate species to recognise specifically and bind to HEV in the mouse declines exponentially with increasing evolutionary separation of the lymphocyte donor from the mouse host. This suggests that, in spite of conservation of the functional interaction in all mammalian species examined[2], the cell-surface structures mediating lymphocyte adherence to HEV have been continuously and randomly altered during evolution.

As originally demonstrated by Gowans and Knight[3], lymphocytes bind to and migrate through the endothelium of post-capillary HEV in lymph nodes during their normal recirculation from blood to lymph. The adherence of lymphocytes to HEV is a highly specific cell–cell interaction: other cells in the blood do not bind to HEV, and lymphocytes do not normally bind to other vessel walls. We have combined short-term *in vivo* homing studies with two previously described *in vitro* methods[4,5] (modified in our laboratory for quantitative analysis[6,7]) to examine the relative ability of lymphocyte populations from several vertebrate species to adhere to HEV in the mouse.

Briefly, the three assay systems used were: (1) an *in vivo* autoradiographic study, comparing the number of radiolabelled lymphocytes localising in HEV 15 min after intravenous injection; (2) a frozen section assay[7] (modified from the method of Stamper and Woodruff[5]), comparing the binding of various lymphocyte populations to HEV in fresh frozen sections of mouse mesenteric node during incubation at 7 °C; and (3) a perfusion assay[6] (modified from Sedgley and Ford[4]), comparing the adherence of lymphocytes to HEV during single pass systemic vascular perfusion at 37 °C. In each system, a specific adherence index (SAI) was calculated relating the observed adherence (to mouse HEV) of sample lymphocytes to that of mouse lymphocytes.

The SAI of splenic lymphocyte populations from several vertebrate species are related directly to the evolutionary distance[8–10] separating the lymphocyte donor from the mouse host (Fig. 1a). Two conclusions can be drawn from these data. First, the *in vitro* and *in vivo* assay systems give similar results. Second, the ability of lymphocytes to adhere to host mouse HEV is inversely related to the evolutionary separation of donor and host, yet is still significantly conserved in the most divergent species tested. (It is remarkable that birds, which are separated

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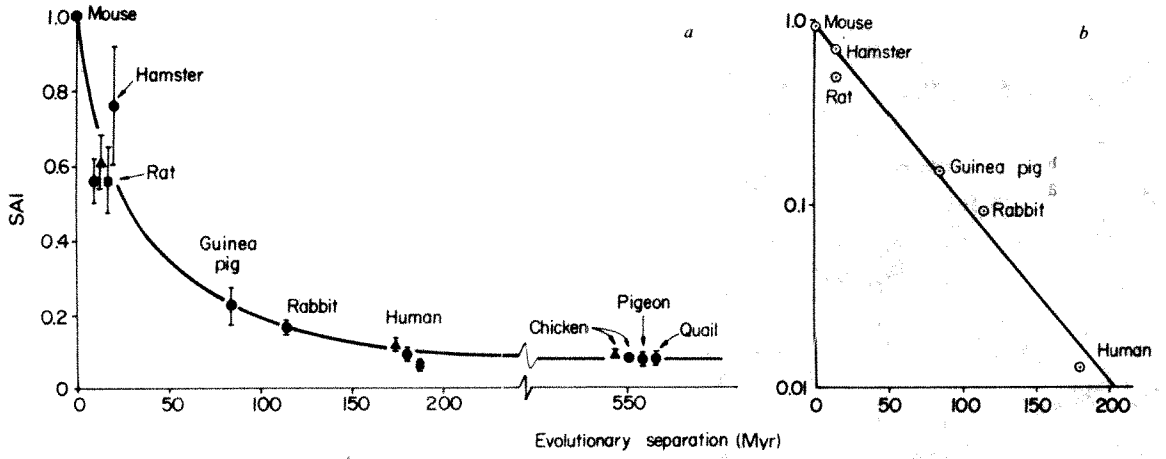


Fig. 1 *a*, Relationship between specific adherence index (SAI) and evolutionary separation of lymphocyte donor from mouse host. Adherence of sample lymphocytes to mouse lymphocyte-binding high endothelial venules (HEV) was measured *in vivo* 15 min after i.v. injection (▲); during single pass systemic vascular perfusion at 37 °C (■), or during incubation on frozen sections of mesenteric lymph nodes at 7 °C (●). The SAI of mouse lymphocytes is unity, and that of negative control cells is 0. Error bars are standard deviations of the ratio estimates. All samples were spleen cells except rat and mouse *in vivo*, which were pooled spleen and mesenteric node cells. 5–8-week-old (BALB/c × C57BL/6 Jax)F_1 mice, Lewis rats, Syrian Gold hamsters, a 6–8-week-old Hartley guinea pig, 6–10-week-old White Leghorn or New Hampshire Red hens, 6-week and 1-year old New Zealand White rabbits, and young adult quail and King pigeons were used. Human spleens were uninvolved laparotomy specimens from young patients with Hodgkins disease. *b*, Demonstration of negative exponential relationship after subtraction of the conserved baseline adherence fraction ($K = 0.080 \pm 0.008$) typified by avian lymphocyte adherence. Data from frozen section assay. *In vivo* system (▲): autoradiographs were made of sections of mesenteric lymph nodes taken from mice killed 15 min after i.v. injection of 10^8 ^{3}H-leucine or ^{3}H-uridine labelled donor lymphocytes. SAI is defined as the average number of sample lymphocytes per ×40 microscope field of lymph node paracortex (either in HEV or already inside the node) divided by the corresponding value for mouse lymphocytes. Background (non-adherent labelled lymphocytes passively trapped in HEV) was less than 3% of even the most poorly adhering sample population. Frozen section assay (●): a monolayer of lymphocytes in Hank's balanced salt solution containing 1% bovine serum albumin was incubated at 7 °C for 30–45 min on freshly cut unfixed frozen sections of mouse mesenteric node. Medium was removed and the section fixed with 1% glutaraldehyde in cold phosphate-buffered saline. After fixation, non-adherent cells were gently rinsed off. To allow quantitative comparisons, an internal standard population of fluorescent lymphocytes labelled by 15 min incubation with 60 μg ml^{-1} fluorescein isothiocyanate (FITC) at 37 °C or 5–50 μg ml^{-1} rhodamine isothiocyanate (RITC) at 25 °C, was mixed with each population before incubation. A reference sample of mouse lymphocytes and a negative control population of erythrocytes or formalin-fixed lymphocytes were included. Data from at least 5 mesenteric node sections were pooled for each sample. The ratio of sample to standard cells in the incubation mixtures (R_I) and adherent to HEV (R_{HEV}) was determined. A specific adherence ratio, R_{HEV}/R_I, was calculated for each sample (SAR_S), for mouse lymphocytes (SAR_M) and for negative control cells (SAR_N). SAI was defined as: $SAI = (SAR_S - SAR_N)/(SAR_M - SAR_N)$. Standard deviations of all ratios were calculated by the delta method. Perfusion assay (■): a mixture of 10^8 ^{3}H-leucine labelled sample spleen cells, 10^8 RITC-labelled standard cells, and 10^8 formalin-fixed FITC-labelled thymocytes (as an internal negative control) in 15 ml medium 199 containing 1% bovine serum albumin was perfused at 37 °C and 2-3 ml per min through the systemic vasculature of an anaesthetised mouse (in via a trocar inserted in the left ventricle, out via an incision in the right atrium). Non-adherent cells were flushed with 10 ml medium. Solitary sample, standard, and negative control cells in HEV were counted in autoradiographs of 6-μm frozen sections of mesenteric nodes from duplicate perfusions. SAI were calculated as for the frozen section assay.

from mice by over 500 million years of evolution and lack morphologically well-developed HEV of the mammalian type[2], nonetheless have lymphocytes capable of recognising and adhering to mouse HEV (Fig. 2).) This decline is not limited to spleen cell populations, as a similar evolutionary decrease in adherence to mouse HEV was observed for mesenteric node lymphocytes from several mammalian species. Furthermore, in all cases examined (mouse, rat, rabbit and chicken) adherence remained a specific property of lymphocyte populations normally capable of entering lymph nodes *in vivo*: immature, non-migrating lymphocytes bound poorly in relation to their mature counterparts[6].

As shown in Fig. 1*b*, the observed relationship between SAI and evolutionary distance approximates a negative exponential function with a decay rate of 2.5 ± 0.3% of the adherence index per million years. Such a clearly defined relationship requires explanation. Although the physical characteristics of cells, such as cell size, surface morphology, surface charge, membrane protein and carbohydrate content or membrane fluidity may well influence certain cellular interactions, it is difficult to invoke such nonspecific parameters in the present case. There is no correlation between lymphocyte size or surface morphology and adherence to HEV—human lymphocytes are larger and exhibit more surface microvilli than either mouse or chicken lymphocytes (when adherent to mouse HEV after vascular perfusion—E. B., van Ewijk, and I. W., unpublished). It is unlikely that either surface charge or carbohydrate content has a significant role, as adherence is unaffected by treatment of lymphocytes with neuraminidase[11,12]. Membrane fluidity is probably not a critical parameter, as it is undoubtedly reduced at 7 °C, a temperature at which lymphocytes bind well to HEV in frozen sections. A possible relationship between overall surface protein content and adherence canot be ruled out, as light trypsinisation of the lymphocyte surface markedly reduces binding[11,12]; it seems more likely, however, that trypsinisation destroys a specific binding protein. Fundamentally, it is difficult to construct a role for gross physical parameters, or a mechanism for their orderly alteration during evolution, that would result in the observed negative exponential relationship between adherence and evolutionary separation.

On the other hand, it is interesting to consider this negative exponential relationship in light of the previously cited studies of the molecular evolution of proteins. Dickerson[1] has reviewed data demonstrating that several proteins (such as cytochrome *c* and haemoglobin) have incorporated amino acid substitutions at characteristic and fairly constant rates during evolution, even though they have continued to serve the same function in diverging lineages. A linear rate of amino acid substitution implies an exponential rate of decline in the fraction of mutable amino acids a protein shares with its progenitor or with diverging proteins (ignoring the minor contribution of reverse mutations back to the original amino acid). Such an exponential decline in shared amino acids could explain a roughly parallel decline in interaction compatibility of the proteins in diverging species. We propose that the observed exponential decline in lymphocyte–HEV adherence compatibility may be the functional result of a linear rate of evolutionary mutation in the components—presumably amino acids—of the cell-surface structures mediating the interaction. Although natural selection would have ensured the continuing compatibility of 'drifting' lymphocyte and HEV surface receptors within a given species, no selective pressure would have prevented progressive development of incompatibility of interaction structures in diverging lineages. Strong selection against abrupt, major changes in the interactive sites of lymphocyte and endothelial cell receptors may have protected certain essential components from mutation, thus explaining the baseline level of interspecies compatibility demonstrated by avian lymphocytes (*K* in Fig. 1*b*).

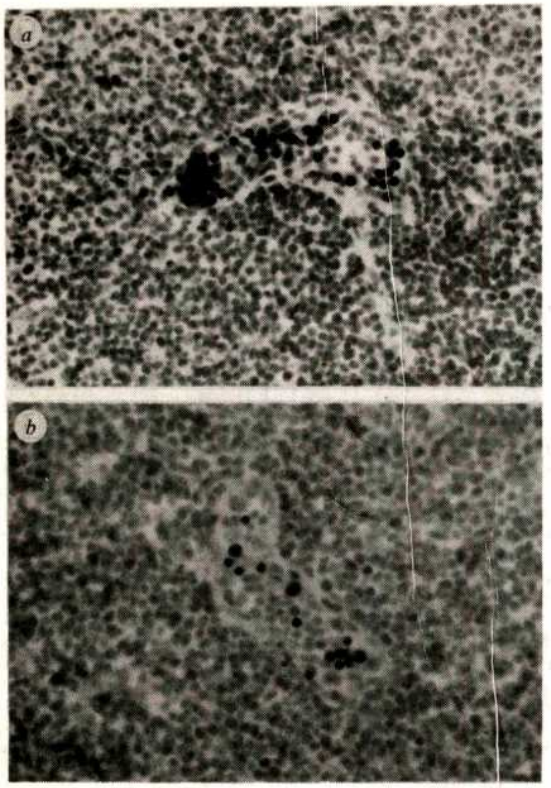


Fig. 2 Mouse (*a*) and chicken (*b*) lymphocytes (darker cells) adhering over HEV in frozen sections of mouse mesenteric lymph node (×200).

In addition to offering a plausible explanation for the observed relationship between SAI and evolutionary distance, this hypothetical model of steady evolutionary drift in interacting macromolecules predicts a similar exponential decline in interaction compatibility with increasing evolutionary separation in other systems. Examination of the ability of other simple, functionally conserved, cellular or molecular interactions to cross species barriers will test the general validity of this model. If verified, the model would imply that preservation of cellular and molecular interactions during evolution must be considered as a dynamic balance between mutation and selection, rather than static conservation.

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Distribution of fibronectin in the ectoderm of gastrulating chick embryos

A GLYCOPROTEIN known as large external transformation-sensitive (LETS) protein, cell surface protein (CSP) or fibronectin[1–3] can be identified on the surface of various cell types[1]. This protein has a molecular weight of 210,000–250,000 and is closely related to the cold-insoluble globulin found in serum and to the factor needed for cell spreading *in vitro*[4,5]. In culture many cells secrete fibronectin into the growth medium and on to the substratum[6–8]; fibroblasts and other cells of mesodermal origin produce particularly large amounts. However, in the early mammalian embryo fibronectin is present in basement membranes before the mesoderm has formed, when the embryo consists only of ectoderm and endoderm[9]. Adult epithelia also produce fibronectin *in vitro*[10], and it forms a component of epithelial basement membranes *in vivo*[11–13]. We have applied an immunofluorescent technique to whole mounts of early chick embryos to examine the distribution and possible morphogenetic function of fibronectin. We discuss our results here, and suggest that fibronectin is involved in morphogenetic movement during early development.

Embryos between Hamburger and Hamilton stages 3 and 6 (ref. 14) were mounted as for New culture[15] and fixed in phosphate-buffered formol saline for at least 12 h. During fixation the endoderm was removed from the area pellucida and parts of the area opaca. In some cases portions of the developing mesoderm were also removed. The embryos were detached from the vitelline membrane and washed overnight with several changes of phosphate-buffered saline. Fibronectin was localised by indirect immunofluorescence[16,17] (Fig. 1).

Fibronectin was detected in association with all parts of the ectoderm and its distribution is shown in Fig. 1. A well defined fibrous meshwork was seen around the entire perimeter of the outer area opaca margins of the blastoderm (Fig. 2) as it expanded across the vitelline membrane. The vitelline membrane itself showed no specific fluorescence. The anterior half of the boundary between the area pellucida and area opaca

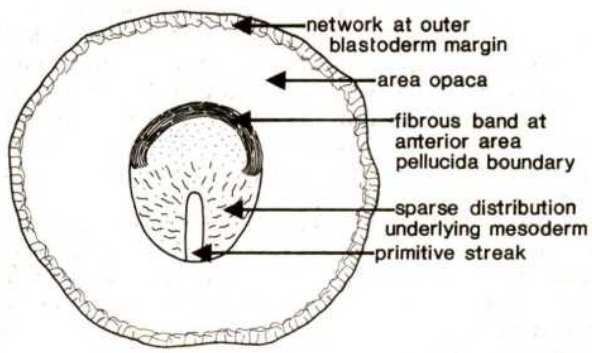


Fig. 1 Diagram to illustrate the overall distribution of fibronectin on the chick ectodermal basement membrane as detected by indirect immunofluorescence. The embryo is shown as if viewed from the ventral surface with the endoderm and mesoderm removed. Embryos were fixed in phosphate-buffered saline (PBS) containing 4% formaldehyde, and the endoderm and part of the mesoderm were removed by microdissection. They were then washed in PBS incubated at 37 °C for 30 min with rabbit anti-fibronectin antisera diluted 1:25 in PBS[16], and washed and incubated for a further 30 min with fluorescein isothiocyanate-labelled goat anti-rabbit (Miles) diluted 1:10. The embryos were washed with PBS, mounted ventral side uppermost on slides in 50% glycerol, and examined in a Zeiss photomicroscope. Phase contrast optics were used to identify particular areas of the embryos and epifluorescence UV illumination to visualise the fibronectin. Photographs were taken using Ilford HP5 film (400 ASA) and the sensitivity increased to 1,600 ASA by developing with Microphen (Ilford). Exposure time was 30–120 s. A total of 23 embryos were examined. Controls included substituting the rabbit anti-fibronectin antisera with pre-immune rabbit sera or using rabbit anti-fibronectin antisera which had been passed through a fibronectin–Sepharose affinity column[17]. In all cases the controls showed only a low background fluorescence.

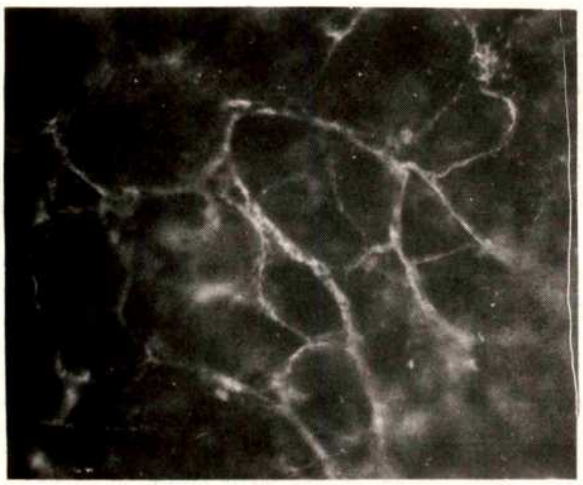


Fig. 2 Immunofluorescence demonstration of fibronectin at the outer margin of the chick blastoderm. Fluorescent fibres form a network (×990).

was outlined by an intensely fluorescent band of parallel fibres. This band was thickest in the midline anteriorly and gradually tapered as it extended posteriorly, terminating on a level with Hensen's node. Fluorescent granular deposits were present between the fibres (Fig. 3). The area pellucida on either side of the primitive streak is normally occupied by mesoderm, overlying the ectoderm. The mesoderm itself showed no specific fluorescence but if it was dissected away fibronectin could be detected on the underlying ectoderm. However, the fibronectin appeared poorly organised, the fluorescence being diffuse, with only occasional long thin fibres running mainly at right angles to the embryonic axis (Fig. 4). Between the mesoderm and the anterior boundary of the area pellucida is the proamnion, which is without mesoderm. Fluorescence there appeared as short thick disconnected rods. A very sparse and weakly fluorescent fibrous network of fibronectin was seen on the endoderm.

The fluorescence profile described above was not observed if rabbit anti-fibronectin was omitted during the staining procedure or if it was substituted with preimmune rabbit serum or rabbit antifibronectin antibodies which had previously been passed through a fibronectin–Sepharose column.

The distribution of fibronectin which we have observed in the early chick embryo can be linked to two known functions of the protein *in vitro*. First, its presence in cultures seems to facilitate cell movement[18], and second, it mediates the spreading of cells on substrates[19] and their attachment to collagen[20], although its function in the last respect may not be absolutely specific[21]. The situations in which we have observed accumulations of fibronectin in the embryo are those particularly associated with



Fig. 3 Immunofluorescence photograph of part of the fibrous band at the anterior area pellucida/area opaca boundary. The fibres are intensely fluorescent, run parallel to each other and are accompanied by particulate fluorescent material (arrows) (×900).

morphogenetic movement. All the outer margins of the blastoderm the area opaca ectoderm is active in locomotion. The outer margins of the blastoderm are responsible for the expansion of the blastoderm across the vitelline membrane so that ultimately the egg yolk is enclosed in the yolk sac[22]. This movement is associated with a high concentration of fibronectin around the marginal ectoderm cells.

The distribution pattern of fibronectin demonstrated by immunofluorescence also coincides closely with a distribution pattern of fibres seen on the ectodermal basement membrane in the area pellucida and area pellucida/area opaca boundary zone by scanning electron microscopy (SEM)[23,24] (Fig. 5). This pattern can be correlated with two separate morphogenetic movements, those of the mesoderm and of the primordial germ cells.

Immunofluorescence demonstrates fibronectin on the ectoderm surface underlying the mesoderm. Mesoderm cells reach their position between the ectoderm and endoderm by descending through the primitive streak and moving laterally out from the streak, across the area pellucida and eventually into the lateral and posterior area opaca, using the ectodermal basement membrane as a substrate[25–27]. Mesoderm cells reach the area opaca in front of the area pellucida by migrating around the anterior boundary between the area pellucida and area

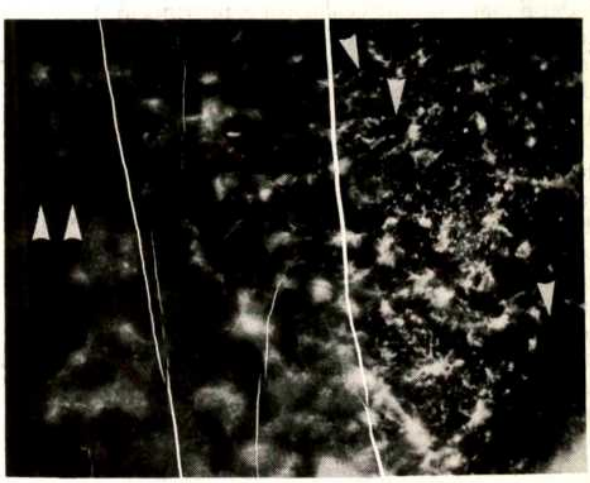


Fig. 4 An area from near the posterior end of the area pellucida. On the left side of the picture the mesoderm is only very faintly and diffusely stained. Removal of mesoderm as on the right reveals bright fluorescence, mainly finely dispersed but also as very thin fibres (arrows). The double arrow marks the embryonic axis (×340).

opaca. This boundary contains a particularly high concentration of fibronectin. The same boundary zone constitutes the germinal crescent. Primordial germ cells form there by detaching from the endoderm. The germ cells then move posteriorly around the germinal crescent to make contact with the developing vitelline vessels, reaching the gonads through the circulation[28,29]. They have therefore moved against the main direction of mesodermal migration around the anterior area pellucida boundary. A high concentration of fibronectin in the germinal crescent may assist the germ cells in this specific migration, and in fact, SEM reveals contacts between primordial germ cells and the fibrous band in the germinal crescent[23]. The developmental stage at which the fibronectin pattern is maximally defined is further evidence that its function is linked to morphogenetic movements. Mesoderm cells enter the anterior boundary zone between the area pellucida and area opaca after Hamburger and Hamilton stage 5 (refs 14, 30). Immediately before this stage the band as seen by immunofluorescence and SEM becomes most prominent[23], but after the entry of mesoderm into the boundary zone it becomes disorganised. Thus, the band has a transient existence coinciding closely with the time at which the anterior border of the area pellucida is a particularly active site of cell movement.

Fibronectin is present in the greatest quantity and in the most highly organised arrangement in the germinal crescent and

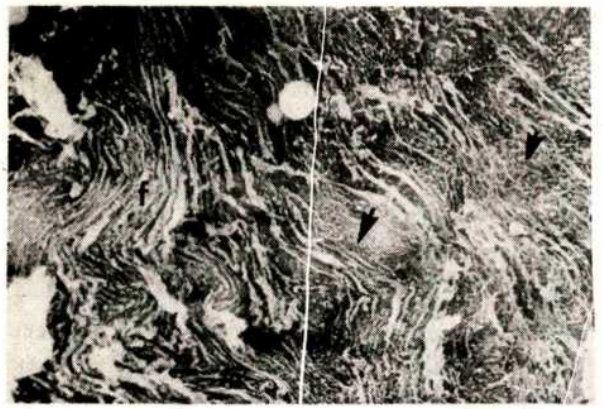


Fig. 5 Scanning electron micrograph of a portion of the fibrous band encircling the anterior boundary between area pellucida and area opaca in a stage 4 embryo. The band consists of rough-surfaced fibres in a parallel arrangement (f). They are accompanied by some loose granular material (arrows) (×1,275).

around the perimeter of the blastoderm. Both these sites are free of mesoderm cells at the time of our observations. At the blastoderm margins only ectoderm is present; in the germinal crescent the ectoderm is associated with endoderm.

We suggest that the fibronectin pattern is laid down by the ectoderm either alone or in association with endoderm and that its function is to facilitate certain morphogenetic movements in early development. First, it is involved in the overall expansion of the blastoderm across the vitelline membrane. Second, fibronectin contributes to a contact guidance system laid down by the ectoderm and utilised by primordial germ cells and mesoderm cells. The cells would tend to move along pathways of fibronectin because of the effects of the protein in favouring cell spreading and locomotion. Further work is in progress to observe these movements directly in living embryos.

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Insulin-receptor antibodies mimic a late insulin effect

AUTOANTIBODIES against the insulin receptor are found in patients with a rare form of extreme insulin resistance associated with acanthosis nigricans[1]. These antibodies are primarily polyclonal IgGs, compete with insulin for binding to its receptor[2,3], immunoprecipitate solubilised insulin receptors[4], and have been used as probes of insulin action. The acute action of the antibodies is to mimic many of insulin's biological effects, particularly those which occur rapidly after hormone binding to its cell-surface receptors[5–9]. In addition, insulin exerts biological effects which appear more slowly. For example, insulin treatment of 3T3-L1 fatty fibroblasts increases the activity of lipoprotein lipase maximally after 2–4 d but is without effect during the first 4 h (refs 10, 11). Some investigators have suggested that these slower effects may be mediated by different mechanisms from those involved in the production of the acute effects[12,13]. We now report that autoantibodies against the insulin receptor mimic this long-term insulin effect on lipoprotein lipase.

3T3-L1 fatty fibroblasts exposed for 24 h to increasing concentrations of partially purified anti-receptor antibodies showed a dose-dependent increase in the activity of lipoprotein lipase (Fig. 1*a*). Significant stimulation of enzyme activity was observed with as little as 3.5 $\mu g\ ml^{-1}$ anti-receptor antibodies and the effect was maximal with 35 $\mu g\ ml^{-1}$ IgG, reaching 65% of the response induced by a maximal stimulatory insulin concentration (1 $\mu g\ ml^{-1}$). In contrast, normal IgG had no stimulatory effect on the activity of the enzyme. However, with very high concentrations of anti-receptor antibodies (350 $\mu g\ ml^{-1}$) the stimulatory activity decreased. This seems to be due to a nonspecific effect of these preparations on the cells or enzyme system, as the same high concentration of normal IgG produced a similar decrease in basal enzyme acitivity (Fig. 1*a*) and diminished the stimulatory effect of high insulin concentrations (data not shown).

As previously demonstrated, the effect of insulin on lipoprotein lipase activity in 3T3-L1 fatty fibroblasts is a slow phenomenon, requiring 12 h for half-maximal insulin stimulation[10,11]. Similarly, the stimulatory effect of the anti-receptor antibodies is characterised by a slow time course of induction (Fig. 1*b*). After 12 h a maximally effective concentration of anti-receptor antibodies (35 $\mu g\ ml^{-1}$) was about equipotent to a maximally effective insulin concentration (1 $\mu g\ ml^{-1}$) in stimulating the lipoprotein lipase activity. The maximal increase in the enzyme activity in the presence of this most potent antibody concentration was observed after 24 h of exposure, and in contrast to the effect of insulin, stimulation by the antibodies then tended to decline. This is probably due to a desensitisation of the biological response at post-receptor sites observed with chronic antibody exposure, but not with insulin. We observed a similar phenomenon when studying the effect of the same antibody preparations on glucose transport and oxidation in 3T3-L1 fatty fibroblasts[7]. Thus, acute exposure of these cells to anti-receptor antibodies resulted in a stimulation of 2-deoxyglucose transport and glucose oxidation, whereas after prolonged exposure basal glucose metabolism returned to control levels. This occurred without change in the ability of the antibodies to inhibit insulin binding. Our interpretation of these data is that despite persistent occupancy of the insulin receptor the anti-receptor antibodies are unable to produce a continuing signal for generation of the biological effects.

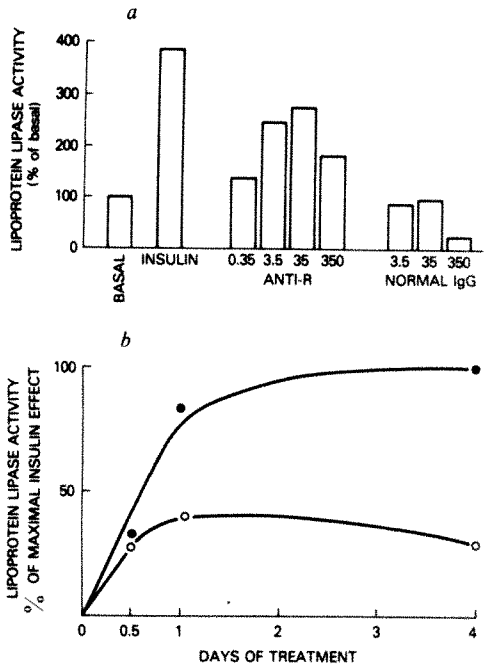


Fig. 1 *a*, Stimulation of lipoprotein lipase in 3T3-L1 fatty fibroblasts by increasing concentrations of antibodies against the receptor for insulin 3T3-L1 cells were isolated by Dr Howard Green and provided by him and Dr Ora M. Rosen[14–16]. Fibroblasts were grown to confluence in Petri dishes with Dulbecco–Vogt's minimal essential medium containing 10% fetal calf serum. After confluence they were treated with media supplemented with 1-methyl-3-isobutylxanthine (0.5 mM) and insulin (1 μg ml^{-1}) for 2 d followed by insulin alone for 4 d to enhance conversion to fatty fibroblasts. Thereafter, cells were cultured for 4 d in Dulbecco–Vogt's minimal essential medium containing 5% fetal calf serum. The fatty fibroblasts were then exposed to insulin (1 μg ml^{-1}), partially purified antibodies against the receptor for insulin, or normal IgG. After 24 h the activity of the enzyme lipoprotein lipase was measured in the cells as described by Spooner *et al.*[17]. One unit of lipoprotein lipase is defined as the amount of enzyme that releases 1 μM of ^{3}H-oleic acid per min at 37 °C from a serum-activated emulsion of ^{3}H-trioleoylglycerol and L-α-phosphatidylcholine. The partially purified antibodies against the receptor for insulin were prepared as follows. Plasma from a patient (patient B-2) with insulin resistance of the type B syndrome[1], containing a high titre of antibodies against the receptor for insulin (1:2,000), was treated with 33% ammonium sulphate. The precipitate was dissolved in and dialysed against 0.05 M sodium phosphate buffer, *p*H 7.4, containing 0.15 M NaCl, to a protein concentration of 10 mg ml^{-1} (based on absorbancy measurements assuming $A^{1\%}_{280\,cm} = 12$). This preparation is referred to as anti-R. The preparation referred to as normal IgG was obtained from plasma from a normal person using the same procedure. Results in this and all subsequent figures represent means of duplicate activity measurements each on three to six individual plates of cells. The data are expressed as per cent of basal lipoprotein lipase activity which was 0.27 ± 0.05 U per mg cell DNA (mean ± s.e.m.; $n = 4$). Lipoprotein lipase activity in the presence of insulin, 1.03 ± 0.06 U per mg DNA ($n = 4$), was statistically different from the basal activity at the $P < 0.001$ level using Student's *t*-test. Activity in the presence of 3.5 and 35 μg ml^{-1} anti-R was 0.69 ± 0.08 ($n = 4$) and 0.77 ± 0.07 U per mg DNA ($n = 4$) respectively; both values are statistically different from basal activity ($P < 0.01$). Activity in the presence of 350 μg ml^{-1} anti-R, 0.49 ± 0.05 ($n = 4$), was marginally significant ($0.05 < P < 0.10$). *b*, Time course of insulin (●) and anti-R (○) induced stimulation of lipoprotein lipase activity in 3T3-L1 fatty fibroblasts. 3T3-L1 fatty fibroblasts were exposed for 0.5, 1 and 4 d to either insulin (1 μg ml^{-1}) or antibodies against the receptor for insulin (35 μg ml^{-1}). At the end of treatment, activity of lipoprotein lipase in cells was measured and expressed as per cent of the maximal insulin stimulation above basal activity. Basal lipoprotein lipase activity was 0.33 ± 0.02 U per mg DNA ($n = 3$). The activity in cells treated with insulin and anti-R was, respectively, 0.65 ± 0.05 ($n = 3$) and 0.59 ± 0.04 U per mg DNA ($n = 3$) after 0.5 d of treatment; 1.16 ± 0.08 ($n = 3$) and 0.72 ± 0.03 U per mg DNA ($n = 3$) after 1 d; 1.33 ± 0.17 ($n = 3$) and 0.60 ± 0.02 U per mg DNA ($n = 3$) after 4 d. Stimulation of lipoprotein lipase activity by insulin and anti-R was statistically significant at all time points ($P < 0.01$).

To determine whether stimulation of lipoprotein lipase by anti-receptor antibody was mediated by mechanisms similar to those involved in the action of insulin, 3T3-L1 fatty fibroblasts were pretreated for 60 min at 37 °C with anti-receptor antibodies and then exposed to increasing concentrations of insulin for 24 h at 37 °C (Fig. 2). As shown above, anti-receptor antibody alone produced a 60% increase in the activity of the enzyme. Addition of insulin at concentrations up to 10^{-6} M produced minimal further effects on the antibody-treated cells. This blockade of the insulin effect suggests that both insulin and the anti-receptor antibody use the same pathway for the production of this biological response. The small increment in the activity of the enzyme observed after the addition of high insulin concentrations is probably due to the fact that in this experiment a low antibody concentration was used, which inhibited insulin binding by only 70%.

Additional evidence that this chronic effect of anti-receptor antibodies is similar to that of insulin was obtained in studies with cycloheximide (Fig. 3). Cycloheximide, in experimental conditions in which protein synthesis was reduced by 90%, inhibited the insulin-induced increase in lipoprotein lipase activity. Similarly, the stimulatory activity of the anti-receptor antibodies on lipoprotein lipase was abolished by cycloheximide. Thus, in addition to their short-term insulin-like effects, the anti-receptor antibodies can mimic a long-term insulin effect.

The present observations have important implications for the mechanism of insulin action. Current studies have suggested two possible pathways to explain the long-term effects of insulin. First, as we have previously suggested[9,24], all the information required for the generation of the biological effects of insulin could be present in the receptor molecule itself. In this case, both insulin and the antibodies against the insulin receptor would generate an activated receptor which would then mediate insulin's effect either directly or by generation of the same second messenger as that for insulin. Alternatively, after binding to the cell-surface receptors, insulin and the anti-receptor antibodies are endocytosed and subsequently the ligands or their degradation product(s) bind to intracellular insulin receptors, which elicit the late insulin effect. The recent observation that insulin and/or the insulin–receptor complex enter target cells[18,19] and the demonstration of specific binding sites for insulin on intracellular organelles[20–23] have been taken as evidence that this last pathway may be more important.

The present results and the above-mentioned data suggest that the second hypothesis is rather unlikely. Indeed, indirect studies have indicated that the antibodies against the insulin receptor bind very poorly to intracellular receptors[25]. Furthermore, it seems very unlikely that two totally different polypeptides, insulin and anti-receptor antibodies, would generate an identical or similar degradation product, which would

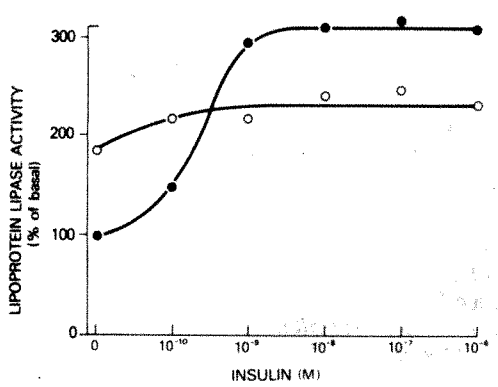


Fig. 2 Effect of antibodies against the receptor for insulin on the insulin-induced stimulation of lipoprotein lipase activity. 3T3-L1 fatty fibroblasts were pretreated for 1 h with either normal IgG (1.75 μg ml^{-1}) or anti-R (1.75 μg ml^{-1}). Increasing concentrations of insulin (0–10^{-6} M) were then added and the incubation continued for a further 24 h. Lipoprotein lipase activity was determined and expressed as per cent of basal. Basal lipase activity was 0.52 ± 0.13 U per mg DNA ($n = 6$). Activities in the presence of 10^{-6} M insulin alone and anti-R alone were 1.61 ± 0.10 ($n = 4$) and 0.95 ± 0.04 U per mg DNA ($n = 6$), respectively. Both values are statistically different from that of basal activity ($P < 0.001$). Lipoprotein lipase activity in cells exposed to anti-R and then 10^{-6} M insulin (1.21 ± 0.07 U per mg DNA, $n = 4$) (○) was statistically different from the activity in cells treated with 10^{-6} M insulin alone ($P < 0.05$) (●).

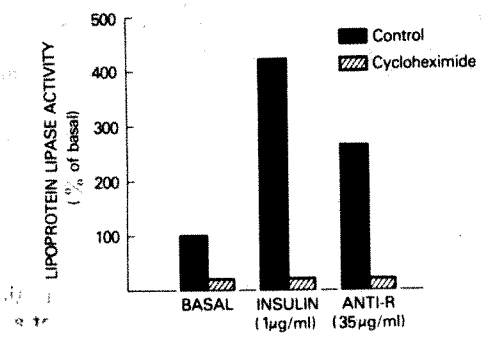


Fig. 3 Effect of cycloheximide on the lipoprotein lipase response to insulin and to antibodies against the receptor for insulin. 3T3-L1 fatty fibroblasts were incubated in basal growth medium with or without insulin (1 $\mu g\ ml^{-1}$) or anti-receptor antibodies (35 $\mu g\ ml^{-1}$) in the absence (control) or presence of cycloheximide (4×10^{-7} M) for 24 h. The data are expressed as per cent of the basal activity observed in control cells. Basal lipoprotein lipase activity in control cells was 0.47 ± 0.05 U per mg DNA ($n = 3$). Activity in the presence of insulin and anti-R was 1.99 ± 0.12 ($n = 3$) and 1.26 ± 0.07 U per mg DNA ($n = 3$), respectively. Both values are significantly different from that of basal activity ($P < 0.001$). In the presence of cycloheximide, lipase activity in insulin and anti-R treated cells was significantly reduced to 0.08 ± 0.01 and 0.09 ± 0.01 U per mg DNA, respectively ($P < 0.001$, $n = 3$).

produce late insulin effects by binding to intracellular insulin receptors. Therefore, our present data favour the idea that both insulin and the anti-receptor antibody generate the same second messenger(s) or alternatively, that they both lead to the endocytosis of the insulin receptor, and the receptor itself contains all the necessary information for the generation of the late effects of the hormone.

Finally, it should be noted that we have dealt here only with acute and chronic 'metabolic' responses to insulin. Insulin, and several other insulin-like peptides, also produce long-term 'growth' responses, such as stimulation of thymidine incorporation into DNA. Whether these effects of insulin are mediated by a different mechanism or even the interaction of insulin with a different membrane receptor, remains to be determined.

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β-Adrenoreceptors determine affinity but not intrinsic activity of adenylate cyclase stimulants

SIGNIFICANT progress has recently been made toward defining and understanding the molecular basis of hormone sensitivity in the β-adrenoreceptor-adenylate cyclase system. Through the use of biochemical[1,2] and genetic[3,4] techniques it has become apparent that this system is comprised of at least three separate components: the β-adrenoreceptor, which is responsible for the binding of β-adrenergic agents; the catalytic unit of the enzyme adenylate cyclase, responsible for the conversion of ATP to cyclic AMP; and a nucleotide regulatory component or components which seems to possess a GTPase activity[5] and to be required for coupling of the β-adrenoreceptor to the adenylate cyclase enzyme[6]. As has been demonstrated by Orly and Schramm[7] and confirmed by Schwarzmeier and Gilman[8] in cell fusion experiments, these components interact to produce the hormone-responsive adenylate cyclase system and this interaction occurs even when the separate functions are donated by heterologous cell types. Frog and turkey erythrocytes each possess a catecholamine-responsive adenylate cyclase. Although similar in some respects these two well-studied systems differ markedly with respect to their drug affinities and intrinsic activities. Intrinsic activity is defined as the maximal ability of a drug to stimulate a biological or biochemical response. To elucidate the role of the β-adrenoreceptor in the expression of these properties, we report here the use of cell fusion techniques to construct a hybrid which possesses the β-adrenoreceptor of the turkey erythrocyte and the adenylate cyclase of the frog erythrocyte. The characteristics of this hybrid indicate that although the receptor determines relative drug affinities (for example, β_1 compared with β_2 subtypes of receptor), it is a component distal to the receptor which determines relative drug intrinsic activities.

Hybrid cells were constructed by fusing frog erythrocytes, the β-adrenoreceptor of which had been inactivated with dicyclohexyl carbodiimide, with turkey erythrocytes, the adenylate cyclase of which had been inactivated by treatment with *N*-ethyl maleimide (Fig. 1 legend). Under the conditions used to inactivate the β-adenoreceptor of the frog erythrocyte the ability of catecholamines to stimulate adenylate cyclase is totally abolished, whereas only 25–30% of basal, fluoride-, and Gpp(NH)p-stimulated adenylate cyclase activity is lost. The fact that the adenylate cyclase in these treated cells remains responsive to Gpp(NH)p indicates the functional presence of the nucleotide regulatory component as well as the catalytic unit of adenylate cyclase.

In the turkey erythrocyte treatment with *N*-ethyl maleimide results in >99% inactivation of control adenylate cyclase activity. However, when assayed for catecholamine-stimulated GTPase activity according to the method of Cassel and

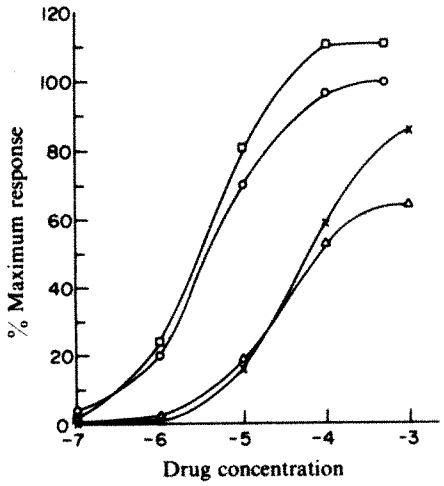


Fig. 1 Dose–response curves of agonists for stimulation of adenylate cyclase in hybrid cell membranes. □, Hydroxybenzylisoproterenol; ○, isoproterenol; ×, adrenaline; △, noradrenaline. The β-adrenoreceptor of the frog erythrocyte was inactivated by treatment of whole cells with 50 M dicyclohexyl carbodiimide (DCCD) for 30 min at 30 °C as described previously[9]. The adenylate cyclase of the turkey erythrocyte was inactivated by treatment of the whole cells with 5 mM *N*-ethyl maleimide (NEM) for 30 min at 0 °C, followed by three washes with buffered saline. Sendai virus was grown according to Watkins[10]. For cell fusion 1×10^9 NEM-treated turkey cells and 2×10^8 DCCD-treated frog cells were mixed in 10 ml buffer containing 135 mM KCl, 5 mM NaCl, 0.8 mM $MgCl_2$, 2 mM $MnCl_2$ and 20 mM Tris-HCl at *p*H 7.4. Approximately 5–10,000 haemagglutinating units of inactivated Sendai virus was added to the cell suspension which was incubated on ice for 10 min and then transferred to a 37 °C water bath for 30–60 min. Normally after microscopical examination of an aliquot of these cells, 50–75% of the nuclei were in polykargons with greater than 95% of the giant cells containing at least one nucleus (determined by relative nucleus size) of each cell type. Membranes were prepared using a freeze-thaw lysis technique[11] and adenylate cyclase activity was assayed as described previously[12,13] with the exception that the final assay mixture contained 0.3 mM ATP and 0.1 mM GTP. For each fusion experiment a 'mixed control' was run in which the appropriate number of DCCD-treated frog and NEM-treated turkey cells were mixed, incubated in fusion buffer without virus, and assayed for adenylate cyclase activity. In no case was isoproterenol-stimulated enzyme activity observed in this preparation. In addition, homologous control fusions of DCCD-treated frog cells and NEM-treated turkey cells did not result in the appearance of hormone-sensitive adenylate cyclase, nor did fusion of wild type frog erythrocytes or wild type turkey erythrocytes with themselves alter the patterns of agonist affinities or intrinsic activities. Maximum response refers to adenylate cyclase activity in the presence of 10^{-4}M isoproterenol and represents an average of 118.3 pmol cyclicAMP per mg protein per 10 min over a basal activity of 45.1 pmol per mg protein per 10 min. The EC_{50} values for the agonists for the activation of adenylate cyclase are: hydroxybenzylisoproterenol 4×10^{-6}M; isoproterenol 4×10^{-6}M; adrenaline 4×10^{-5}M; noradrenaline 3×10^{-5}M. EC_{50} is defined as the concentration of agonist required to elicit 50% of the maximal stimulation of adenylate cyclase produced by that particular drug. The curves shown are the average of four separate experiments.

Sellinger[14], membranes derived from these treated cells exhibited only a 15–20% loss of catecholamine-stimulated GTPase activity. Thus, these cells seem to possess both the β-adrenoreceptor and the nucleotide regulatory protein.

In the turkey erythrocyte catecholamine agonists demonstrate a β_1 order of potency (isoproterenol > noradrenaline ≈ adrenaline) for the activation of adenylate cyclase[15]. In the frog erythrocyte, however, a β_2 pattern (isoproterenol > adrenaline > noradrenaline) of catecholamine agonist potencies is observed[16]. This definition of β-adrenoreceptor subtypes has been set forth by Lands[17]. Shown in Fig. 1 are the dose–response curves of four β-adrenoreceptor agonists for stimulating adenylate cyclase activity in membranes derived from hybrid cells. The preparation demonstrates a typical β_1 adrenoreceptor specificity for these agonists. Isoproterenol is more potent than noradrenaline and adrenaline which are approximately equipotent. In addition, hydroxybenzylisoproterenol, a β_2-specific agonist, which is tenfold more potent than isoproterenol in the frog β_2 system[18] exhibits the β_1 characteristic of equipotency with isoproterenol in the hybrid cells (L. J. Pike and R. J. Lefkowitz, unpublished results).

As the parental turkey erythrocyte adenylate cyclase and the frog erythrocyte β-adrenoreceptor have been completely inactivated, the observation that β- agonists can stimulate adenylate cyclase activity in this system suggests that a hybrid receptor–adenylate cyclase has been constructed. The β_1 specificity of the agonist response indicates that the relative affinities of β-adrenergic agents are determined by the receptor protein itself.

To determine whether the receptor was also responsible for determining the intrinsic activities exhibited by agonist drugs, the maximal ability of a series of adrenergic drugs to stimulate the adenylate cyclase in hybrid membrane preparations was examined. The results of these studies are shown in Table 1.

It can be observed that in all cases the intrinsic activities in the hybrid closely resemble those of the frog system. Particularly striking differences between agonist intrinsic activities in the turkey and frog erythrocyte are seen for noradrenaline, cobefrin and isoetharine. In each of these instances the hybrid system's response is similar to that of the frog. For all the drugs tested, the hybrid intrinsic activities are not significantly different from the frog erythrocyte intrinsic activities (on the basis of Student's *t* tests), whereas the hybrid intrinsic activities are significantly different ($P < 0.01$) from those of the turkey erythrocyte. As the intrinsic activities parallel those of the adenylate cyclase donor rather than the receptor donor, it seems that some component distal to the receptor is responsible for determining intrinsic activity.

In our current conceptualisation of the system this distal component might be either the nucleotide regulatory component, catalytic unit of adenylate cyclase, or both. It is evident that only the frog erythrocyte catalytic adenylate cyclase unit is active in the hybrids. However, the hybrids seem to contain both turkey and frog nucleotide components. Thus, it is unclear what role the nucleotide regulatory component(s) might play in the determination of intrinsic activity, as one or both might be functioning in the hybrid.

Table 1 Intrinsic activities of β-adrenergic agents in membrane preparations of frog, turkey and hybrid erythrocytes

Agonist	Intrinsic activity		
	Hybrid	Frog	Turkey
(−)Isoproterenol	1.00 (9)	1.00 (4)	1.00 (4)
(−)Adrenaline	0.87 ± 0.03 (9)	0.95 ± 0.02 (3)	1.00 ± 0.01 (3)
(−)Noradrenaline	0.62 ± 0.03 (9)	0.54 ± 0.02 (4)	1.00 ± 0.02 (4)
(±)Cobefrin	0.50 ± 0.01 (4)	0.47 ± 0.03 (4)	0.92 ± 0.03 (4)
(±)Isoetharine	0.35 ± 0.04 (5)	0.42 ± 0.01 (3)	0.71 ± 0.02 (3)
(−)Soterenol	0.13 ± 0.02 (5)	0.11 ± 0.01 (3)	0.44 ± 0.04 (3)
Dobutamine	0.07 ± 0.01 (5)	0.07 ± 0.01 (3)	0.19 ± 0.01 (3)

Control frog and turkey intrinsic activities were assessed using fused frog and fused turkey erythrocytes prepared in the same manner as the hybrid erythrocytes. To determine intrinsic activities several concentrations of each drug over a 20-fold range bracketing the saturation point were assayed for stimulation of adenylate cyclase activity. The highest activity observed (minus basal) for each was divided by the maximal activity (minus basal) observed in the presence of saturating concentrations of isoproterenol and the values are reported as this fraction. Activities were (pmol cyclicAMP per mg protein per 10 min): basal 183; isoproterenol 3108 in the frog; basal 7.6; isoproterenol 99.1 in the turkey; and basal 45.1; isoproterenol 118.3 in the hybrids. The values given are the mean ± g.e.m. of the number of experiments shown in parentheses.

The construction of a hormone-sensitive adenylate cyclase system containing components from the frog and turkey erythrocyte systems has allowed us to assess the possible role of the β-adrenoreceptor in the determination of the affinities and intrinsic activities of drugs. As has been previously observed by others[7,8,19] we find that the receptor molecule seems to confer β-adrenoreceptor specificity to an adenylate cyclase system. In addition, however, the receptor also determines the relative affinities of β-adrenergic agents. Thus, the specificity observed in the β_1/β_2 subtypes most likely arises from slight differences in receptor protein structure. Interestingly, however, these data indicate that it is not the receptor but rather some component(s) distal to it that is responsible for the determination of intrinsic activity. Characterisation of the reciprocal hybrid (*N*-ethyl maleimide frog cell crossed with dicyclohexyl carbodiimide treated turkey cell) would further elucidate this point. However, to date all attempts to construct a functional hybrid of this composition have been unsuccessful.

The reconstitution of a catecholamine-stimulated adenylate cyclase system using cell fusion of these two different but complementary cell types is a potentially powerful tool that will be useful in defining the molecular components responsible for a variety of system characteristics such as desensitisation and the differing patterns of nucleotide regulation of adenylate cyclase activation.

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Acetylcholinesterase like that of skeletal muscle in smooth muscle reinnervated by a motor nerve

SKELETAL muscles contain several molecular forms of acetylcholinesterase (AChE) characterised by different sedimentation coefficients[1–4]. In rat muscle[1,2] (but not human[3] and chicken[5] muscle) the heavy form of AChE is exclusively present at the motor endplate regions. In contrast, the heavy form of AChE has not been detected in cat[6] or rat[2] smooth muscles. The endplate AChE of rat skeletal muscle is selectively detached by treatment with collagenase[1,7], an enzyme which has been shown specifically to digest the collagen-like tail of the heavy form of AChE from several sources[8,9]. The presence of endplate AChE is neurally regulated[10], and there is evidence that this effect is partially mediated by muscle activity[11] and by a trophic factor conveyed by axoplasmic transport[12,13]. Previous results from our laboratory have shown that reinnervation of the cat nictitating membrane smooth muscle by a motor nerve results in an increase of AChE activity[14]. We report here that collagenase treatment detaches active AChE from cat smooth muscle reinnervated by a cholinergic motor nerve, and that a new AChE form with a high sedimentation coefficient is induced in these muscles. Our results suggest that the presence of the motor nerve determines, in reinnervated smooth muscle, the appearance of an AChE similar to that found in skeletal muscle.

The superior cervical sympathetic ganglion was removed from adult cats, the hypoglossal nerve was sectioned and the central stump sutured to the postganglionic fibres of the superior cervical ganglion. Details of the experimental procedure are given elsewhere[14]. Seven months post-operation, both the hypoglossal and the preganglionic nerves were stimulated to confirm that smooth muscles had been reinnervated exclusively by the hypoglossal. In all cases, the nictitating membrane and the pupil responded only to hypoglossal stimulation. The inferior smooth muscles of both nictitating membranes were then removed for AChE assay[15]. In one series, control and reinnervated muscles were incubated *in vitro* at 37 °C in a medium[16] containing 0.2 mg ml^{-1} collagenase (preparation type III, 400 U mg^{-1}, Sigma). We used the collagenase digestion technique which is known to detach selectively AChE from the motor endplates, leaving the non-endplate AChE intact[7]. In another series of experiments, homogenates of reinnervated and normal smooth muscle were sedimented in a linear (5–20%) sucrose gradient following the method of Martin and Ames[17]. Sucrose solutions (Schwarz–Mann, special enzyme grade) containing NaCl-Triton X-100 were used[1]. The specific activity of AChE in control muscles was 0.57 nmol ^{3}H-acetate formed per min per mg protein, while in reinnervated muscles it increased to 1.23. Smooth muscles reinnervated for a longer period (10 months) showed a higher AChE level, reaching a specific activity of up to 3.1 (ref. 18).

Reinnervated smooth muscles incubated with collagenase released an amount of AChE proportional to the time of incubation whereas control muscles showed no solubilised AChE activity (Fig. 1). It is known that collagenase disrupts the muscle fibre basement membrane[19,20], and releases motor endplate AChE which is partly localised within the basal lamina at the neuromuscular junction[21]. The enzymatic detachment of active AChE from reinnervated smooth muscle suggests that this new enzyme form is probably anchored to a basal lamina-like material—as has been proposed for AChE forms which present sensitivity to collagenase[8]—and would correspond to a high sedimentation coefficient form, similar to the AChE form specific for rat motor endplate[1]. The AChE release experiments, however, do not yield definite structural information since commercial collagenases are contaminated with several proteases[22]. The molecular forms of AChE present in reinnervated smooth muscle were therefore identified by velocity sedimentation analysis. Figure 2 shows that a new heavy form of AChE appears in smooth muscle reinnervated by a cholinergic motor nerve. This heavy form of AChE which probably corresponds to the asymmetric form A_{12} of Bon *et al.*[8], accounts for 9% of the total activity and represents ~25% of the total AChE increase in reinnervated muscles. As the heavy form of AChE is not present in normal smooth muscle[6], it is likely that the species detached by collagenase corresponds to this high sedimentation coefficient form[23]. The present results show that a motor nerve can induce in smooth muscle a form of AChE similar to that found in skeletal muscle. As innervation by a cholinergic motor nerve is a common factor for reinnervated smooth muscle and skeletal muscle, our results suggest that the motor nerve is able to regulate AChE in any musculature it innervates.

Several different mechanisms could be involved in neural AChE regulation: (1) muscle activity; (2) acetylcholine itself; (3)

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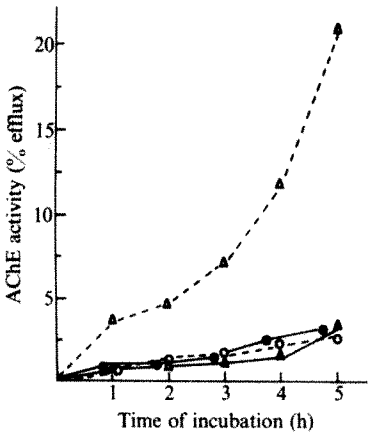


Fig. 1 Release of AChE from inferior smooth muscles of the nictitating membrane as a function of time of incubation. Experimental conditions: normal smooth muscle of the nictitating membrane with collagenase (○) and without collagenase (●), reinnervated smooth muscle of the nictitating membrane with collagenase (△) and without collagenase (▲). Smooth muscle samples (15–20 mg) were incubated at 37 °C in 300 μl of a medium containing 138 mM NaCl, 4 mM KCl, 1 mM $MgSO_4$, 50 mM sodium phosphate (*p*H 7.5), 0.1 mg ml^{-1} chloramphenicol, and 0 or 5 μM BW 284C51 dibromide. Collagenase (0.2 mg ml^{-1}) was added to the incubation medium of some of the muscle samples. At various times, duplicate aliquots of 10 μl incubation medium were removed for AChE assay[15]. Subsequently, the muscle samples were homogenised 1/15 (w/v) in ice-cold buffer *p*H 7.3 (1 M NaCl, 0 1% Triton X-100, 0.05 M Tris-HCl, 0.2 mM EDTA) to determine AChE activity. Untreated muscle samples were also homogenised and assayed for AChE. The absolute amount of AChE in incubated samples is not the same for the different experimental situations studied, therefore to evaluate comparatively the AChE release in each case[16] the initial amount of enzyme present in the samples must be taken into account. % AChE efflux:

$$\frac{(\text{AChE medium} \times 100\%)}{(\text{AChE medium} + \text{AChE in homogenate})}$$

release of AChE from the nerve terminal; and (4) neurotrophic control. These four mechanisms are discussed below:

(1) Muscle activity has been shown to play a role in modulating AChE in chick muscle culture[24], and stimulation of muscular activity can induce AChE spots in rat muscle[11]. The 16S AChE form reappears at the original endplates in denervated muscles, after reinnervation at a different site[25]. A possible explanation for this is that muscle activity stimulates synthesis or stabilisation of 16S AChE at sites on the muscle previously determined by nerve contact[25,26]. Lømo and Slater[11] suggest that muscle activity might turn on synthesis of junctional AChE in addition to turning off synthesis of extrajunctional acetylcholine receptors in rat skeletal muscle. We know that reinnervation of the nictitating membrane smooth muscle by a motor cholinergic nerve determines both a decrease in the hypersensitivity to acetylcholine[27], and the induction of a heavy form of AChE; hence, the effect suggested for muscle activity in skeletal muscle[11] might also apply in smooth muscle reinnervated by a motor nerve. Recently, however, it has been shown that, in the rat, the endplate 16S AChE form is influenced by the length of the nerve stump[28]. As muscles become inactive at the time of nerve section[29] regardless of the nerve stump length, it follows that the endplate 16S AChE form also depends on an activity-independent mechanism[28].

(2) Acetylcholine *per se* does not seem to be an important factor in AChE regulation: during reinnervation of skeletal muscle with preganglionic parasympathetic cholinergic fibres, AChE decreased and almost disappeared when reinnervation was completed, despite normal acetylcholine release[30].

(3) Release of neural AChE has been demonstrated in rat phrenic nerve–hemidiaphragm[31]. Although the motor nerve contains a heavy form of AChE[32,33], only the 10S form of AChE has been shown to be released from nerve terminals. Evidence that a heavy form of AChE is conveyed by axonal flow was obtained by studying the accumulation of material at the proximal stump of a damaged nerve[32], so it is possible that the heavy form of AChE is produced locally either in Schwann or other extra-axonal cells, at the site of the injured stump. In this context, it has been recently demonstrated that glial cell acid phosphatase is transported to damaged axons[34]. In any case, as the axonal flow is an important mediator in the neurotrophic regulation of junctional AChE[12], the motor nerve innervating the smooth muscle of the nictitating membrane could provide a source for the heavy form of AChE.

(4) AChE trophic substances[35,36] are conveyed by axoplasmic transport[12,13] and in motor nerves a trophic factor has been detected which is capable of maintaining the level of AChE in cultures of aneural embryonic muscle or denervated adult chicken muscle[36]. Moreover, a trophic factor, other than AChE, isolated from rat phrenic nerve and transported by axonal flow, has been shown to maintain the levels of endplate AChE in rat diaphragm tissue culture[13]. This finding strongly suggests that a substance different from AChE, conveyed by axonal flow to the nerve terminal, would be a major factor in the neurotrophic regulation of endplate AChE. The effect we observed in reinnervated smooth muscle may be partly mediated by a similar factor. Thus motor nerves regulate AchE, but parasympathetic

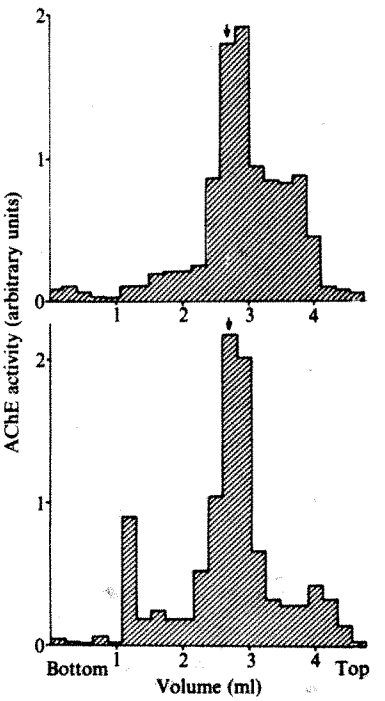


Fig. 2 Velocity sedimentation of smooth muscle extracts in a sucrose gradient. AChE activity profile of: *a*, control smooth muscle; *b*, reinnervated smooth muscle. Smooth muscles (~10 mg) were homogenised in ice-cold NaCl–Triton buffer, *p*H 7.3 (1M NaCl, 0.5% Triton X-100, 0.05M Tris-HCl, 0.2 mM EDTA). After various times of enzyme extraction, samples of 0.2 ml (2.4 mg protein) were layered on linear (5–20%) sucrose gradients, in the presence of the same buffer as for homogenisation and subjected to velocity sedimentation. Ultracentrifugation was performed in a Beckman L5-65 ultracentrifuge, with a SW 65 rotor at 39,000 r.p.m. for 12 h at 20 °C. A volume of 0.05 ml of each fraction was assayed for AChE activity. Activity was measured by a radiochemical procedure in which release of ^{3}H-acetate from ^{3}H-ACh iodide (NEN, 50 mCi $mmol^{-1}$) was quantified by ion-exchange chromatography and scintillation spectrometry[15,16]. AChE activity is expressed in arbitrary units. In our standard assay conditions the hydrolysis of acetylcholine was inhibited by 80% with 5 μM BW 284C51 dibromide, a specific inhibitor of AChE activity. Catalase, used as a marker enzyme (11.3S), was determined colorimetrically[38] and proteins by the method of Lowry *et al.*[39]. The arrow represents the position of catalase.

nerves[30] do not possess this capacity. In addition, motor nerves can induce high sedimentation coefficient AChE, similar to the specific form of rat motor endplates, as has been reported for denervated muscle endplates[2], non-synaptic segments of skeletal muscles[2], original endplates in denervated muscles after reinnervation at a different site[25], primary cultures of rat muscle cells[37], and smooth muscle (this paper).

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Aequorin luminescence during activation of single isolated smooth muscle cells

INCREASED cytoplasmic Ca^{2+} supposedly couples excitation to contraction in smooth muscle. But there have been no direct measurements of such an increase and there is little information about the kinetics of changes in intracellular Ca^{2+} during individual contractions of smooth muscle. Thus the basic aspects of excitation–contraction (EC) coupling in smooth muscle are not known with the same degree of certainty that exists for striated muscle[1]. For example, there is a long delay between stimulation and contraction in smooth muscle; this delay might result from a slow onset of Ca^{2+} release after a change in surface membrane potential or from the slowness of some other process[2]. If it were possible to correlate measurements of transmembrane potential, intracellular Ca^{2+} and contraction in the same single cell the mechanism of EC coupling in smooth muscle might be better understood. Some progress in this direction has been made by the development of methods for enzymatic isolation of smooth muscle cells and measuring electrical activity and force production or shortening in these single cells[2–6]. We report here a further approach to the problem by (1) microinjecting the Ca^{2+} indicator aequorin into smooth muscle and (2) directly measuring changes in intracellular Ca^{2+} during EC coupling in single isolated smooth muscle cells. Cytoplasmic Ca^{2+} evidently began to rise without detectable delay following a single electrical stimulus applied to a cell injected with aequorin and reached a peak well before contraction presumably began. The long delay, therefore, between stimulation and contraction seems inherent in the mechanism by which the contractile proteins of smooth muscle are activated.

All experiments were performed on single smooth muscle cells isolated from the stomach muscle of the toad, *Bufo marinus*, by an enzymatic digestion technique that involved the use of trypsin and collagenase. Isolated cells obtained by this technique exhibit physiological, pharmacological, metabolic and ultrastructural properties similar to those of cells in the isolated tissue[4–6]. But they do not exhibit slow rhythmic spontaneous potential changes once the nerve endings are removed[3].

Photomicrographs made during injection of a single smooth muscle cell are shown in Fig. 1. The pipette was left in the cell following the shortening that accompanied insertion. Small volumes of the aequorin solution were then injected by applying gaseous pressure to the butt end of the pipette. The relaxation that resulted when the injected liquid contained EGTA or EDTA was sustained until the cell was stimulated by a current pulse[7]. The swelling that indicated an injection of liquid rapidly subsided when the pressure was released. A cell could typically be injected several times without the swelling itself inducing contraction. The pipette was then withdrawn from a cell, removed from the bath, the quartz–iodine lamp was turned off, and a 25-mm diameter light guide, which extended to the shutter of a photomultiplier[8], was centred above the cell. Thus, stimulation occurred several minutes after injection whereas aequorin distribution in the cross-section of the injected segment was presumably uniform in only a few seconds (assuming a diffusion coefficient of aequorin of 5×10^{-8} cm^2 s^{-1} (ref. 8); the maximum diameter of 11 cells that were fixed when fully relaxed and subsequently measured by scanning electron microscopy was 4.34 ± 0.32 μm). Withdrawal of the pipette did not cause contraction (Fig. 1) and an electrical stimulus could elicit shortening and relaxation after the entire procedure that was not detectably different from a shortening response elicited before the procedure.

The pre-stimulus level of light emission from an aequorin-injected cell was not detectably different from the light recorded during stimulation of a non-injected cell (that is, there seemed to be no 'resting glow' and no change in anode current from the photomultiplier associated with the electrical stimuli themselves). But when a single electrical stimulus was applied to an injected cell a rapid rise in light output was observed. Light output reached a peak within about 10 ms of the onset of stimulation in Fig. 2 for example. The average (± s.e.m.) time to peak was 4.8 ± 0.6 ms in 27 of the cells. The response was sometimes large enough to saturate the recording system and the time to peak was not estimated in those cases. Light emission in Fig. 2 initially decayed as a single exponential with a rate constant of 37 s^{-1} but it was still above the pre-stimulus level until at least 250 ms after the onset of the stimulus. Therefore, the rise and most of the fall in luminescence was complete well before mechanical events presumably even began[2]. This is in striking contrast to the behaviour of amphibian skeletal muscle cells where the onset of force development lags no more than a few milliseconds behind the onset of light and light emission declines to only about half its peak level by the time peak force has developed[8].

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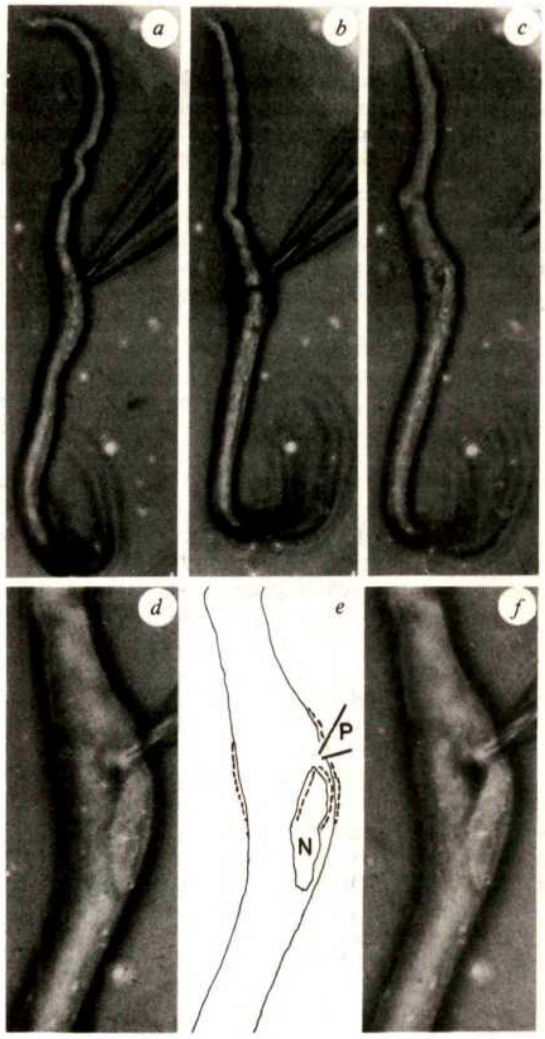


Fig. 1 Injection of aequorin into an isolated smooth muscle cell. Photomicrographs made from selected frames of a 16-mm cine film (12 frames per s) taken through an inverted microscope with a Zeiss ×40 phase contrast objective. *Upper row*: Magnification ×928. *a*, The cell before insertion of the micropipette containing aequorin; *b*, 5 s after the insertion of the micropipette; insertion triggered slight shortening of the cell probably caused by a momentary leakage of Ca^{2+} along a shunt resistance in parallel with the input impedance of the cell; *c*, 2 s after withdrawal of the micropipette. Twice between *b* and *c* pressure was applied briefly (~1 s) to the micropipette; liquid apparently entered the cell each time since each pressure pulse was accompanied by perceptible but evanescent swelling. The cell remained relaxed following withdrawal but when electrically stimulated it contracted. *Lower row*: Magnification ×1,740, *d*, The same cell after insertion of the micropipette but just before injection of liquid; *e*, the superimposed outlines of the cell before (continuous lines) and during (dashed lines) injection (that is, *d* set on *f*); *f*, during injection of aequorin. The micropipette (P) was always inserted into the uppermost part of the middle of a cell, and when liquid was injected the cine films showed that the most conspicuous distention was usually in a plane perpendicular to the page; furthermore, the nucleus (*N*) shifted during injection. We also recorded membrane potential responses to electrical stimulation from some isolated cells, and responses ranged from being slow and regenerative during long-duration pulses (see ref. 3) to fast and graded following short-duration pulses (Fig. 2). The entire range of responses could be obtained reproducibly from the same cell and showed that microinjection *per se* did not necessarily abolish the characteristic ability of these cells[3] to respond to depolarisation. We also photographed shortening and relaxation of injected cells[7], which was apparently unchanged and reproducible. This showed that microinjection *per se* did not necessarily change normal EC coupling in these cells. Aequorin was dissolved in either 33 mM EGTA (*p*H 7.0), 50 mM EDTA (*p*H 8.0), or quartz distilled water (*p*H 7.4). Chelating agents can influence aequorin results and their presence may be undesirable as a general rule[17]. But EDTA or EGTA in a micropipette prevented or reversed the tendency for a cell sometimes to shorten irreversibly during pipette insertion (Fig. 1). Thus a chelator was often present in our aequorin solutions (for example, the aequorin solutions used to obtain Figs 1 and 3 contained EDTA whereas the solution used to obtain Fig. 2 contained only quartz distilled water). Another advantage to the presence of EDTA or EGTA was that a buffer should oppose a change in $[Ca^{2+}]$ if it remained undiluted or did not become saturated with Ca^{2+} once inside the cell. Aequorin would then have either detected no change in Ca^{2+} or have indicated a delayed, slow rise as the buffer became saturated. On the contrary, we saw a rapid rise in light emission. Furthermore, the cells contracted and relaxed normally in response to punctate electrical stimulation delivered less than a minute after the injection of EDTA or EGTA which supports the idea that the chelators assume little functional importance after injection[7]. Aequorin solutions were put into filament containing glass micropipettes and pressure injected into individual cells by means similar to those already described for skeltal muscle[8]. But some of the differences in procedure were as follows: (1) the resistances of the micropipettes ranged from 15 to 50 MΩ when filled with 3 M KCl; (2) phase-contrast optics and a quartz–iodine lamp were used for both visual observations and cine-micrography; and (3) cells were stimulated either by a transverse electric field, or by the passage of current between 5 M NaCl in a blunt glass pipette next to a cell and a platinum wire anode positioned at the periphery of the liquid bath. In all the experiments reported here (34 cells) the cells were bathed in a high Ca^{2+} Ringer solution (about 30× normal) that contained (mM) calcium 57, potassium 3.0, magnesium 0.98, sodium 45, chloride 162, sulphate 0.98, Tris(hydroxymethyl)aminomethane 2 (*p*H 7.4), glucose 11, and 6 mg l^{-1} gentamycin. A high external $[Ca^{2+}]$ facilities microelectrode impalements[3] and does not change the delay between an electrical stimulus and the onset of contraction[2]. The temperature of the bath ranged from 25° to 34 °C.

The cause for the falling phase of an aequorin response is not certain, but several possibilities can probably be eliminated. First, the decay does not represent total depletion of aequorin since a second stimulus could induce a second flash of light after only one injection; furthermore, the rate constant for the decay of aequorin luminescence from the cells (for example, Fig. 2) was about 20 times higher than the rate constant for decay when aequorin is consumed *in vitro* by rapid mixing with a maximally effective concentration of Ca^{2+} (ref. 9). Second, the rate of quenching of luminescence when aequorin is exposed *in vitro* to Ca^{2+} chelating agents[9] is about five times higher than the rate constant for decay of light emission from these cells. Thus, the falling phase (Fig. 2) was not determined by the maximum rate imposed by the kinetics of the aequorin reaction. It is also unlikely that the fall of an aequorin response is caused by movement or by a change in the intrinsic optical properties of a cell because the maximum length changes or birefringence changes occur several thousand milliseconds after the maximum change in light emission following electrical stimulation[5,6]. Calcium binding to sites within the cell and/or the removal of Ca^{2+} from the cytoplasm by membrane associated reuptake mechanisms most likely account for the decay of light[1].

Luminescent responses were not always of the type shown in Fig. 2 (see for example, Fig. 3). This particular atypical response was much larger and more complex than the type in Fig. 2 and exhibited three phases. Other cells showed only the first phase (I), which could be reproduced by subsequent stimuli[10]. The second phase (II) had a rate of decay that was similar to the rate of decay of the response in Fig. 2. The final phase (III) decayed slowly and had a time course consistent with the overall consumption of aequorin[9]. Our hypothesis is that the first and last types of luminescent responses were not only atypical aequorin responses but were associated with atypical membrane responses, that is, responses different from those in Fig. 2 or in ref. 3. Atypical responses might be due to cell injury before or during a stimulus. For example, a cell segment or an entire cell can become inexcitable as a consequence of micropipette penetration or pressure injection yet undergo little or no significant decrease in resting potential[11–14]. An electrical stimulus, particularly an excessively intense stimulus, could change the membrane potential of such a cell and thus elicit a luminescent response. The total light emitted during responses of the last type (III) were largest and most readily detected but were probably associated with an atypically large rise in intracellular Ca^{2+}. Subsequent stimulation did not elicit further light emission which supports the idea that this type of response (III) is due to overall aequorin consumption. Successive stimuli applied to several such cells in a syncytium could elicit apparently reproducible responses but from different cells.

Observations obtained from single isolated cells preclude this last complexity.

Earlier studies left open the possibility that there might be a long delay between stimulation and the onset of intracellular Ca^{2+} release in smooth muscle[2]. But the rapid rises in light reported here suggest that the delay in EC coupling is not likely to be due to this possibility. Nor is it likely that the delay can be ascribed to the time for diffusion of Ca^{2+} through the cytoplasm. The small diameter of these cells should permit radial gradients in the intracellular $[Ca^{2+}]$ to be dissipated within 3 to 4 ms after the onset of a stimulus if diffusion is the major factor determining Ca^{2+} distribution. Calcium may carry the current producing a change in membrane potential in these cells[3]. Therefore, Ca^{2+} entry could account for part if not most of the aequorin signal. The source of the Ca^{2+} cannot be inferred from the present results alone. But one can now discount the possibility that the latency for contraction in smooth muscle results from a delay in the exposure of the contractile filaments to Ca^{2+}. Thus the rate limiting step seems to be imposed by a slow reaction during which the contractile proteins in smooth muscle are activated. Others have suggested that a sequence of Ca^{2+}-sensitive phosphorylation reactions are required for the activation of smooth muscle[15,16], and our results support the idea that these reactions impose the characteristically long delay in the activation of smooth muscle contraction.

We need more information to infer the sites responsible for both the rise and fall in intracellular Ca^{2+} following stimulation of these cells. But now that one can inject aequorin into smooth muscle cells these and other questions about the mechanism of EC coupling in smooth muscle can perhaps be answered.

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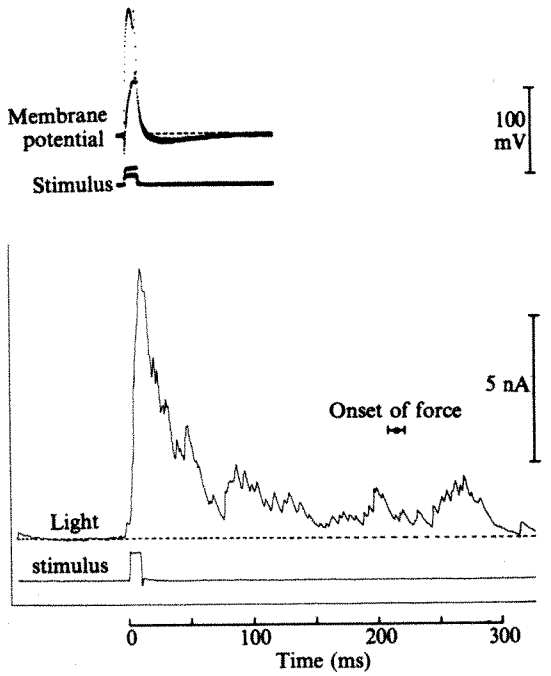


Fig. 2 Light emission and membrane potential change from single, isolated smooth muscle cells stimulated by 10-ms pulses. *Lower traces*: Light emission from a single cell following pressure micro-injection of aequorin and electrical stimulation. The aequorin response was initiated by a pulse applied via wires lying parallel to one another along either side of the cell[2]. The filled circle and bar labelled onset of force indicates the average delay (± s.e.m.) between a single electrical pulse and the first detectable sign of mechanical activity[2]. *Upper traces*: Membrane potentials recorded through the same microelectrode (filled with 3 M KCl) that was used to inject the stimulus current into the cell. Field stimulation, on the other hand, either distorted the membrane response recorded via a microelectrode or saturated the recording amplifier. The stimulus duration, solution composition, temperature, and other experimental conditions were the same as those used to obtain the lower aequorin traces.

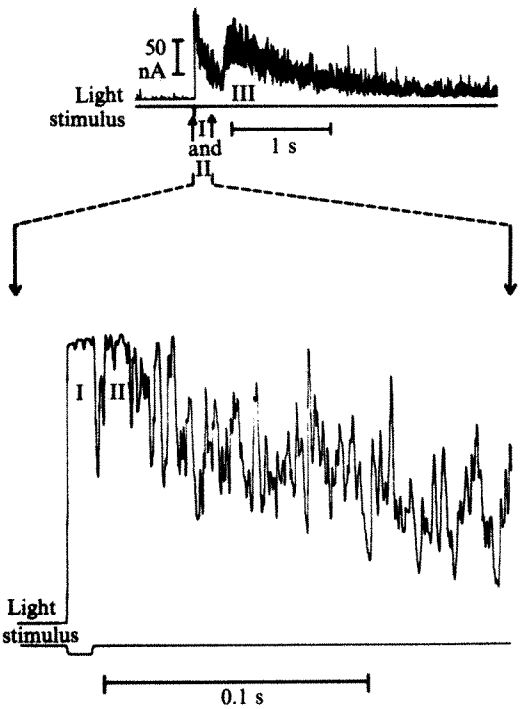


Fig. 3 An unusually large and complex light response from an isolated smooth muscle cell injected with aequorin and then stimulated by a single 10-ms pulse. The entire reponse is shown on the slower time scale (upper traces). The initial part of the same response is shown on a faster time scale in the lower traces. The initial rise and fall in light (I) is associated with the period of the stimulus. The second rise and fall in light (II) has a time course similar to the response shown in Fig. 2. The third rise and fall in light (III) is the slowest of the three, and has a time course consistent with the partial or total consumption of the aequorin in the cell[9].

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Light-activated drug confirms a mechanism of ion channel blockade

A VARIETY of mechanisms underlie the pharmacological blockade of membrane excitability. Some drugs seem to reduce the frequency at which ion channels open; a good example is the effect of curare on acetylcholine receptor channels at normal resting potentials. Another sort of mechanism may account for the action of many local anaesthetics and related drugs containing charged ammonium groups. It is postulated that such molecules block transmembrane currents as they bind to sites within open ion channels, much like a plug in a drain, with the important difference that the events occur on a millisecond time scale. This model, which we shall call 'open-channel blockade', was first applied to the effect of internal tetraethylammonium ions on K^+ channels in squid axon[1] and more recently to similar actions of local anaesthetics on acetylcholine receptor channels[2–4] and on electrically excitable Na^+ channels[5]. (Curare seems to exert an additional open-channel blockade at high negative potentials[6,7].) The concept of open-channel blockade would receive direct experimental support from the demonstration that the blockade is exerted even if the blocking molecule is not bound to the channel (or indeed is not present at all) until after the channel opens. Such a demonstration is made possible by a drug that (1) blocks acetylcholine receptor channels in *Electrophorus* electroplaques and (2) is created, in less than a millisecond, by a flash of light.

Bieth *et al.*[8] have described the photoisomerisable compound, *N-p*-phenylazophenylcarbamylcholine iodide (EW-1, Fig. 1). The *cis* isomer can be formed from the *trans* isomer by exposure to light of wavelength 300–500 nm and spontaneously re-isomerises with a time constant of approximately 120 s at room temperature and neutral *p*H. A solution can be maintained in a predominantly *cis* state by steady exposure to 360-nm light. When *cis*-EW-1 is applied to the innervated face of a voltage-clamped *Electrophorus* electroplaque, neurally evoked postsynaptic currents (PSCs)[9–11] decay more rapidly than normal,

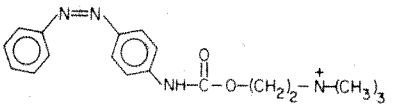


Fig. 1 *N-p*-phenylazophenylcarbamylcholine (EW-1), a light-activated cholinergic blocking drug.

the effect reaching fourfold at a concentration of 50 μM. *cis*-EW-1 also affects the response to bath-applied agonist, causing diminution of agonist-induced currents at equilibrium and rapid components in voltage-jump relaxations. For both PSCs and equilibrium agonist-induced currents, the blockade is more pronounced at more negative membrane potentials. Such effects typify the blockade of acetylcholine receptors by local anaesthetics and other suspected open-channel blockers[12–17]. Similar, but much weaker, effects are seen with *trans*-EW-1.

The light-flash experiments made use of the xenon flash tube and the optical system described by Nass *et al.*[18]; the flash was 90% complete within 0.5 ms (individual molecules probably isomerise within less than a microsecond after absorbing a photon). The preparation was bathed in *trans*-EW-1 and kept in the dark between flashes. In experiments on neurally evoked PSCs, a flash accelerated the decay phase of the PSC (Fig. 2). Very few channels open during the decay phase[19–22]; therefore the newly created *cis*-EW-1 molecules blocked channels that were already open. After the flash, the PSC decayed with at least two exponential components. We have quantitative data for only the fastest component, which accounted for more than half the decay amplitude in all conditions. This component's rate constant increases at higher flash intensities, suggesting that it arises from binding of newly created *cis*-EW-1 molecules. Furthermore, the increase is linearly proportional to the number of photoisomerisations (Fig. 3), as expected if each channel can be blocked by the binding of just one *cis*-EW-1 molecule.

Steady conductances are induced by bath-applied agonist. If EW-1 was present as well in the bathing solution, a flash produced a relaxation to a smaller conductance (Fig. 4). As in the PSC experiments, the light-flash relaxation had at least two exponential components. The amplitude of the fastest component was graded with the flash intensity and its rate

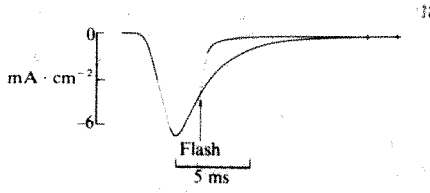


Fig. 2 A light-flash experiment with neurally evoked PSCs. Superimposed traces from two successive voltage-clamp episodes, at an interval of 0.3 s, in 10 μM *trans*-EW-1. During the second episode, a flash was delivered, isomerising at least 80% of the *trans*-EW-1 molecules to the *cis* isomer. Voltage −150 mV. Temperature 16 °C. Cell 57-42-T34.

constant was greater than that of a voltage-jump relaxation just before the flash, again showing that the blockade arises from an increased rate of channel termination rather than from a decreased rate of channel opening. As a percentage decrease of the conductance, the fast component had the relative size 1.0:0.6:0.35 for the agonists suberyldicholine, acetylcholine, and carbachol. (The experiments were carried out after pretreatment with methanesulphonyl fluoride to block acetylcholinesterase[10].) Thus, the initial component of the blockade is greater for agonists with a greater channel duration[10,23], as expected if the newly created *cis*-EW-1 molecules suddenly increase the rate constant for channel closing rate by a fixed amount (in ms^{-1}), with no dependence on the nature of the bound agonist.

The light-flash relaxations had a spectral dependence similar to that of the *trans* → *cis* photomerisation of EW-1. When the experiments were carried out using *cis*-EW-1 (rather than *trans*-EW-1), flashes had little additional effect. The signals are specific to acetylcholine receptor channels: light flashes have no effect on electrically excitable Na currents, even in the presence of EW-1.

There are interesting complications in these experiments. (1) The flash-induced blockade in a given *trans*-EW-1 solution is

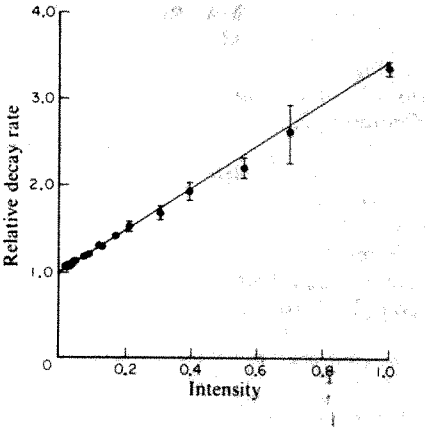


Fig. 3 PSC kinetics as a function of flash intensity. Data from trials like that of Fig. 3, in 5 μM EW-1. Vertical axis, rate constant of fastest PSC component after the flash, normalised to the value for the immediately preceding trial without a flash. Flash intensity was varied with neutral-density filters; intensity of 1.0 corresponds to 40% *trans* → *cis* photoisomerisation. Error bars show ±s.d. Temperature 10 °C. Cell 57-61, 62.

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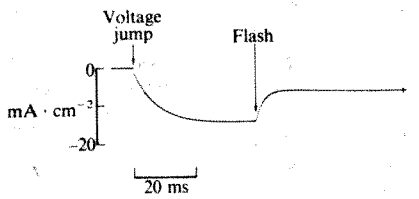


Fig. 4 Voltage-jump and light-flash relaxations in the presence of acetylcholine and 5 μM EW-1. Agonist-induced current is shown; passive and capacitative currents have been subtracted. Seven milliseconds after the start of the episode, the voltage was jumped from +50 to −150 mV. At about the midpoint of the episode, a flash was delivered, producing a conductance decrease. Flash intensity was the same as for the experiment of Fig. 2. Temperature 10 °C. Cell 61-72, T70, 71.

much greater than the steady-state blockade in a nearly pure *cis*-EW-1 solution of equal concentration. (2) Most of the blockade is transient, disappearing with a time constant of about 300 ms after the flash, even though *cis*-EW-1 has a lifetime of at least 40 s over the *p*H range from 2–13. These facts suggest that *trans*-EW-1 accumulates in the synaptic cleft; the flash would transiently produce a local *cis*-EW-1 concentration in excess of that in the bathing solution. The site of accumulation is probably not the catalytic site of acetylcholinesterase[8], because in experiments with carbachol, the light-flash relaxations did not change significantly after the enzyme was inactivated with diisopropyl fluorophosphate[9] or with methanesulphonyl fluoride[10]. The site of accumulation could be the peripheral anionic site of acetylcholinesterase[24], the acetylcholine receptors themselves, or some other component of the membrane or synaptic cleft. We do not know whether *cis*-EW-1 accumulates at the same site.

We have obtained very similar results using *N*-*p*-phenylazophenyl-*N*-methyl carbamylcholine and *N*-*p*-phenylazophenyl-*N*-phenyl carbamylcholine[25]. Like other experiments with light-activated compounds, these studies extend our knowledge of drug-receptor mechanisms[18,25,29]. With similar techniques it may be possible to test another interesting facet of the 'open-channel blockade' model, namely that the channel conducts again as the blocking drug leaves its binding site[4,16].

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Occlusion of K ions in the unphosphorylated sodium pump

POST, Hegyvary and Kume[1] found that the rate of rephosphorylation of $(Na^+ + K^+)$ATPase that had just been dephosphorylated depended on which congener of K (Rb or Li) had been used to catalyse the hydrolysis of the phosphoenzyme, even when the experiments were done in such a way that the composition of the medium during the rephosphorylation was constant. To explain the ability of the enzyme to 'remember' which kind of ion had catalysed the hydrolysis, they postulated that during the hydrolysis the catalysing ions became occluded within the enzyme, and were released only later after a slow conformational change. Furthermore, as ATP in high concentrations accelerated the rephosphorylation, they supposed that the slow conformational change was accelerated by the binding of ATP to a low-affinity site on the enzyme. Karlish, Yates and I.M.G.[2] suggested that the hypothetical 'occluded-K form' of Post *et al.* was identical with the form of unphosphorylated $(Na^+ + K^+)$ATPase that predominates in K-containing Na-free media; they found that the conversion of that form (E_2K) into the form (E_1Na) that predominates in high-Na media was very slow ($k \approx 0.25\ s^{-1}$ at 20 °C), and was accelerated by nucleotides acting with a low affinity and without phosphorylation. Karlish *et al.* also studied the conformational change in the reverse direction—$E_1Na \rightarrow E_2K$—and concluded that K ions combine with the E_1 form of the enzyme at sites with a relatively low affinity. They therefore suggested that the occluded-K form could be generated either following the binding of K^+ ions at low-affinity sites, probably on the intracellular surface of the membrane, or by the hydrolysis of phosphoenzyme catalysed by K ions bound to high-affinity sites on the extracellular surface of the membrane[2,3] (see Fig. 1). They pointed out that, if their hypothesis were correct, it could account for inward and outward movements of K ions through the sodium pump, and could explain why orthophosphate and ATP (or its non-phosphorylating analogues) are required for K–K exchange. However, the similarities between the properties of the occluded-K form and the form that predominates in K-containing, Na-free media could be coincidental. Furthermore, the idea of an occluded-K form is also open to doubt, as the experiments of Post *et al.* prove only that the product of the hydrolysis of phosphoenzyme differs depending on whether Rb or Li ions catalyse the hydrolysis, not that the ions become trapped inside. Both problems could be resolved if it were possible to show that, in Na-free media, K ions or Rb ions binding to the unphosphorylated form of the enzyme were indeed occluded. We report here experiments showing that they are.

Briefly, the procedure followed in these experiments was (1) to suspend a partially purified suspension of $(Na^+ + K^+)$ATPase in a medium free of Na and Mg and containing 100 μM ^{86}RbCl, with or without nucleotide; (2) to pass the suspension rapidly down a cation-exchange column loaded with Na; (3) to measure

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Table 1 Retention of Rb by $(Na^+ + K^+)$ATPase after the removal of free Rb by passage of the enzyme through a cation-exchange resin

	Nucleotide in suspension medium	Protein content of effluent (mg)	Rb content of effluent (nmol)	Extra Rb in absence of nucleotide (nmol)	Extra Rb (nmol per mg protein)
Expt 1	None	0.628 ±0.025	0.992 ±0.051	0.699 ±0.051	1.12 ±0.09
	2 mM ATP	0.657 ±0.027	0.293 ±0.003	—	—
Expt 2	None	0.568 ±0.019	1.160 ±0.042	0.825* ±0.044	1.45 ±0.09
	2 mM ATP	0.572 ±0.018	0.351 ±0.025	—	—
	2 mM ADP	0.582 ±0.010	0.318 ±0.012	—	—

One-mg quantities of $(Na^+ + K^+)$ATPase (specific activity 12 μmol per mg per min), suspended in 0.5-ml portions of a medium containing 100 mM Tris/Tris Cl (*p*H 7.4 at 20 °C), 15 mM histidine, 1 mM EDTA (Tris salt) and 100 μM ^{86}RbCl, with or without nucleotide, were passed through columns of Biorad Biorex 70 carboxylic resin (50–100 mesh) in the Na form. Each column was prepared by putting the resin, suspended in 100 mM HEPES (Na salt, *p*H 7.4), in the barrel of a 1-ml disposable tuberculin syringe (cross-sectional area 0.17 cm^2) whose tip was loosely plugged with glass fibre and fitted with a nylon tap. The final bed volume was 0.5 ml, and the total capacity was 1.65 mEquiv. After the resin had packed down, the tap was opened until the surface of the Na HEPES solution reached the top of the resin. The upper part of the syringe was then filled with water, and acid-washed sand was allowed to settle through the water to form a layer 1 mm thick on top of the resin. This was to prevent contamination of the enzyme suspension with Na from the resin. The water above the sand was removed by aspiration, and the upper part of the syringe was washed twice with water. The enzyme suspension was slowly run into the syringe without disturbing the sand layer, and the syringe was mounted vertically on the moveable platform of a Palmer stand, whose coarse screw (pitch 5 mm) could be turned at predetermined rates by an electric motor acting through a friction clutch. The piston was inserted into the barrel so that about 0.25 ml of air was trapped above the enzyme suspension; a small hole drilled through the wall of the barrel, at a point corresponding to 1.3 ml on the (extrapolated) scale, allowed this to be done without any significant rise in pressure. The tap was opened and the motor switched on. Suitable stops had been arranged so that, as the syringe was raised by the motor, the piston was pushed in, the movement beginning only after the motor had reached its set speed, and terminating abruptly just before the piston reached the surface of the resin. The flow rate was 0.22 ml s^{-1} and the temperature 21 °C. The effluents were analysed for total protein[7] and for ^{86}Rb. The figures in the second and third columns of the table are each the mean of three determinations ±s.e.m.

* Calculated by subtracting the average of the Rb contents of the ATP and ADP samples from the average Rb content of the nucleotide-free samples.

the amounts of radioactivity and of protein emerging from the column. Rb was used rather than K, partly because ^{86}Rb has a more convenient half life than ^{42}K, and partly because the data of Post *et al.*[1] suggest that Rb is released from the occluded form slightly more slowly than K.

Details of the procedure are given in Table 1, but it is convenient to consider some of them here. The main difficulty was to find conditions which allowed the column to remove free Rb from the suspension completely enough for the small amount of Rb occluded within the enzyme not to be masked, and quickly enough for the enzyme to emerge before too much of the occluded Rb had been released. $(Na^+ + K^+)$ATPase from pig kidney outer medulla was used because it can be prepared conveniently about 30% pure in reasonably large quantities[4], and the purified preparation is not vesiculate. When the suspending media were to contain ATP, the ATP was added only 1 or 2 min before the experiment, so that hydrolysis (in the absence of Mg and Na, and in the presence of 1 mM EDTA) should have been negligible. With 100 μM Rb, 2 mM ATP or ADP is more than sufficient to displace the equilibrium between E_2 and E_1 forms of the enzyme virtually completely in favour of the E_1 form[5]; in the presence of nucleotide, any occluded Rb would therefore have been released before the enzyme was added to the column.

Preliminary experiments showed that, with the conditions chosen: (1) in the absence of enzyme, the column removed more than 99% of ^{86}Rb from 0.5 ml of a solution containing 100 μM ^{86}RbCl, 100 mM Tris/Tris Cl (*p*H 7.4) and 1 mM EDTA (Tris salt); the small fraction of Rb remaining in the effluent was not significantly affected if the solution also contained 2 mM ATP; (2) when 1 mg of enzyme was added to the solution, 60 ± 4% of the enzyme was recovered in the effluent; the presence of 2 mM ATP did not significantly affect the recovery of enzyme.

The results of two separate experiments, each carried out in triplicate, are summarised in Table 1. In the presence of nucleotides the amounts of Rb that emerged from the columns were about 0.6% of the total, and not significantly different from the amounts of Rb that emerged from the columns in control experiments without enzyme. In the absence of nucleotides, an extra amount of Rb was carried through the columns, which is just what we should expect if the enzyme contained occluded

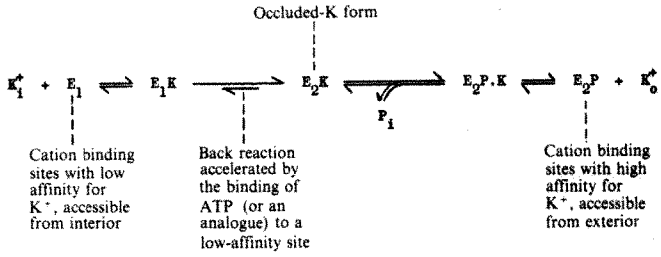


Fig. 1 Scheme showing the hypothesis of Karlish, Yates and I.M.G.[2], in which two routes to the occluded-K form are coupled back-to-back to produce a chain of reactions capable of carrying K ions through the membrane. Note: (1) E_2P in this scheme is identical with E_2P in the Albers–Post scheme (see Fig. 1 of ref. 8); E_2K in this scheme is identical with E_2K in Fig. 4 of ref. 2, and also with $E_2(K)$ in Fig. 5 of ref. 2 and Fig. 1 of ref. 8. (2) In the present experiments we are concerned only with the unphosphorylated forms of the enzyme. (When ATP was present, phosphorylation was prevented by the lack of Mg and, for most of the time, also of Na.)

Rb. Before we accept that explanation, however, we must see how this extra amount compares with the amount of occluded Rb that the enzyme might be expected to carry through the columns.

In the last column of Table 1, the extra Rb that appeared in the effluents in the absence of nucleotide has been expressed as nmol Rb per mg protein in each effluent. The specific activity of the enzyme used in these experiments was 12 μmol per mg protein per min, at 37 °C in standard conditions, and as enzyme approaching 100% purity has a specific activity of 40 μmol per mg protein per min in identical conditions, the purity of our enzyme must have been about 30%. If the molecular weight of the enzyme is assumed to be 250,000, our preparation must have contained 1.2 nmol of enzyme per mg protein. If each enzyme molecule can occlude two Rb ions, the maximum amount of occluded Rb would therefore be 2.4 nmol per mg protein. The amount of extra Rb to be expected in the column effluents is, however, less than this for two reasons: first, at an Rb concentration of 100 μM, not all the enzyme would have been in the E_2 form initially, and second, some of the occluded Rb would have been released during the time the enzyme was passing down the column. The relevant data for Rb are not available, but if we assume that Rb behaves like K, the fraction of enzyme in the E_2 form initially would have been about two-thirds[6]. The rate constant for the conversion of E_2K to E_1K, which is thought to be the rate-limiting step for the release of occluded K, has

been estimated to be about 0.25 s^{-1} at 20 °C in the absence of nucleotides[2,4]. Using the data in Table 1 of ref. 1, we calculate that the rate constant for the conversion of E_2Rb to E_1Rb is probably about 0.64 times as great. The amount of extra Rb to be expected per mg protein in the effluent is therefore $2.4 \times 0.67 \times \exp(-0.25 \times 0.64t)$ nmol, where t is the average time that enzyme in the effluent has spent passing through the resin. We cannot give a precise value for t, but it was almost certainly between 0.8 and 2.0 s. The expected amount of extra Rb is therefore between 1.17 and 1.41 nmol per mg protein. Given the uncertainties in the calculation, and the experimental errors, the agreement between the predicted and the observed values is remarkably good. We therefore conclude that the extra Rb carried through the columns with the enzyme in the absence of nucleotides does represent Rb occluded within the E_2 form of the enzyme.

We are now trying to adapt the rapid ion-exchange technique used in the above experiments to test the hypothesis[2,3] that Na ions are occluded within the phosphorylated $(Na^+ + K^+)$ATPase when it is in the E_1P conformation, the conformation believed to be the immediate product of phosphorylation of the protein by ATP.

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Highly active cyclic and bicyclic somatostatin analogues of reduced ring size

THE biological activity of an organic compound represents the sum of several properties, including solubility, absorption, transport, plasma protein binding, metabolism and receptor binding. The degree of molecular flexibility may affect these properties in different ways. Our approach to the design of somatostatin analogues with reduced susceptibility to metabolic inactivation has been both to eliminate those amino acids which are not required for biological activity and to increase the rigidity of the molecule. We report here the preparation of conformationally constrained analogues of somatostatin (Fig. 1, II*a* and III) which are highly active inhibitors of the release of insulin, glucagon and growth hormone *in vivo*. Analogue III (Fig. 1), which retains only four of the amino acids of the natural hormone (sequence 7–10), is relatively resistant to the action of trypsin *in vitro*.

We have already reported[1] the preparation of analogue I, a highly active bicyclic analogue of somatostatin that has a covalent bridge between the β-carbons of amino acids 5 and 12 (numbers assigned to amino acid residues of the analogues correspond to those of somatostatin). On the basis of the high biological activity of analogue I, we concluded that the corresponding β-carbons in somatostatin are in close proximity when somatostatin is bound to the receptors involved in the inhibition of the release of insulin, glucagon and growth hormone. Reduction of the disulphide bridge to give the monocyclic, dihydro derivative did not explain the high activity of analogue I *in vitro* and *in vivo*[1]. At that time our attention was focused on amino acids 7–10 of somatostatin. These are important in the interaction with receptors whereas amino acids 5, 6, 11 and 12 are not. It had been reported earlier that amino acids 4 and 13 could be deleted individually while retaining biological activity[2]. These observations suggested that the amino acids to the left of the disulphide bridge of analogue I could be deleted and the disulphide bridge replaced by methylene groups while retaining biological activity. We synthesised analogue II*a*, cyclo-(Aha-Phe-Phe-D-Trp-Lys-Thr-Phe), also designed as an analogue of I incapable of *in vivo* reduction[3,4] (Aha is ω-aminoheptanoic acid). A disulphide-containing analogue of ring size similar to that of analogue II*a* has been reported to have significant biological activity[5].

The minimum requirements for biological activity can be tested further by replacing phenylalanines 6 and 11 of analogue II*a* by cystine, as we have reported for an analogue with the full ring size of somatostatin[1]. Therefore, we also synthesised the bicyclic analogue III, cyclo-(Aha-Cys-Phe-D-Trp-Lys-Thr-Cys), which retains only amino acids 7–10 of somatostatin.

These analogues were prepared as outlined in Fig. 2. The L-Trp analogue of II*a*, cyclo-(Aha-Phe-Phe-Trp-Lys-Thr-Phe) (II*c*), was prepared in a similar way. Analytical results and physical properties are summarised in Table 1. The NMR spectra at 300 MHz supported the structures by demonstrating

Table 1 Characterisation data for analogues

	Amino acid analysis	$[\alpha]_{589}^{24}$ (conc.)†	R_F‡ (solvent system)§
II*a*	Lys 1.00 Thr 0.98 Phe 3.05 Trp 0.97 Aha 0.99*	−49.2 (0.15)	0.79 (A), 0.32 (B)
II*b*	Lys 1.00 Thr 0.70‖ Phe 0.98 Trp 1.00¶ Aha 1.01 Cys* 1.78‖	−35.5 (0.2)	0.58 (A), 0.13 (B)
II*c*	Lys 1.00 Thr 0.96 Phe 3.04 Trp 0.97 Aha 1.00*	−43.2 (0.1)	0.79 (A), 0.35 (B)
III	Lys 0.99# Thr 1.01 Phe 1.00 Trp 1.00¶ Aha 1.03 Cys 1.89	−64.7 (0.25)	0.69 (A), 0.23 (B)

* Methanesulphonic acid-catalysed hydrolysis for 20 h at 110 °C. † In 50% aqueous AcOH. ‡ Silica gel, Analtech. § (A) 10:5:1:3, AcEtO/pyridine/AcOH/H_2O; (B) 80:20:2, $CHCl_3$/MeOH/conc. NH_4OH. ‖ When Thr (or Ser) precede cysteine in a peptide we have observed simultaneous destruction of both amino acids. Racemisation of Ile in the sequence Ile-Cys has been reported to involve thiazoline formation[10]. Presumably, thiazoline formation would allow further reactions when a β-hydroxyl, capable of elimination, is present. ¶ Determined by UV. # after 20 hours 6M HCl hydrolysis at 110 °C.

Table 2 Inhibition of release (relative potency of somatostatin = 1)

Compound	Glucagon	Insulin	Growth hormone *In vitro*	Growth hormone *In vivo*	Gastric secretion
I	1.4 (0.26–12.40)	1.53 (0.92–2.66)	0.37 (0.29–0.48)	1.88 (0.35–8.33)	0.10
II*a*	0.86 (0.44–1.53)	0.88 (0.30–2.45)	0.93 (0.69–1.2)	0.65 (0.20–4.61)	0.03
II*b*		0.020 (0.003–0.060)	0.03 (0.02–0.04)	0.14 (0.05–0.29)	0.03
II*c*	0.041 (0.004–0.150)	0.10 (0.05–0.19)	0.14 (0.12–0.16)	—	<0.01
III	2.66 (1.32–6.10)	3.50 (2.31–6.38)	1.24 (0.81–1.88)	2.55 (0.99–11.1)	0.05

The biological effects were measured using methods described previously[1]. Inhibition of glucagon and insulin release was measured in urethane anaesthetised rats. Growth hormone release inhibition *in vivo* was measured in pentobarbital-treated rats. *In vitro* inhibition of growth hormone release was measured using dispersed pituitary cells. Inhibition of gastric secretion was measured in dogs stimulated by pentagastrin. 95% confidence limits are given in parentheses. For glucagon release by analogue II*b*, a low level of activity was observed, with no dose response.

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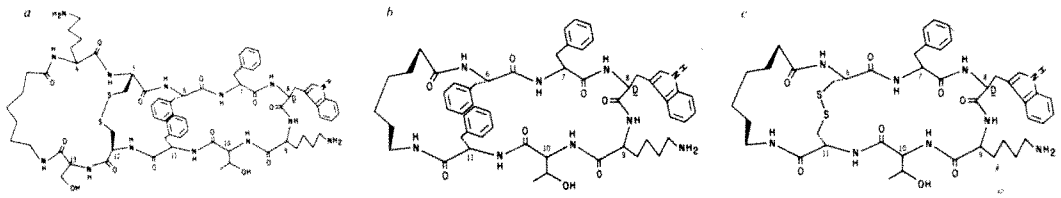


Fig. 1 Structure of analogues I, II*a* and III.

normal resonances for all the amino acids, with no unassignable resonances. The only abnormal resonance was the γ-CH_2 of Lys 9, shifted upfield about 1 p.p.m. in analogues II*a*, II*b* and III, a shift we have attributed to a proximity of the side chains of Trp 8 and Lys 9 in analogues with D-Trp in position 8. The high field resonance in these new analogues lacking Lys 4 permits unequivocal assignment to the CH_2 of Lys 9 as previously proposed[6].

The biological potencies of the analogues are summarised in Table 2, with the results previously reported for analogue I. In these respects analogues I, II*a* and somatostatin are nearly the same except that I and II*a* are less potent inhibitors of gastric secretion. It is clear from these and previous studies[1] that inhibition of gastric secretion involves unique structural requirements. The high potency of analogue II*a* provides additional evidence that *in vivo* disulphide reduction is not a prerequisite for the biological activity of analogue I.

We have suggested that the roles of Phe 6 and Phe 11 are related, at least in part, to stabilising a peptide conformation through the formation of a hydrophobic bond[1]. This conclusion is further strengthened by the high biological activity of analogue III relative to II*b*. The disulphide bridge of analogue III seems to be a prerequisite of high biological potency because the lack of bonding between amino acids 6 and 11, either covalent (III) or hydrophobic (II*a*), results in lowered potency, as seen in analogue II*b*. Once again, the D-Trp 8 analogue II*a* is more potent than the L-Trp diastereomer (II*c*), as was first observed with somatostatin[7] and later reported also in the dicarba-series[8]. We have interpreted this higher potency in the D-Trp series as representing a stabilisation of the biologically active conformation[6]. The high activity of analogues II*a* and III indicates that the binding of somatostatin to receptors is due chiefly to amino acids 7–10. The potencies reported in Table 2 not only reflect changes in receptor binding but are also a summation of *in vivo* effects, including metabolism. The *in vitro* growth hormone release data give the closest estimation of receptor binding because potential metabolism has been minimised by the use of conditions previously developed for a successful pituitary receptor model[9].

The bicyclic analogue III was more resistant to enzymatic cleavage, as demonstrated by its relative resistance to trypsin hydrolysis between lysine and threonine *in vitro*. The monocyclic analogue II*a* was cleaved by trypsin at about 100 times the rate of hydrolysis of III in identical conditions (1.5×10^{-3}M peptide). The course of hydrolysis was followed using high pressure liquid chromatography (HPLC) to assess the ratio of substrate to product at various intervals. It is interesting that in the presence of 1.5×10^{-3}M mercaptoethanol, analogue III was hydrolysed at about the same rate as II*a*. Thus, the disulphide bond adds enzymatic stability apparently through conformational or steric constraint. The lower rate of tryptic hydrolysis was due to reduced binding to trypsin because analogue III did not inhibit tryptic hydrolysis of II*a* even when present in a concentration 10-fold higher than that of analogue II*a*. The introduction of conformational constraint seems mainly to have increased K_M rather than prevented some needed conformational change along the pathway of hydrolysis.

There was an increased duration of action after subcutaneous administration of analogue III relative to II*a* and somatostatin (Fig. 3): the data in Fig. 3 show that 75 min after injection, both II*a* and III significantly inhibit the release of growth hormone, whereas somatostatin does not. At 135 min after injection, only analogue III shows significant inhibition of growth hormone release. Indeed, III significantly inhibited growth hormone release in this system up to 5 h after subcutaneous administration (to be reported in detail elsewhere). Although it seems reasonable that the greater duration of action is a consequence of the enhanced enzymatic stability of analogue III, it may be related in part to solubility differences, giving effectively depot forms of the analogue[8].

In conclusion, we believe that amino acids 7–10 of somatostatin, when constrained in the correct conformation, contain receptor binding elements sufficient to express the total activity of somatostatin for suppression of the release of insulin, glucagon and growth hormone. Our studies suggest that conformationally constrained structures can be designed to

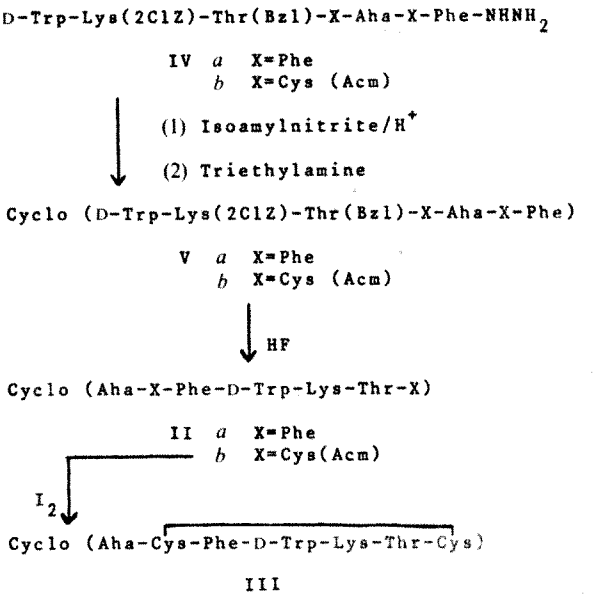


Fig. 2 Route for synthesis of analogues. The experimental details of the synthetic procedure are described in ref. 1 for larger ring monocyclic and bicyclic analogues of somatostatin. II*a* was prepared without purification of intermediates. The final product (III) was purified by chromatography on silica 60 using CH_2Cl_2/isopropanol/NH_4OH (concentrated) (60:40:5) as eluant. Isolated yield was 42% of product plus 22% of material showing trace impurities (melting point 234–236). In this study I_2 oxidation of analogue II*b* was carried out at a concentration of 3.2 mM in dimethylformamide using 6 equivalents of I_2. Excess I_2 was removed by filtration through silica gel 60, the I_2 being eluted with $CHCl_3$ and 10% MeOH/$CHCl_3$ while the peptide was adsorbed. III was eluted with $CHCl_3$/MeOH/H_2O (6:4:1) and the crude peptide product further purified by filtration through Sephadex G-50F, 50% AcOH, to give an isolated yield of 35%. III was generally prepared without purification of any intermediates. Purified samples of the intermediate II*b* were obtained by chromatography on silica gel, eluted with $CHCl_3$/MeOH/NH_4OH (70:30:3) followed by filtration through Sephadex G-25F (2M AcOH). The properties and analytical data obtained on these synthetic products and cyclo-(Aha-Phe-Phe-Trp-Lys-Thr-Phe) (II*c*) are summarised in Table 1. Final products had a purity of ≥98% as analysed by TLC and HPLC.

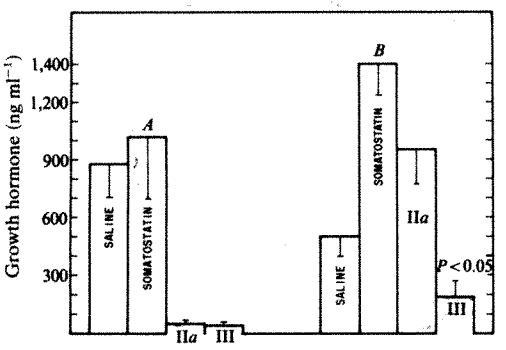


Fig. 3 Duration of action of somatostatin and analogues on the inhibition of growth hormone release. Male Sprague–Dawley rats (190–200 g) were injected with saline or compound subcutaneously. After 1 or 2 h sodium pentobarbital (17 mg per kg intravenously) was injected. The rats were bled 15 min later from the orbital sinus. The plasma was collected and assayed for growth hormone content by radioimmunoassay. The data are presented as the mean ± s.e.m. (six rats per group). Doses of somatostatin (50 μg) and analogues II*a* (50 μg) and III (25 μg) were chosen to attain equipotence. *A*, 75 min after administration; *B*, 135 min after administration.

decrease susceptibility to metabolism while retaining biological activity. Because analogue III shows greater stability both *in vivo* and *in vitro*, we believe that the incorporation of conformational constraint is a viable approach to the design of long acting somatostatin analogues.

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δ-Aminolaevulinic acid is a potent agonist for GABA autoreceptors

ACUTE attacks of the hereditary hepatic porphyrias are frequently accompanied by neuropsychiatric symptoms. The presentation of nervous system involvement varies; in decreasing order of frequency, there may be motor neuropathy, confusion, psychiatric manifestations, hyperexcitability and epileptic-type seizures[1]. Psychiatric manifestations include depression, anxiety, insomnia and an organic brain syndrome[2]. Although the aetiology of the neural dysfunction in the acute attack is unknown, several mechanisms have been suggested at a neurochemical level. It is possible that porphyrin precursors, which are produced in excess by the liver during acute episodes, gain access to the nervous system and exert direct neurotoxic effects[3–5]; alternatively, a metabolic defect in haem biosynthesis in nerve cells might result in a functional haemoprotein deficiency which becomes critical at the time of the acute attack[6]. The porphyrin precursor, δ-aminolaevulinic acid (δ-ALA), exhibits various effects in mammalian central nervous tissue preparations. It inhibits high-affinity uptake and increases efflux of γ-aminobutyric acid (GABA)[3] and L-glutamate[4] in rat cortical synaptosomes; it also inhibits the $(Na^+ + K^+)$ ATPase isolated from rabbit brain and red blood cells[7]. These effects occur only at relatively high concentrations of δ-ALA (at least 10^{-4}M), and it is very unlikely that such concentrations are reached in the brain during acute porphyric attacks[8]. Recently, there has been strong evidence that release of GABA is subject to negative feedback control through presynaptic receptors on GABAergic terminals[9–11]. Here we report that δ-ALA inhibits potassium-stimulated release of GABA from preloaded synaptosomes, probably by acting as an agonist at these presynaptic receptors. This effect is evident at concentrations of δ-ALA (10^{-6}M) which are well within levels recorded in the cerebrospinal fluid of porphyric patients showing neuropsychiatric symptomatology[12].

The method used has been published previously[13]. Briefly, fresh synaptosomes prepared from the cerebral cortices of adult male Wistar rats were resuspended in 0.32 M glucose to give a protein concentration of about 5 mg ml^{-1} and diluted 1:10 with ice-cold incubation medium (final concentrations: 128 mM NaCl; 5 mM KCl; 2.7 mM $CaCl_2$; 1.2 mM $MgSO_4$; 0.1 mM aminooxyacetic acid to prevent GABA catabolism; 10 mM Tris-HCl buffer at *p*H 7.35). One-ml aliquots of the suspension were preincubated at 37 °C for 15 min in a water bath in an air atmosphere. [2,3−^{3}H]GABA of specific activity 54 Ci mmol^{-1} (Radiochemical Centre) was diluted with unlabelled GABA to give a specific activity of 10 Ci mmol^{-1} and a small volume (1/100 of the final volume) added to the incubation tubes to a final concentration of 0.5×10^{-6} M. Incubation was continued for a further 10 min after which the synaptosome suspensions were layered on Millipore filters (0.45 μm pore) resting on filter supports constituting the bottoms of four parallel superfusion chambers[14], and thermostatically maintained at 37 °C. The chambers were connected to a multichannel peristaltic pump, the excess medium drawn off at maximum flow rate and the filters washed with 10 ml of medium (concentrations as described above) containing 30 mM glucose. The flow rate was adjusted to 0.5 ml min^{-1} and 1-min fractions were collected directly into scintillation counting vials. Superfusion was continued for 10 min with plain medium to establish a

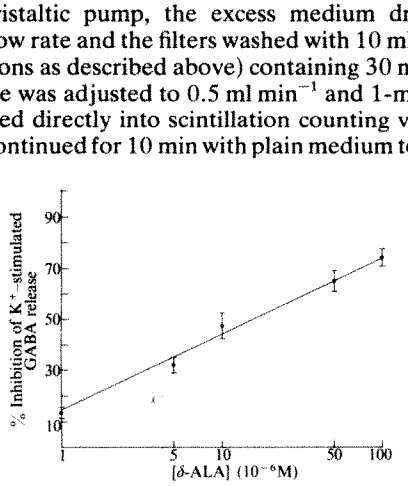


Fig. 1 A dose–response curve for the inhibition of potassium-induced GABA release by δ-ALA. The horizontal axis gives concentration of δ-ALA ($\times 10^{-6}$ M) on a logarithmic scale. Represented on the vertical axis is the % inhibition of potassium-stimulated (55 mM) GABA release. Each point is the mean % inhibition ± s.e.m. ($n = 4$). The curve is the best fit to the data by the method of least squares ($r^2 = 0.97$). It was not possible to produce 100% inhibition of the stimulated release as δ-ALA alone at concentration $>10^{-4}$ M caused increased efflux of radioactivity from the synaptosomes[3].

Table 1 Drug effects on the potassium-stimulated release of GABA from preloaded synaptosomes

Drug present	Induced release (% increase over baseline control)	Mean drug effect on induced release
55 mK K^+ alone (6)	188 ± 26.9*	
10^{-4} M δ-ALA alone (4)	23 ± 9.9	
55 mM K^+ 10^{-4} M δ-ALA (4)	48 ± 6.4†	−74.5%
55 mM K^+ + 10^{-4}M δ-ALA + 10^{-6} M bicuculline (4)	191 ± 13.2	+1.6%
55 mM K^+ + 10^{-5} M δ-ALA (4)	92 ± 13.5†	−51.1%
55 mM K^+ + 10^{-5} M δ-ALA + 10^{-6} M picrotoxin (4)	187 ± 10.2	−0.5%
55 mM K^+ + 10^{-6}M δ-ALA (4)	163 ± 7.5‡†	−13.0%
55 mM K^+ + 10^{-6}Mδ-ALA + 10^{-6} M bicuculline (4)	197 ± 13.1	+4.8%

Experiments were carried out as described in the text. The values in parentheses indicate the number of times the experimental conditions in the first column were repeated. Baseline efflux was calculated as % total tissue stores released per fraction (0.57 ± 0.068%, $n = 7$). The second column refers to the % increase in efflux over this control baseline level; in each case the mean ± s.e.m. is given. Statistical significance was calculated using the two-sided Student t-test. The third column represents the average drug-induced change compared with the release caused by 55 mM potassium alone. δ-ALA at concentrations $<10^{-4}$M had no effect on the basal release of radioactivity from the synaptosomes. Bicuculline and picrotoxin alone (10^{-6} M or 10^{-5} M) did not facilitate the release of GABA, presumably because the flow of superfusion fluid removed any GABA as it was released. In the absence of negative feedback effects to antagonise, the GABA receptor anagonists has no effect on basal efflux.

* Substitution of Mg^{2+} for Ca^{2+} in the medium did not significantly affect basal release of radioactivity, although 76 ± 2.2% ($n = 4$) of the K^+ (55 mM) induced release was Ca^{2+} dependent.

† $P < 0.001$, significant difference from 55 mM K^+ alone.

‡ $P < 0.05$, significant difference from 55 mM K^+ alone.

baseline rate of GABA efflux; the plain medium was then exchanged for the test medium containing the drugs listed in Table 1 and superfusion continued for a further 15 min. Aquagel I liquid scintillant, 5 ml, was added to each vial and the radioactivity of the vials was counted in a Packard Tri-Carb spectrometer at 37% efficiency. Filters were solubilised in 10 ml of Aquagel I and counted similarly. Stimulation of GABA release was calculated as the percentage increase in efflux over the baseline unstimulated level. The data obtained were analysed using a two-sided Student t-test and the results are shown in Table 1.

δ-ALA dose dependently reduced the potassium depolarisation-induced release of GABA from the nerve endings (Fig. 1). The reduction was significant at a δ-ALA concentration of 10^{-6} M, and at a concentration of 10^{-4} M 75% of the stimulated release was abolished. The concentration of δ-ALA which inhibited 50% of the potassium-stimulated release was approximately 10^{-5} M. In all cases the reduction was prevented by the GABA receptor antagonists bicuculline or picrotoxin (10^{-6} M) (Table 1). δ-ALA at 10^{-6} M or 10^{-5} M exhibited no effects on the basal release of GABA although, at 10^{-4} M, GABA efflux was slightly stimulated (Table 1). Neither bicuculline nor picrotoxin alone (10^{-6} M or 10^{-5} M) modified the basal release of radioactivity in the conditions used here. This is expected, as we consider that any GABA released from the tissue is rapidly removed from the superfusion chamber by the flow of medium. The concentration of GABA in the medium does not therefore reach the levels necessary to stimulate presynaptic receptors and hence the bicuculline or picrotoxin has no negative feedback effects to antagonise.

The evidence presented shows that δ-ALA, an ω-amino acid with a five-carbon chain similar in structure to GABA, reduces the amount of radioactivity released as a result of potassium stimulation, and that this effect is prevented by the GABA receptor antagonists bicuculline and picrotoxin. This is consistent with the idea that δ-ALA acts as an agonist at presynaptic receptors in the mammalian central nervous system. Indeed, it seems to be a fairly powerful agonist, inhibiting 50% of the stimulated release at a concentration of 10^{-5} M.

Although the influx of δ-ALA into the brain is limited[8], it has been demonstrated that δ-ALA can penetrate the blood–brain barrier of the rat at plasma concentrations known to occur in acute intermittent porphyria and appear in the cerebrospinal fluid in micromolar concentrations[15]. In addition, cerebrospinal fluid levels of $1.6–2.0 \times 10^{-5}$ M have been recorded in patients during acute attacks characterised by hyperexcitability and convulsions[12]. Our findings strongly support a direct mechanism whereby δ-ALA acts to inhibit GABA release from inhibitory nerve endings. This effect is evident *in vitro* at concentrations as low as and lower than those already recorded in the central nervous system during acute porphyric episodes.

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Correlation of local stability of DNA during melting with environmental conditions

DNA MELTING studies have revealed fine structural features in the melting profiles of many natural DNAs[1–4]. Restriction fragments have been used to assign modes showing up in melting profiles to the denaturation of well delineated segments inside the DNA molecule[1,3] and possible correlations of these segments with biological functions have been suggested[1,5,6]. However, when the exact sequence of natural DNAs became available, known genetic or regulatory features extending over tens to hundreds of base pairs did not appear to be characterized systematically by simple physical parameters. For example, the base pair composition fluctuation of ΦX174 DNA is almost smooth[6,7], whereas several distinct modes are seen in experimental melting profiles[8,9], each reflecting separate melting of segments of hundreds of base pairs. Attempts have been made to calculate the melting profiles of DNA from its sequence[10–13], and two calculated melting profiles have been published for ΦX174 DNA. Both calculated curves show fine structure, but comparison with the experimental profiles showed 'no obvious

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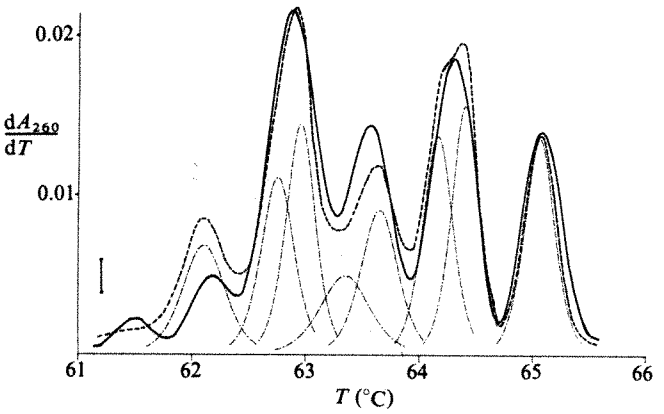


Fig. 1 Melting profile of a restriction fragment of SV40 DNA, extending from base pair 1,967 to 3,409 (origin: *Eco*RI restriction site). Solid line : experimental melting profile, obtained in 10 mm Na^+ (citrate), 10^{-4} EDTA; temperature incremented stepwise by 0.05 °C, mean heating rate 4 °C per h (see ref. 1 for experimental setup and procedure, and ref. 19 for data processing). Dashed line: calculated melting curve, for the sequence published by Fiers *et al.*[20]. Parameters used: $T_{AT} = 44.8$ °C, $T_{GC} = 93.9$ °C, $W = 4.07$, $b = 1.114$ (loop entropy factor), $\beta = 0.034$ (mode form factor); see text and refs 14 and 16 for definition of these parameters. Dashed-dotted curves correspond to the melting profiles of individual, cooperatively melting segments (only segments with 100 or more base pairs are shown).

systematic relationship' (ref. 8). The very different profiles observed for the same ΦX174 DNA in two different ionic strengths[8] could not be accounted for by the theory used. Thus the melting profile is not determined by the DNA sequence alone. However, recently good agreement between the calculated and experimental melting profile of ΦX174 DNA was obtained by Azbel[14–16]. Azbel's model is based on the observation that nucleation of the helix–coil transition requires much higher energies than that exchanged between base pairs, so that behaviour of DNA is mainly correlated to its ground state at a given temperature inside the melting range (see ref. 16). We have applied Azbel's model to another DNA of known sequence (a restriction fragment of SV40 DNA) and ΦX174 DNA in two different environmental conditions, and we obtained good agreement between predictions and experimental observations.

It can be seen from Figs 1 and 2*b*, *c* that the agreement between calculated melting profiles and experimental curves, obtained in this laboratory or published by Vizard *et al.*[8], is satisfactory both in terms of the number of modes and the shapes of the various profiles. Minor differences (most are within the experimental error bar) may be due to linear, first order approximation to Azbel's model.

An interesting aspect of the model is the fact that a phase-diagram can be constructed to show the variation of the ground state as a function of two dimensionless parameters which are related to standard thermodynamic functions. The phase diagram shown in Fig. 2*a* predicts the state of ΦX174 DNA, inside its melting range, for a range of $W = V/(f_1 - f_0)$, where V is the boundary energy between helix and coil regions, and $f_1 - f_0$

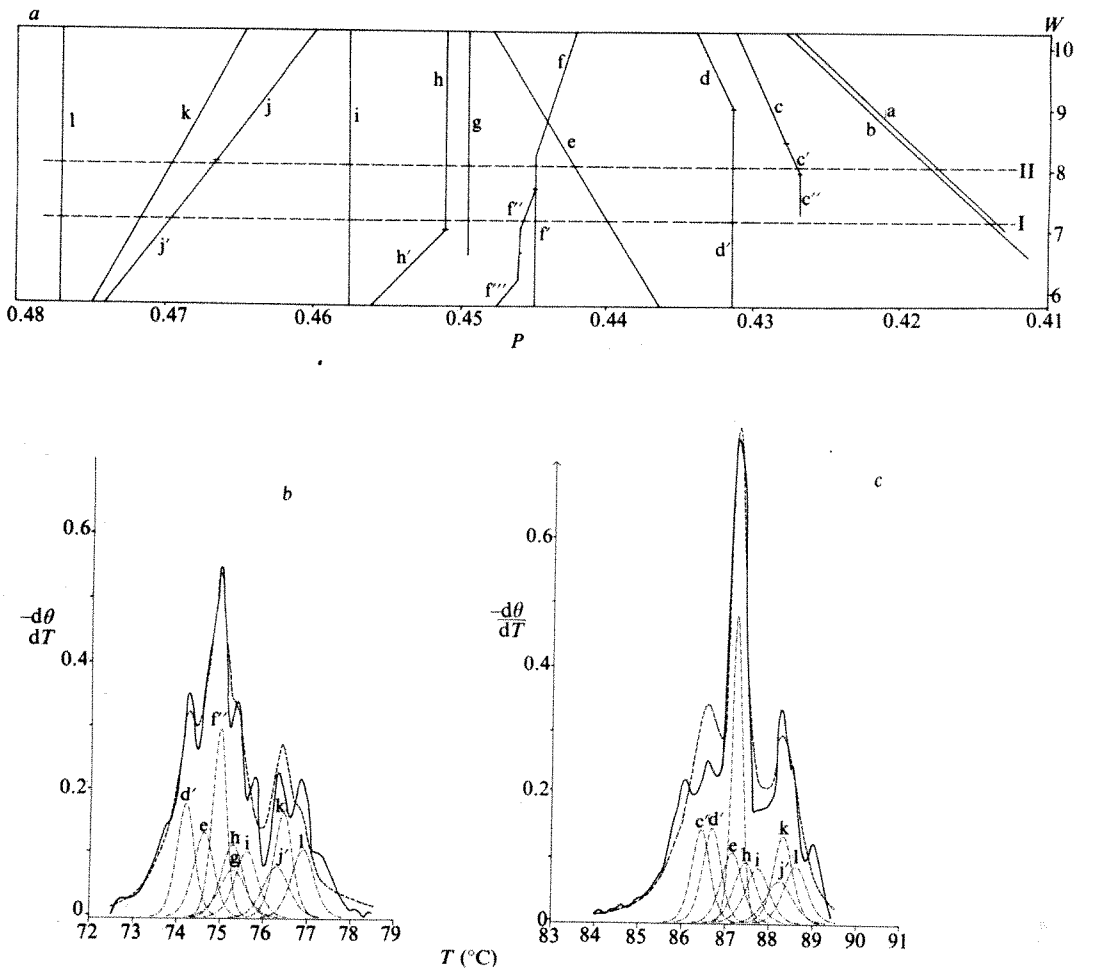


Fig. 2 *a*, Part of the phase diagram of ΦX174 DNA computed from its sequence[21]. Ordinate: $W = V/(f_1 - f_0)$, abscissa: $p = (T - T_{AT})/(T_{GC} - T_{AT})$ (see text and ref. 16). The lines divide domains having different composition of melted and helical segments in the ground state. Neighbouring domains differ by the state of a given stretch of base pairs (only domains differing by the state of at least 200 base pairs are shown). The base pair sequences changing state on crossing the boundaries are: (letters refer to letters drawn in the diagram; numbers indicate base pairs, the origin being the end of gene H (ref. 21)).

(a) 2,648–2,880
(b) 3,841–4,056
(c) 1,412–1,990
(c′) 1,440–1,880
(c″) 1,579–1,880
(d) 4,839–5,386
(d′) 4,839–5,283
(e) 22–405
(f) 2,880–3,678
(f′) 3,451–3,678
(f″) 2,880–3,451
(f‴) 3,016–3,451
(g) 4,127–4,414
(h) 1,057–1,412
(h′) 1,177–1,412
(i) 405–744
(j) 744–1,057
(j′) 759–1,057
(k) 4,414–4,839
(l) 1,990–2,334

b, Solid line: experimental ΦX174 DNA melting profile, 30 mM Na^+ (from ref. 8). Dashed line: computed curve corresponding to intercept I($W = 7.17$) of the phase diagram. Other parameters: $T_{AT} = 55.4$ °C, $T_{GC} = 104.5$ °C, $\beta = 0.084$, $b = 1.114$. This curve differs slightly from that obtained by Azbel[14] because we have used a somewhat different, more accurate sequence[21]. Dashed-dotted curves: melting profiles of individual segments (only the most prominent modes are depicted). *c*, Full line: experimental ΦX174 melting profile, 195 mM Na^+ (from ref. 8). Dashed line: computed curve, corresponding to intercept II ($W = 8.02$) of the phase diagram. Other parameters: $T_{AT} = 68.1$ °C, $T_{GC} = 111.2$ °C, $\beta = 0.081$, $b = 1.114$. Dashed-dotted curves as in *b*.

is the difference in free energy between AT and GC pairs, the abscissa of the phase diagram is given by $p = (T - T_{AT})/(T_{GC} - T_{AT})$ (ref. 16).

As W varies, the size and behaviour of some of the segments remain invariant, such as segment 405–744. Others change, however, such as segment 2,880–3,678 which changes its state at $p = 0.445$ when $W \geq 7.71$, is split into two segments when $W < 7.71$; segment 3,451–3,678 changes state at $p = 0.445$ as above, and 2,880–3,451 changing state at higher p (temperature). Thus size, location, and relative stability of homostable (cooperatively melting) segments inside DNA may be modified by changes in W. These can be induced by various environmental changes. A complete catalogue of the solution parameters acting on W is not yet established, but experimental evidence shows that ionic strength[17,18], *pH* and type of counterion[18] act on the thermodynamic functions entering W and can modify the melting profile of a given DNA.

Extending the phase diagram concept to DNA *in situ*, we may consider changes in W induced by the interaction of DNA with metabolites, activators, histones, polyamines, and so on. These could modify the size, location and relative stability of homostable cooperatively-behaving segments inside the DNA. Such stability switching and shifting could account, at least in part, for the regulatory activity of these agents. Attempts to correlate the complete phase diagram with known genetic features are under way.

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Environmental factors affecting the quantity of alcohol dehydrogenase in *Drosophila melanogaster*

THE alcohol dehydrogenase gene in *Drosophila melanogaster* has recently been the subject of intense study because it has properties that make it almost ideal for the investigation of genetic fine structure and control in a higher eukaryote[1–3], and for research on the population genetics of enzyme polymorphism[4,5]. There have been reports of genetic variation in alcohol dehydrogenase activity within and between electromorphs[5–10], and of a putative control mutant with half the 'normal' activity[11]. The studies described here show that environmental factors can change the amount of ADH protein in a fly by at least fourfold. They invalidate conventional methods of correcting for body size in studies of the activities and amounts of enzymes in *Drosophila*, and therefore cast doubt on much recent work. They also raise the possibility of an inducible eukaryotic enzyme that is suitable for refined genetic analysis.

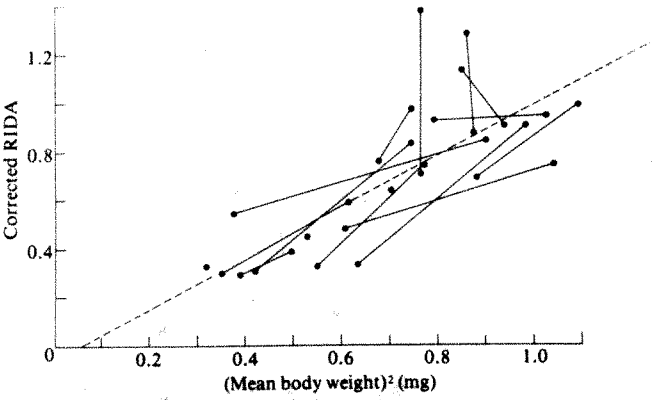


Fig. 1 The regression of corrected radial immunodiffusion area (RIDA) on the square of the mean body weight in batches of 25 4-day old male flies. The dotted line represents the overall regression ($y = 1.045x - 0.051$, $P < 0.001$). Measurements made on the same inbred line in different generations are joined by continuous lines. Inbred lines on which only single measurements were made appear as disconnected spots. The corrected RIDA is the area of the sample divided by the area of a standard extract (a mass homogenate of flies from a *white-honey* stock, used throughout the experiment) measured on the same gel. The flies came from 15 inbred lines, all homozygous for the Adh^S allele, derived from the Edinburgh Kaduna population (established with flies collected in Nigeria in 1949 and maintained since then in population cages). The lines were grown in one-third-of-a-pint milk bottles containing dead-yeast–cornmeal–molasses medium (11 g of yeast per l of vkm) at 25 °C. Adults were isolated as they emerged from pupae. 25 4-day old males were homogenised in phosphate buffer (*p*H 8.0), and the homogenates were passed through a Millipore filter. 4-μl samples of filtrate were introduced into the wells of a 1% agarose gel (*p*H 8.6) containing 2% rabbit antiserum against homogenised *D. melanogaster*. The gels were incubated for 48 h at 25 °C and the precipitin rings stained for ADH with nitroblue tetrazolium–methylene blue[13]. Each point represents the mean of two replicate gels.

The first observation, originally seen in experiments carried out by P. C. Hillier, is that both the activity of the enzyme (measured spectrophotometrically) and its quantity (assayed by single radial immunodiffusion[12]) can rise disproportionately with body weight. This is illustrated in Fig. 1, which shows the relationship between the area of the precipitin ring (strictly proportional, in our gels, to the quantity of enzyme) and body weight in a series of inbred lines homozygous for the Adh^S (slow) allele derived from the Kaduna population[13]. There is a fourfold range in the quantity of enzyme in flies from different cultures. That this is not due solely to genetic variation between the inbred lines is shown by the large differences between generations in one line. Indeed, there is no evidence that the various *SS* lines derived from Kaduna differ genetically in their quantities of ADH. The differences are not due to errors of measurement; the control areas (from the standard extract) are relatively constant from gel to gel, and the average difference between corrected replicate measurements is 11.5%.

The increase in the quantity of ADH with body weight is not linear. In the Kaduna lines it rises proportionally to the square of the body weight (fitting the power curve $y = ax^b$ to the data gives $b = 2.24$). Figure 2 shows that this phenomenon can occur within one inbred line. It gives measurements of single male flies from the Montgomery strain. In this case the amount of ADH is

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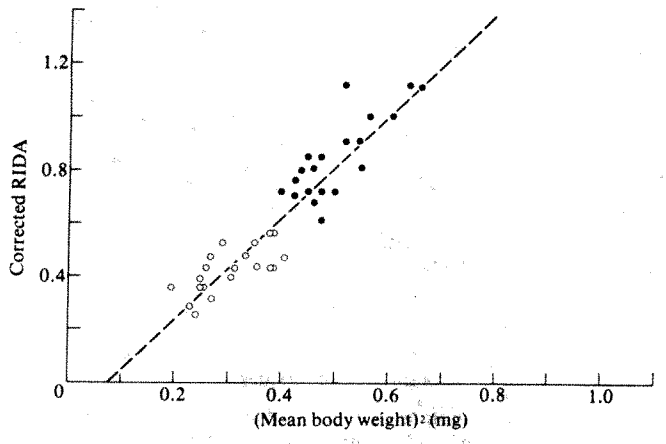


Fig. 2 The regression of corrected RIDA on the square of body weight in single 4-day old male flies from the Montgomery inbred line (homozygous for the Adh^S allele). The dotted line represents the overall regression ($y = 1.915 - 0.144$, $P < 0.001$) which is estimated for comparison with Fig. 1. The measurements were made in two separate generations, represented by open and closed circles. The difference between the generations, due to uncontrolled environmental differences, is very clear. The methods were identical to those given in Fig. 1 legend, except that individual flies were homogenised, and the homogenates centrifuged cold at 13,000*g* for 2 min, and that the gels contained 3% sheep antiserum. Each point represents the mean of two replicate gels.

approximately proportional to the cube of the weight ($b = 3.13$), and once more there is a fourfold range. The significant difference between the slopes of the regressions shown in Figs 1 and 2 suggests that the lines differ in their responses to the environmental factor that increases the quantity of ADH, but this remains to be confirmed.

Because the weight of adult flies is known to be influenced by the conditions of larval growth, we introduced newly hatched first instar Kaduna *SS* larvae into 2.5-cm shell vials containing 10 ml of dead-yeast–cornmeal–molasses medium with various quantities of dead yeast. Four vials had 50 g of yeast per l of medium, four had 10 g, four had 2 g and four had no yeast at all. The quantities of ADH in single 4-day-old adult males that emerged from these vials were measured by radial immunodiffusion. The results, given in Table 1, show that although the progressive addition of yeast increased the mean body weight by about 30%, it more than doubled the average quantity of ADH. Within the treatment groups there was only a slight association between body weight and ADH. It did not depart from linearity, and was significant in only one group (10 g yeast per l). Between the groups, however, the association is very striking, and the differences are highly significant ($P < 0.001$).

Table 1 The effects of different concentrations of dead yeast on the amount of ADH protein

Amount of dead yeast (g per l medium)	No. of vials	No. of 4-day male flies measured	Mean weight mg (s.e.)	Mean RIDA mm^2 (s.e.)
0	4	18	0.719(0.015)	30.13(1.37)*
2	4	17	0.791(0.021)	47.88(2.89)*
10	4	18	0.901(0.015)	81.83(2.02)
50	4	20	0.906(0.018)	80.70(1.73)

1st instar larvae were obtained from eggs deposited by Kaduma Adh^S homozygotes on laying dishes, and were grown in 2.5-cm shell vials containing 10 ml of medium (50 larvae per vial) at 25 °C. The methods were otherwise identical to those given in Fig. 2 legend. The asterisks represent means based on RIDAs that were significantly heterogeneous between vials ($P < 0.05$). Despite this heterogeneity the differences in RIDA between treatments are highly significant ($P < 0.001$).

These findings have several implications. The amount of enzyme is 'allometric' with body weight, rather than strictly proportional to it. This means that conventional ways of correcting for body size (dividing the activity or quantity of enzyme either by the body weight itself or by the total soluble protein, which is proportional to body weight) may give seriously misleading results. Supposed genetic differences in the activity or quantity of enzyme may merely reflect persistent disparities of body weight and/or the conditions of culture. This uncertainty applies to the report of a twofold difference in the quantity of ADH between genotypes[7], and to the recent claim that a 30% difference between a line selected for resistance to ethanol and an unselected control was due to the action of 'regulatory' genes[14]. It may also apply to work on other *Drosophila* enzymes, for which the effects of variations in the medium have not been studied. Our results suggest that screening for activity mutants may be greatly improved by a careful control of the medium. We note that disparities between lines in the conditions of culture may seem to be genetic because microorganisms can be passed on from the medium of the parents to that of their offspring.

The causes of the changes in the quantity of ADH are unknown. They could be due to an increased synthesis of enzyme in favourable conditions, to a decreased breakdown, or to changes in the time course of synthesis or breakdown. These possibilities are being studied, but in any event there is evidence that the precipitating factor is some part of the larval environment correlated with the quantity of yeast. A constituent or product of the yeast is the most obvious candidate. Because the flies were raised on dead yeast the factor is unlikely to be ethanol. Two per cent ethanol added to medium containing 2 g yeast per l does not increase the quantity of ADH (unpublished observation). If the changes prove to be a case of induction, and if the inducing substance can be identified, these observations will offer interesting possibilities for the study of eukaryotic gene control.

We thank the MRC and SRC for financial support.

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Erratum

In the letter 'Influence of leaves on sporophore production by fungi forming sheathing mycorrhizas with *Betula* spp.' by F. T. Last *et al.*, *Nature* **280**, 168–169, in paragraph 2 line 5, for '*Betula pendula* collected by Roth', read '*Betula pendula* Roth collected from . . . .'

Corrigendum

In the letter 'Non-selective isolation of human somatic cell hybrids by unit-gravity sedimentation' by P. L. Chang *et al.*, *Nature* **278**, 168–169, in paragraph 4 lines 9–10 the letters A and B referring to Fig. 1 have been transposed. It should read '. . . 2.4 units (A) . . . compared to 4.1 units (B) . . .'

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The Mathematics of Finite Elements and Applications III, MAFELAP 1978

edited by J. R. Whiteman

July/August 1979, c 520pp., £15.00/$31.50 0.12.747253.3

This volume records the Proceedings of the Third Conference on the Mathematics of Finite Elements and Applications, which was held at Brunel University in 1978. The decision to hold the conference followed the success of the previous two. The book contains the 10 main invited papers and 30 contributed papers, which were presented as lectures at the meeting, together with the abstracts of some 30 other papers that were presented in poster sessions. As before, the aim of the conference was to bridge the gap between the mathematical theory of finite elements and their applications. In particular, the main invited lecturers and the contributed papers were chosen so as to give coverage to as many as possible of the developments that have taken place in the finite element field in the three years since the last conference. Several chapters contain suggestions by the authors as to possible future paths that finite element techniques may take.

Frontiers of Speech Communication Research

edited by B. Lindblom and S. Öhman

August 1979, xxvi + 394pp., £18.80/$39.50 0.12.449850.7

The variety of new theories and related research is mirrored by the range of topics covered in this book: the quantitive modelling of the human vocal tract and its acoustical properties; acoustic phonetic description of various languages; the perception of speech and the development of objective measures of speech quality; applications of speech research; and the development of distinctive feature frameworks for linguistic theory. These original research reports, together with a number of review articles, provide a broad, up-to-date survey that makes the book a valuable reference work. The scope of the contributions will attract students and professionals engaged in research into speech communication, and those involved in the related fields of speech pathology, psychology, physiology and acoustics, and relevant areas of computer science and engineering.

Cognition and Perception Series

Decision Processes in Visual Perception

D. Vickers

August/September 1979, viii + 406pp., £21.60/$45.50 0.12.721550.6

The principal aim of this book is to present a theory of some of the fundamental mechanisms involved in the quantitative processing of visual information by human beings. In developing the theory, the book provides a detailed, constructive review of the various experiments and alternative hypotheses in each field of research. Throughout, the author relates his theory to other approaches, and presents it as the outcome of a logical sequence of alternative hypotheses. The various theoretical notions are dealt with in a concrete, intuitive way rather than a purely mathematical one, but abundant references are given to enable the reader to evaluate the argument in a more rigorous manner. The book will have a wide appeal to advanced undergraduates and researchers in experimental and cognitive psychology, particularly in the areas of perception, psychophysics and human information processing.

Drug Carriers in Biology and Medicine

edited by G. Gregoriadis

August/September 1979, c 300pp., £21.80/$46.00 0.12.301050.0

In theory, target selectivity for drugs can be achieved either on the basis of differential sensitivity or differential accessibility. However, it has only been in the last few years that the latter method has been considered practicable, by taking advantage of structures on the cell surface to bring about selective concentration of drugs by means of appropriate carriers. This is the first book to bring together work on a variety of drug carriers, and its publication now is evidence of the large amount of work already done and the success that has been achieved. The contributors survey the various approaches to the problem that have attempted using different types of carrier, and speculate on the possibilities of future research. The carriers dealt with include macromolecules and cells with homing properties harvested from biological systems, and synthetic carriers such as liposomes tailored to fulfil particular needs.

Electronic Imaging

edited by T. P. McLean and P. Schagen

August 1979, xviii + 522pp., £29.00/$61.00 0.12.485050.2

This book consists of the papers presented at an International Symposium sponsored and organised by the Rank Prize Funds, and held at the Royal Society, in September 1979. The aim of this symposium was to bring together experts on all aspects of electronic imaging, to exchange ideas and to benefit from their differing approaches to problems in this field. All the papers were given by invitation, which provided the opportunity for a balanced coverage of the many aspects of the subject: the pick-up display and recording of images of visible scenes, the processing of such images by electronic means, the sensing and use of images originating from non-visible radiation, such as infrared, microwave or ultrasonic and the interpretation by the eye-brain complex of the images presented to it.

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British Coastal Shrimps and Prawns

Keys and Notes for the Identification of the Species

G. Smaldon

July 1979, vi + 126pp., £4.50/$9.50 0.12.659260.6

British Coastal Shrimps and Prawns is a delightfully illustrated synopsis, which should be of wide interest. Searching for these well-known animals is a popular summer occupation for many and a livelihood for a few, as the larger ones are of culinary importance. There are over 40 different species to be found around British shores. With this synopsis it will now be possible to identify them accurately, thanks to the general accounts of the biology of each species aided by the illustrations, which carefully portray characters of systematic importance without taking away any of the fascination of these lively crustaceans.

Like previous synopsis, this one is designed to be a working field and laboratory guide for professional and amateur biologists. It bridges the gap between popular field-guides and rare taxonomic treatises and monographs. This book will be of considerable value to fish and wild life biologists, natural historians, conservationists, oceanographers and marine scientists.

Cobalt in Biology and Biochemistry

R. S. Young

August/September 1979, x + 148pp., £11.80/$25.00 0.12.772750.7

Cobalt is in a unique position amongst the rarer heavy metals found in biological systems. It plays an essential role in ruminant nutrition, functions as a key element in Vitamin B_{12} and there is extensive evidence on the beneficial effects of small quantities of this metal on growth and development in many plants, animals and micro-organisms. With over forty years of experience of all aspects of cobalt, Dr. Young is eminently placed to make a critical assessment of such published investigations. This book takes the form of a review of the literature of cobalt and a reference manual that will enable researchers to benefit from an insight into the techniques employed and data obtained by others working in different areas of science. The cobalt content of fertilizers, waters and plants, cobalt in human and animal nutrition and the effects of cobalt on enzymes and micro-organisms are the subjects of just some of the chapters. The text is well documented with over a thousand references, allowing the agricultural, medical, biological and chemical researcher or student ample scope to pursue his own particular interest and to aquire ideas from other areas of science which may be applicable to his own.

The Design and Analysis of Longitudinal Studies

Their Role in the Measurement of Change

Harvey Goldstein

August/September 1979, xvi + 200pp., £11.80/$25.00 0.12.289580.0

The principal aim of this book is to set out the theoretical and practical problems of longitudinal studies within the general framework of studies concerned with understanding the process of change, in particular with developmental studies of children. The seven chapters are designed to lead the reader through the various stages of study design and analysis. They bring together in a systematic manner a widely scattered literature and assemble it in a coherent form. The topics dealt with include the practical problems of planning a study, the problems of sampling, the choice of suitable instruments to measure change, the development of statistical models for analysing change, the setting up of norms or standards over time and the design of computer programmes for processing longitudinal data. This will make stimulating and enlightening reading for students and research workers concerned with longitudinal studies of human populations as well as experimental studies, for example, of animals and plants involving longitudinal data.

Applied Problems in Memory

edited by M. M. Gruneberg and P. E. Morris

August/September 1979, xiv + 290pp., £12.40/$26.50 0.12.305150.9

Memory plays a vital part in all human activities and a proper understanding of how it works in real life is of great importance to many practical and social problems. However, for many years there has been less emphasis on research into applied problems of memory than into theoretical aspects. The present book examines applications of memory research with contributions from eminent workers in a number of areas. The book covers an impressive range of problems in which psychologists have recently been engaged, from the more obvious improvements in techniques for better remembering and in education, to less evident areas such as reading, memory in the aged, effects of stress, memory for skills and disorders of memory. The wealth of practical examples given throughout the text contribute to make a highly readable account of the subject. Within its wide scope, the book has a great deal to offer, not only to cognitive psychologists and workers in educational, clinical and industrial psychology, and in ergonomics, but also to anyone concerned with practical problems of memory at all levels.

Bacteria and Mineral Cycling

T. M. Fenchel and T. H. Blackburn

August/September 1979, xii + 266pp., £14.60/$31.00 0.12.252750.X

This book describes the influences of bacterial metabolism on the chemical environment of the biosphere. Its readers will include general microbiologists who want to find out about the ecological significance of micro-organisms; ecologists whose microbiological background is limited yet who wish to understand the role of bacteria in natural ecosystems; postgraducates in microbial ecology; and, finally, applied scientists — such as soil scientists or environmental engineers — involved with microbial ecosystems. The discussion of the energetics of bacterial processes serves as a general structure for most of the topics in the book since such considerations may afford insight into which processes are important in natural ecosystems. Within this basic framework, two major aspects of bacterial metabolism are stressed. Firstly, the chemical evolution of the biosphere and secondly, the manner in which different element cycles interconnect in natural ecosystems. Appendices are included with information that will enable those with only elementary knowledge of microbiology, energetics and ecology to understand the

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This new interdisciplinary journal concerns appetite — the tendency to seek and consume substances, be they food, water or any other ingestable materials — in all its aspects. It will cover the basic appetitive and ingestatory mechanisms of interest, including neural, systematic physiological, biochemical, pharmacological, sensory, psychological, social, cultural and economic processes. The applied study of appetite may be *clinical* (weight control, obesity, dietary health risks, anorexia nervosa and malnutrition), *industrial* (sensory evaluation, consumer research on foods and drinks and feed regulation for animal production) or *zoological* (conservation and pest control). Papers are invited from any of these areas, although material submitted should have substantial relevance to intake or attitudes to ingestable substances. An important aim of the journal will be to publish innovatory papers crossing the traditional boundaries between disciplines: reviews, comments and mini reviews with an integrative bias are welcomed as well as reports of new investigations. There will be a place for book reviews, abstracts or reports, summaries of papers published elsewhere, bibliographies and announcements of relevant meetings — indeed anything appropriate to the general area of appetite. This major new publication, while not affiliated to any society, is intended for researchers in a wide range of disciplines including psychology, anthropology, zoology, pharmacology, psychiatry, medical research, nutrition, food science and applied social science.

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reviews

Selection in the social insects

Brian Charlesworth

Caste and Ecology in the Social Insects. By G. F. Oster and E. O. Wilson. Pp. 352. (Princeton University Press: Princeton, New Jersey, and Guildford, UK, 1979.) Hardback £12.50; paperback £4.70.

In the *Origin of Species*, Darwin wrote: ". . . I believe that natural selection, by acting on the fertile ants . . . could form a species which should regularly produce neuters, all of large size with one form of jaw, or all of small size with widely different jaws; or lastly, and this is the greatest difficulty, one set of workers of one size and structure, and simultaneously another set of workers of a different size and structure". He attributed this to "family selection": "slight modifications of structure or of instinct, correlated with the sterile condition of certain members of the community, have proved advantageous: consequently the fertile males and females have flourished, and transmitted to their fertile offspring a tendency to produce sterile members with the same modifications". Apart from the brief formulations of kin selection by Fisher and Haldane in the 1930s, relatively little was added to this wonderful insight of Darwin's about the evolution of the social insects until W. D. Hamilton's seminal papers in the early 1960s. Since then, interest in this fascinating subject has steadily grown.

Oster and Wilson's book is a synthesis of recent work, with particular emphasis on the problem of the selective factors affecting the numbers and kinds of sterile castes. Professor Oster is well-known for his outstanding contributions to mathematical ecology, and Professor Wilson for his leadership in the field of sociobiology. One would therefore expect this book to be a formidable combination of theoretical skills and wide knowledge of the natural history of the social insects. This expectation is certainly fulfilled. The book alternates between theoretical analyses and discussions of the relevant biological facts. Although the mathematical derivations are relegated to appendices, I suspect that many biologists will, like myself, find the theory rather heavy going. Nonetheless, many important questions are discussed, and workers on the evolution of social behaviour will need to study this book with care.

Chapter 1 provides an introduction to the problem of explaining the varied behavioural repertoires of the workers, and (in some groups) the existence of distinct physical castes. The authors state their intention of attacking the problem mainly through the concept that "castes and division of labour are subject to optimisation by natural selection at the colony level". (By colony-level selection, they mean Darwin's family selection; curiously enough, Darwin's contribution is never mentioned.)

Chapter 2 reviews the extensive data on different types of colony life-cycles. Some interesting suggestions are put forward concerning the selective factors which may influence the ways in which colonies can be founded (swarming as opposed to foundation by reproductives only, single as opposed to multiple queens, and so on), and there is a detailed theoretical analysis of the optimal allocation of resources between workers and reproductives during the development of a colony. This seems to provide a convincing explanation for the fact that, in temperate-zone species, reproductives are usually produced as a burst towards the end of the season, with the production of workers being simultaneously terminated. To my mind, this is the most successful application of the theory to a real-life problem in the whole book, as the theoretical predictions are not intuitively obvious, but follow from a plausible model. A note of caution is in order, however, as recent theoretical work suggests that the frequency of workers within a colony may be subject to control by kin selection or parental manipulation. A colony-level selection approach to this problem may not, therefore, be entirely satisfactory.

Chapter 3 is a discussion of the genetic basis of selection on social insects, particularly in relation to the sex-ratio problems originally treated by Trivers and Hare. I found this chapter somewhat disappointing. It assumes that selection should act to maximise either the fitness of the colony, the inclusive fitness of workers, or the inclusive fitness of the queen. It is not at all clear what the relevance of the colony's fitness is to a question such as the optimal allocation of resources between male and female reproductives, as evolutionary change in this variable must proceed by the substitution of genes which express themselves in individuals (queens or workers). Furthermore, considerable algebraic contortions have to be gone through in order to define the fitness parameters correctly, and to obtain solutions to the maximisation problems. A simpler approach would have been to use the standard population genetics methods for determining the values of the population strategies which are immune to invasion by rare genes causing deviant strategies — Maynard Smith's concept of the Evolutionarily Stable Strategy (ESS). These methods have been successfully applied to the sex-ratio problem by E. L. Charnov and others, and have the advantages both of simplicity and of being based firmly on gene frequency analysis. Chapter 3 also contains a rather misleading account of the ESS concept, which conveys the impression that an ESS is equivalent to a Nash equilibrium in standard games theory. But in the animal conflict models originally studied by Maynard Smith, members of a population playing a strategy S_1 have the fitness associated with contests between pairs of S_1 individuals. A rare mutant playing a different strategy S_2 will, to a good approximation, have the fitness associated with contests between S_2 and S_1. S_1 is an ESS if the former fitness exceeds the latter for all values of S_2. This has nothing in common with a Nash equilibrium, which involves a pair of opponents with different interests. Nash equilibria may be relevant to situations where different members of the population have conflicting interests (for example, the queen-worker conflict studied by Oster and Wilson), but the ESS concept is broader.

Chapter 4 gives a lucid account of the major facts about temporal and physical castes. Chapter 5 outlines the concepts underpinning the theoretical models of caste evolution developed in later chapters, the central idea being that of the "caste distribution function" or CDF, which specifies the distribution of the numbers of workers with respect to physical and behavioural caste, as a function of time, throughout the colony's development. The CDF is assumed to be adjusted by colony-level selection such that the total number of reproductives is maximised. Chapter 6 develops ths model, using dynamic optimisation theory to solve for the optimal CDF. As the authors make clear, the problem is not so much why there should be distinct castes, but why there are usually so few. They claim that, in

general, a size distribution of workers which precisely matches the distribution of size of available resource items is not to be expected. Their argument relies heavily on the concept of "fidelity cost", which assumes that an abrupt increase in the number of individuals in a given class creates an energy cost to the colony. This is an interesting idea, but seems rather difficult to evaluate empirically, in view of the necessarily arbitrary way in which the fidelity cost is introduced into the models. Chapter 7 is concerned with foraging strategies of workers. Chapter 8 is a useful discussion of the role of optimisation theory in evolutionary biology, and Chapter 9 is a brief survey of unsolved problems. As the authors make clear, both here and in the preface, the major factors determining the evolution of the detailed features of caste ratios and behavioural flexibility remain largely unknown. Nevertheless, their use of quantitative models highlights the problems very clearly and should prove very stimulating to research in this area. □

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Affinity chromatography

An Introduction to Affinity Chromatography. By C.R. Lowe. Pp. 253. (North-Holland: Amsterdam, New York and Oxford, 1979.) $29.25; Dfl60.

THIS volume is part of a series on laboratory techniques. The first four chapters consist of an introduction to chromatography, and accounts of basic concepts, chemistry and techniques of affinity separations. Chapter 5 is a selection of applications (protein-enzyme area), and a separate chapter covers regulatory macromolecules and more complex structures (although how antibody-antigen systems fit this category escapes me). The last two chapters are concerned with analytical applications and special techniques (very useful). The book covers most of the earlier work in the field: 9 references out of the 240 are 1977 or later.

The author has many thoughtful remarks to make for users of affinity chromatography. Rather than present a flawless up-to-the-minute review, Dr Lowe provides a valuable set of guidelines which should inspire rather than weary the reader. Because the technique is developing so rapidly some parts of the book are incomplete. Thus, it is now clear that triazine dyes are widely used as affinity ligands, their main advantage being the combination of capacity and selectivity. A slightly misleading comment on p455 suggests that all albumins will bind to Cibacron Blue columns when this property refers to human albumin only.

In the next edition, I would like to see some of the following recent advances discussed: the receptor purification advocated by Baulieu, involving alteration of the molecular weight of a protein using soluble dextran-ligand conjugates, electrophoretic desorption of high affinity biospecific adsorbents, light-reversible affinity ligands, and high performance affinity chromatography.

The sections on ligand concentration, temperature effects, the importance of ligand preassembly and the concept of group specific affinity matrices did not do justice to the author's work in these areas. I would also have liked to see a warning of the toxicity of *s*-trichlorotriazine (cyanuric chloride).

Pride of place in accounts of affinity chromatography goes to the now famous use of immobilised oxamate to adsorb lactate dehydrogenase in the presence of NAD(H). Dr Lowe's book leaves this bastion of theoretical preconception unassaulted, although Wood *et al.* have convincingly shown (1977) that replacing CNBr with triazine chemistry abolishes the biospecificity of the oxamate column.

Apart from the above minor criticisms, I found the balance between commercials, laboratory methods (for which Dr Lowe has an obvious flair) and theory enjoyable and well thought out. **P.D.G. Dean**

P.D.G. Dean is Senior Lecturer in the Department of Biochemistry at the University of Liverpool, UK.

Biological semiconduction

Dielectric and Electronic Properties of Biological Materials. By R. Pethig. Pp.376. (Wiley: Chichester, UK, and New York, 1979.) £15.

THE possibility that electronic energy bands exist in certain solid-state biological systems, playing a role in energy migration (Möglich and Schön; Jordan 1938) and electron transfer (Szent-Györgyi, 1941) has been around for over 40 years. During this time there has occurred a small but steady amount of theoretical and experimental progress on biological semiconduction, that is, on the electron transfer problem. Earlier work tended to show the conductivities were too low for biological significance, but with passage of time, the effects of hydration, and of charge-transfer, in inducing appreciable electronic conduction in proteins, all go towards increasing the prospects of a role of semiconduction in certain biological processes. The recent demonstration of high conductivity in reduced cytochrome c_3 by Inokuchi and coworkers, is another step in the same direction. Dr Pethig's book is aimed at introducing newcomers from a variety of disciplines to the field, and these are to be welcomed, as the need to increase the ratio of facts to speculations is rather urgent.

Very sensibly Pethig's first three chapters deal successively with dielectric theory, the intrinsic dielectric properties of biopolymers, and the effects of solvation on the dielectric properties. In these he established the basic solution properties, and then goes on in his fourth chapter to the effects of water on the solid-state behaviour, dealing with X-ray studies and water adsorption. In his fifth chapter Pethig outlines interfacial dielectric phenomena, the Maxwell-Wagner effect and its developments. Although this treatment is clear, I believe it could usefully have been a greater length, as the separation of Maxwell-Wagner from dipolar and hopping loss mechanisms is a fundamental problem in this field. Per contra, the sixth chapter dealing with dielectrophoretic studies is mainly of interest in connection with preparative separation methods, and is slightly off the main theme of the book. Chapter 7 deals with biological membranes and tissues, with brief notes on black lipid membranes, and includes three pages on the sometimes controversial effects of electromagnetic fields. With the final two chapters, 8 and 9, we come to the main part of the book, in which the author's own research interests are to be found. Chapter 8, on electrons, energy levels and energy bands, provides the basic background for crystalline and amorphous semiconductors, and includes an account of band gap and band width calculations for biopolymers, from the pioneer work of M.G. Evans and J. Gergely (1949) to the present. Chapter 9 sets out the experimental data on semiconductivity, Hall effects, charge-transfer interactions, piezoelectric effects and so on for proteins, DNA and other biological molecules. Photoconductivity is mentioned, but rather too briefly in view of its possible importance in vision and photosynthesis. Chapters 8 and 9, however, could scarcely be comprehensive in the space available, and Pethig's approach has been to take a few selected topics in depth. So, for example, there is a thoughtful appraisal of the effects of adsorbed water on conductivity, and an account of Pethig's own work on the methylglyoxal-protein system, in connection with Szent-Györgyi's recent suggestions concerning carcinogensis.

I believe this book gives an attractive introduction to its field. Mathematics are kept within the bounds necessary for a general readership, and in spite of the author's engineering background the emphasis is always on molecules rather than equivalent circuits. It is logically arranged, reads easily, and there is an ample supply of references. As a personal preference, I think a chapter summarising

the well-established field of ionic conduction in membranes, might have added to the value of the book, and formed a bridge to already well-established areas of electrophysiology. Where ionic and electronic conductivity exist side by side, there is a real problem in distinguishing one from the other. But Dr Pethig was probably wise not to trespass beyond his brief of electronic properties, since this is a neglected area of biophysics at present, and I believe this book makes a valuable contribution towards remedying this neglect.

D.D. Eley

D.D.Eley is Professor of Physical Chemistry at the University of Nottingham, UK.

Photochemistry of small molecules

Photochemistry of Small Molecules. By Hideo Okabe. Pp. 431. (Wiley: Chichester, UK, 1978.) £24.55.

ADVANCES in techniques, made over the past decade or so, have greatly facilitated detection of photochemical primary products and made possible detailed studies of photodissociation dynamics. The information obtained, coupled with much new experimental data on the reactivities of ground state and excited atoms and radicals, has provided the basis for an improved understanding of photochemical processes. Dr Okabe's book has been written to cover the progress made in the gas phase photochemistry of molecules containing up to five atoms.

The contents of the book fall essentially into three parts. Two chapters deal with the principles of spectroscopy and of the primary photochemical process; the introductory section is concluded with a chapter on experimental techniques. The author has chosen here to emphasise certain topics that are of interest to himself; and they are topics of importance often ignored elsewhere. The thermal contribution to photodissociation, atomic resonance absorption and emission, and deviations from the Beer-Lambert Law are dealt with in detail, as are energy and angular distributions in photofragments, and the determination of dissociation energies. The chapter on techniques is similarly concerned with certain special aspects rather than with a comprehensive overview. The order of presentation is sometimes unexpected, with detail preceding generalisations. For example, a mathematical treatment of band intensities is given in a section before, and unrelated to, that in which observations of intensity distributions are reported and the Franck-Condon principle stated.

The central section of the book consists of four chapters dealing with the quenching and reactions of excited atoms, and the photochemistry of diatomic to pentatomic molecules. For some 80 molecules, the spectroscopy and photochemistry are discussed in the light of information available up to July 1977. Although the huge amount of factual material presented necessarily precludes a deeply critical approach, the review is structured and readable.

A final chapter of the book is devoted to some topics related to photochemistry: isotopic enrichment, photochemical air pollution of the troposphere and stratosphere, and the photochemistry of the atmospheres of Mars, Venus and Jupiter. No more than an introduction can be given to these subjects in a total of 35 pages, but the chapter will provide a stimulus to the reader, as well as guiding him to more detailed references.

The author states that his book is aimed at the physical chemist, spectroscopist and atmospheric scientist interested in photochemistry. The level and content seem about right for this readership. A non-specialist would probably find the first chapters inadequate, and the review part too detailed, for the book to provide an introduction to photochemistry. But, for the practising photochemist, Dr Okabe has provided a most valuable reference book.

R. P. Wayne

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Asbestos sourcebook

Asbestos. Vol.1: *Properties, Applications and Hazards.* Edited by L. Michaels and S. S. Chissick. Pp. 553. (Wiley: Chichester, UK, and New York, 1979.) £25.

IN 1976, Dr Michaels and Dr Chissick, of the University of London, could discover no comprehensive source book on all aspects of asbestos. This first of two volumes is the result of a useful, and at times entertaining, attempt to provide such a book. The volume covers the mineralogy, chemistry and physics of asbestos, its use, monitoring, identification, and substitute materials; and five chapters are devoted to asbestos-related diseases. There are also contributions on attitudes to asbestos, and dealing with asbestos problems.

Each chapter was commissioned with an obviously careful brief to be comprehensive, not too technical and independent of other chapters. This has led to some repetition, for example, of the different varieties of asbestos and of the history of asbestos-related diseases. But this does not detract from any individual chapter and is only a slight annoyance to reviewers or other cover-to-cover readers.

Clearly, the brief that the editors gave themselves was to seek moderation. The contributors were carefully selected to exclude members of the asbestos industry and its declared opponents. This has worked remarkably well, as the contributions are well balanced without the extreme views often expressed on the emotive subject of the hazards of asbestos. This balance has been maintained effectively except in the chapter on attitudes to asbestos. There, the moderate discussion of attitudes of governments, industry and unions becomes more extreme itself when considering the extreme viewpoints of pressure groups and in the media. It was a pleasure to see the publication of such a positive response.

It was also a pleasure to see aired (for example, on page 385) certain of the inter-professional disputes which have been well-known among research workers, but have rarely been made public. This is a good move towards more openness in science.

In a series of commissioned chapters, not all can be of uniform standard. The introduction contains definitions (misleadingly headed Bibliography) of some terms. The first is of asbestos bodies, but the definition is of a fibre; the second contains one of those misprints prized by collectors — asbestosis is characterised by diffuse *intestinal* fibrosis — although it is argued on page 421 that the fibrosis is not "interstitial"; the radiographic classification given was issued by the International Labour Office in 1958, but the first version specifically to cover the radiographic changes related to asbestos exposure was not issued until 1971. The chapter on the clinical features of asbestos-related disease seems to treat the subject most superficially, particularly by comparison with the adjacent chapters. There were also a few other odd statements such as road death statistics are a hazard to health (page 330), and asbestos is the only known naturally occurring fibrous mineral (page 306). More seriously, there was no specific mention of the Medical Research Council as a source of information despite the many years of research on asbestos undertaken by its staff.

However, the overall impression is of a most comprehensive source book, useful to almost everybody who needs to discover information about asbestos. It goes a long way towards the editors' aims of public education. The only missing information seems to be the contents list for volume two.

Charles E. Rossiter

Charles E. Rossiter is Head of the Division of Computing and Statistics at the Clinical Research Centre, Harrow, UK.

Desert ecosystems

Arid-Land Ecosystems: Structure, Functioning and Management. Volume 1. Edited by D.W. Goodall, R.A. Perry and K.M.W. Howes. Pp. 881. (Cambridge University Press: Cambridge, 1979.) £45.

THIS volume is one of two that will give an overall account of arid-land studies, one of the themes of the International Biological Programme. Although much of the official IBP work was carried out in the USA, a considerable amount of similar work was also carried out in other countries at the same time, and much of this information has also been included. Unfortunately, and in spite of efforts by the editors, it has not proved possible to provide global coverage of arid regions. There is no account of South American deserts and the Sudan, a country with one of the largest desert areas, is only mentioned in passing. However, the areas that are described include North America, northern Africa, southern Africa, Australia, and South-West and Central Asia. The climate, soils, geomorphology, hydrology, flora and fauna are covered and the processes that operate within, and control, the ecosystem are dealt with under component, atmospheric, soil, plant and animal processes.

The editors' task must have been a nightmare for in spite of international cooperation investigators still seem to have operated within their own sub-set of published work and conventions, often set by linguistic or old colonial spheres of influence. This can give rise to reliance on a very distorted range of literature or undue emphasis on a personal field of interest in a review article. Thus, one regional account emphasises productivity, another phytosociology, and in some accounts animals receive scant attention. The characterisation of regional climate is similarly diversely treated. In one account simplified 'Walter Klimadiagramm' are given, another resorts to rainfall tables, a third makes use of maps with isohyets, and others give the 'Walter Klimadiagramm' in full. Standardisation of representation would have allowed comparison and integration to be made more easily.

The part of the volume devoted to processes is more satisfactory and includes an excellent chapter on diversity and niche structure in desert communities by E.R. Pianka. Chapters on radiation, precipitation and soil processes deal well with specifically desert conditions. Other chapters treat topics more generally.

This book has not managed to distil the knowledge accumulated in the IBP studies on arid ecosystem structure and function in a unified way. It would be unfair, however, to be too critical as it is difficult to give constructive suggestions on how a more satisfactory account could have been achieved. It is perhaps best to accept the volume for what it is — a mammoth compendium of information on many of the world's desert ecosystems.

M. J. Chadwick

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Prosimian behaviour

The Study of Prosimian Behavior. Edited by G.A. Doyle and R.D. Martin. Pp. 696. (Academic: New York, San Francisco and London, 1979.) $49.50.

The Study of Prosimian Behaviour is that rare pleasure: a book which fully achieves what it sets out to do. Its articles survey current knowledge of the prosimians, tabulate that knowledge by species and subject, and point out important hypotheses or conclusions for future study. From the opening chapter, in which Petter and Petter-Rousseaux give a revised classification, as well as the first full set of species distribution maps since Hill's tome *Primates: Comparative Anatomy and Taxamony,* Vol.1 (Edinburgh University Press, 1953), it is clear that this is now the standard reference for most aspects of prosimian behaviour. As such, it belongs in any university library.

The editors are to be congratulated on assembling articles of fairly standard format, with a minimum of repetition, although gestation lengths are given in three of the chapters and Martin and Charles-Dominque's basic decoding of home range and social structure in nocturnal forms enters at least three others. One might quibble that social structure of the diurnal species is under-represented, but this has been dealt with in recent books by A.F. Richard *(Behavioural Variation: Case Study of a Malagasy Lemur;* Bucknell University Press, Lewisburg; Associated University Presses, London, 1978 and edited by I. Tattersall and R.W. Sussman (*Lemur Biology*; Plenum, New York, 1975).

Chapters cover classification (J-J. Petter and A. Petter-Rouksseaux), phylogenetic and allometric aspects of behaviour (R.D. Martin), reproduction (R.N. Van Horn and G.G. Eaton), maternal behaviour (P.H. Klopfer and K.J. Boskoff), development (G.A. Doyle), learning (B.J. Wilkerson and D.M. Rumbaugh), vocal communication (J-J. Petter and C.M. Hladik), diet (C.M. Hladik), spatial distribution (J.I. Pollock), vision (G. Pariente), olfaction (A. Schilling), locomotion (A. Walker), field studies of lorisids (P. Charles-Dominque and S.K. Bearder), and a study of *Tarsius bancanus* (C. Niemitz).

One theme which runs through the book is the importance of nocturnal life, where the prosimians may give us clues to our common ancestor. Charles-Dominique argues that small diurnal primates would compete with diurnal birds, which explains why small primates are nocturnal, and only larger ones diurnal. This size constraint then reappears in Doyle's and Martin's treatments of allometry in brain size and developmental rates.

The reader may trace his own interests. One such might be the "psychology of place". Wilson and Rumbaugh stress that prosimians approach formal learning tests by position, not by attention to objects. Then Charles-Dominique remarks that rainforest galagos and pottos can be trapped twice running if the trap is moved to a nearby site, for "It would seem the traumatic effect of capture is associated more with the place . . . then with the trap itself". Schilling then shows how scent-marking identifies places in both individual and social contexts — again, the recourse of a more nocturnal, less visually oriented evolutionary lineage. This, in turn, ties in with the views of Pollock, Bearder, and Charles-Dominque on ranging, "detour problems" for slow-climbing forms in the forest canopy, and social organisation by the overlapping of home ranges. For many prosimians, social life itself depends on position in the forest, cued by scent marks, rather than on vocal or visual contact with other animals.

One notable contribution is Georges Pariente's study of prosimian vision. Pariente presents photography of the living retina, electrophysiological studies, measurements of light intensity in Madagascar forest conditions, and activity of nocturnal species in relation to ambient light. He argues that "nocturnal life is more a shift in thresholds of sensitivity than a completely new, 'inversed' mode of existence. The notion of a night when vision is impossible only exists for strict diurnal species . . . Nocturnal prosimians can move around perfectly well in full daylight, using their vision in the usual way". He concludes that if the prosimian eye retains this degree of plasticity, one might propose a "primate ancestor which needed to see wellby both day and night . . . small bodies, perhaps arboreal, with an appreciable amount of activity in the daytime . . . This ancestral form would have already been able to decode both in the intensity and frequency of light".

It is wholly appropriate that *The Study of Prosimian Behaviour* is dedicated to Georges Parente, who was tragically killed in a traffic accident shortly after completing his article for this book.

Alison Jolly

Alison Jolly is Research Associate in the School of Biological Sciences at the University of Sussex, Brighton, UK.

announcements

Appointments

The Royal Society has awarded a Senior Research Fellowship to **Dr D.T. Edmonds,** university lecturer in physics in the University of Oxford and fellow of Wadham College, Oxford, for five years from 1 October 1979.

Amoz I. Chernoff, has been appointed Director of the Division of Blood Diseases and Resources in the National Heart, Lung, and Blood Institute.

Dr Aksel C Wiin-Nielsen (Denmark), has been appointed Secretary-General of the World Meteorological Organization (WMO) for a period of four years commencing 1 January 1980.

Geoffrey Caston, has been appointed Secretary General of the Committee of Vice-Chancellors and Principals in succession to **Sir Roy Marshall** who is taking up the Vice-Chancellorship of the University of Hull on 1 October 1979.

The following have been elected as Junior Beit Fellows as from 1 October 1979. **Farzin Farzaneh; Simon Brian Fishel; Virginia Jane Sandford Karlsson; Andrew John Parker; John Douglas Waterfield.**

Dr Jacob A. Brody, Associate Director, Epidemiology, Demography and Biometry Program, National Institute on Aging, National Institutes of Health, has been elected Vice-President of the American Epidemiological Society.

Dr Iain M. L. Donaldson has been appointed to the Chair of Applied Zoology in the University of Hull with effect from 1 October 1979.

Dr John W. Neale of the Department of Geology, University of Hull, appointed to a personal professorship with effect from 6 April 1979.

Dr Frank M Leslie, Reader in Mathematics in the University of Strathclyde since 1971, has been appointed to a Personal Professorship of Mathematics in the University.

Professor John Stuart, has been appointed Head of a new Department of Haematology in the University of Birmingham from 1 October 1979.

Robin Butlin has been appointed professor and head of Loughborough University's Department of Geography.

Neil W. Ashcroft, professor of physics at Cornell University, has been elected director of the University's Laboratory of Atomic and Solid State Physics for a five-year term with effect from 1 July 1979.

Dr Frank Wilkinson reader in physical chemistry at the University of East Anglia, has been appointed professor of physical chemistry in Loughborough University's Department of Chemistry.

Prof Michel Revel, Head of the Weizmann Institute's Virology Department is the first incumbent of the Ruth and Jerome A. Siegel and Freda and Edward M. Siegel Chair in Virology.

Dr R.F. Sellers, has been appointed Director of the Animal Virus Research Institute in succession to **Dr J.B. Brooksby,** who retires in December 1979.

Professor Bryan E. Richards has been appointed to the Mechan Chair of Aeronautics and Fluid Mechanics in the University of Glasgow, with effect from January 1980.

Dr Christopher Arme, Principal lecturer and Head of the Biology Unit at the North Staffordshire Polytechnic, has been appointed to a new Chair of Zoology in the Department of Biological Sciences at the University of Keele, from 1 October 1979.

Meetings

15-17 August, **20th Canadian High Polymer Forum,** Quebec (Dr Ian McEwan, Secretary Treasurer, 20th Canadian High Polymer Forum, Canadian Industries Limited, 1330 Castlefield Avenue, Toronto, Ontario, Canada).

21-24 August, **Cryogenic Engineering and International Cryogenic Materials Conferences,** Madison (Mrs Dee Belsher, CEC Administrator, National Bureau of Standards, Boulder, Colorado 80303).

9-14 September, **8th International Congress on Biometerology,** Tel Aviv (World Meteorological Organization, Geneva, Switzerland).

13-15 September, **1979 Midwest Meeting of the American Geophysical Union,** Ohio Florida Avenue, N.W., Washington, D.C 20009).

17-18 September, **26th Pacific Northwest Regional Meeting,** Oregon (American Geophysical Union, 2000 Florida Avenue, N.W., Washington, D.C. 20009).

19-26 September, **World Telecommunication Forum,** Geneva (Mrs Rita Glita, American Express Travel Service, 7 rue de Mont-Blanc, Boite postale 859, 1211 Geneve 1, Switzerland).

20 September, **The Oral Cavity: Disease and Drug Treatment,** London (Secretariat, c/o Institute of Biology, 41 Queen's Gate, London SW7, UK).

23-28 September, **A short course on Computer Ergonomics,** Loughborough (J.S. Wilcox, Administrative Assistant, Department of Human Sciences, University of Technology, Loughborough, Leicestershire, UK).

26-27 September, **584th meeting of the Biochemical Society,** Swansea (Meetings Officer, The Biochemical Society, 7 Warwick Court, High Holborn, London WC1, UK).

5 October, **The Nature of the Glassy State,** London (N. Rivier, Blackett Laboratory, Imperial College, London SW7, UK).

31 October, **5th Electronics Update Colloquim,** London (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

15 November, **Techniques of Teaching Crystallography,** London (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

3-7 December, **1979 Fall Meeting,** San Francisco (American Geophysical Union, 2000 Florida Avenue, N.W., Washington, D.C. 20009).

18 December, **Synthetic Developments in the Prostanoid Field,** London (Secretariat, c/o Institute of Biology, 41 Queen's Gate, London SW7, UK).

1980

2-4 January 1980, **17th Annual Solid State Physics Conference,** Coventry (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

24-26 January, **European Meeting on Intensive Care,** Paris (SOCFI, 7 rue Michel-Ange, F75016 Paris, France).

10-14 March, **1980 Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy,** Atlantic City (Frank W. Plankey, Department of Chemistry, University of Pittsburgh, Pennsylvania 15260).

17-18 March, **Modelling Dispersion of Transport Pollution,** Cambridge(The Institute of Machematics and its Applications, Maitland House, Southend-on-Sea, Essex, UK).

18-20 March, **International Symposium on the Genetic Control of Natural Resistance to Infection and Malignancy,** Montreal (Dr Emil Skamene, Montreal General Hospital, Montreal, Quebec, Canada).
24-28 March, **Vacuum Devices Conference,** Cambridge (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).
31 March-2 April, **3rd International Conference on Physicochemical Hydrodynamics,** Madrid (Prof. M.G. Velarde, Univ. Autonoma de Madrid, Cantoblanco (Madrid) Spain).
14-16 April, **Semi Insulating III-V Materials,** Nottingham (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW7, UK).
18-20 April, **1980 Annual Conference of the Education Group of the Institute of Physics,** Birmingham (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).
26-27 April, **International Symposium on Bipolymers,** Birmingham (Dr H. Warson, 284 Warwick Road, Solihull, West Midlands, UK).
26-28 April, **6th Congress of German Cybernetics Society,** Mainz (Prof. I.W. v. Seelen, Institut für Zoologie, Universität Mainz, Saarstr. 21, D-6500 Mainz, FRG).
5-10 May, **1st International Conference on the Behaviour of Marine Animals, Ecology and Pollution,** Concarneau (Dr Yves Rouger, Marine Biology Laboratory, Collège de France, Concarneau 29100, France).
6-9 May, **9th World Congress on the Prevention of Occupational Accidents and Diseases,** Amsterdam (Benelux Organising Committee, Veiligheidsinstituut, Postbox 5665, 1007 AR Amsterdam, Netherlands).
27-30 May, **Joint meeting of the Institution of Mining and Metallurgy, the Society of Mining Engineers of AIME and the Metallurgical Society of AIME,** London (The Secretary, Institution of Mining and Metallurgy, 44 Portland Place, London W1, UK).
28-31 May, **IFAC Symposium on Water and Related Land Resource Systems,** Ohio (IFAC-Water Systems 1980, American Geophysical Union, 1909 K Street, N.W., Washington D.C. 20006).
9-14 June, **9th International Symposium on Organic Sulphur Chemistry,** Riga (The Organizing Committee, Institute of Organic Synthesis of the Latvian SSR Academy of Sciences, Aizkraukles 21, 226006 Riga, USSR).
10-12 June, **2nd International Conference on Developments in Power-System Protection,** London (The Press Office, Institution of Electrical Engineers, Savoy Place, London WC2, UK).
16-20 June, **9th International Coffee Science Symposium,** London (R.J. Clarke, General Foods Limited, Banbury, Oxon, UK).
24-26 June, **International Conference on Power System Monitoring and Control,** London (The Press Office, Institute of Electrical Engineers, Savoy Place, London WC2, UK).
25 June, **Symposium on Trench and Fore-arc Sedimentation and Tectonics in Modern and Ancient Subduction Zones,** London (J.K. Leggett, Geological Society of London, Burlington House, London W1, UK).
29 June-6 July, **1980 International Palynological Congress on Diagenesis of Sedimentary Organic Matter,** Organic Preservation and Fossil Fuel Exploration, Cambridge (Dr Jim Brooks, Exploration Department, British National Oil Corporation, 150 St Vincent Street, Glasgow, UK).
21-26 June, **4th International Congress of Immunology,** Paris (Congres-Services, 1 rue Jules Lefebvre, 75009 Paris, France).
30 June-4 July, **13th International Symposium on Chromatography,** Cannes (G.A.M.S. 88 boulvard Malesherbes, 75008 Paris, France).
7-11 July, **2nd International Congress on Toxicology,** Brussels (SdR Associated, 16 Avenue de Abeilles, 1050 Brussels, Belgium).
7-12 July, **8th International Congress of Primatology,** Florence (B. Chiarelli, Institute of Anthropology, Via del Proconsolo 12, 50127 Firenze, Italy).
7-17 July, **26th International Geological Congress,** Paris (Secrétariat Général due 26eme Congrès Géologique International, Maison de la Géologie, 77-79 rue Claude Bernard, 75005 Paris, France.).
14-19 July, **9th International Conference on General Relativity and Gravitation,** Jena (Professor E. Schmutzer, GR9 Sektion Physik, Friedrich-Schiller-Universität, DDR-69 Jena, Max-Wien-Platz 1, FRG).
14-24 July, **3rd International Symposium on Water Rock Interaction,** Edmonton (Dr Brian Hitchon, Secretary-General, WRI-3, Alberta Research Council, 11315-87 Avenue, Edmonton, Alberta, Canada).
22-26 July, **4th International Conference on Cyclic Nucleotides,** Brussels (J.E. Dumont, I.R.I.B.H.N., School of Medicine, 2 rue Evers, B-1000 Brussels, Belgium).
4-9 August, **7th International Conference on Raman Spectroscopy,** Ottawa (Ken Charbonneau, Conference Services, National Research Council of Canada, Ottawa, Ontairo, Canada).
2-5 September, **7th International Symposium on Medicinal Chemistry,** Madrid (7th International Symposium on Medicinal Chemistry, 142-144 Oxford Road, Cowley, Oxford, UK).
3-5 September, **Microbial Adhesion to Surfaces,** Reading (Dr P.R. Rutter, c/o The Conference Secretariat, Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).
5-7 September, **17th Symposium of European Association against Virus Diseases,** Munich (Professor F. Deinhardt, Max-von-Pettenkofer-Institut, Pettenkoferstrabe 9a, D-8000 München 2, FRG).

Reports & publications

UK & Ireland — June

Air Pollution — Its Despersion and Effects. Pp. 59. £4. Water Pollution — Its Dispersion and Effects. Pp. 49. £4. (Atkins Research and Development Monographs.) (Epsom, Surrey: Atkins Research and Development, Woodcote Grove, Ashley Road, 1979.) [46

Health Service Commissioner. First Report for Session 1979-80: Investigations completed — December 1978 to March 1979. Pp. 216. (London: HMSO, 1979.) £3.25 net. [66

Health and Safety Executive. Isocyanates: Toxic Hazards and Precautions. (Guidance Note EH 16). Pp. 8. (London: HMSO, 1979.) 38p plus postage. [66

Review Body on Doctors' and Dentists' Remuneration — Ninth Report, 1979. Chairman: Sir Ernest Woodroofe. Pp. v + 127. (Cmnd. 7574). (London: HMSO, 1979.) £2.25 net. [76

Department of the Environment. Nineteenth Progress Report of the Standing Technical Committee on Synthetic Detergents. Pp. iv + 16. (London: HMSO, 1979.) 75p net. [86

Clinical and Laboratory Haematology, Vol. 1, No. 1, 1979. Edited by J.M. England. Pp. 1-73. Published quarterly. Annual subscription: £21 (UK), £26 (overseas, and $57-50 (USA and Canada). (Oxford and London: Blackwell Scientific Publications, 1979.) [86

Natural Environment Research Council: Institute of Terrestrial Ecology. Sampling. By J.N.R. Jeffers. (Statistical Checklist, 2). Pp. 7. (Cambridge: Institute of Terrestrial Ecology, 68 Hills Road, 1979.) [116

Freshwater Biological Association. 1929-1979: The First Fifty Years. By G.E. Fogg. Pp. 39 (Ambleside: Freshwater Biological Association, 1979.) [116

The Wellcome Trust, 1976-78: Twelfth Report. Pp. 113. (London: The Wellcome Trust, 1 Park Square West, 1979.) [116

Council for Science and Society, The Outer Circle Policy Unit. The Big Public Inquiry. (A Proposed New Procedure for the Impartial Investigation of Projects with Major National Implications.) Chairman of Working Party: Paul Sieghart. Pp. iii + 72. (London: The Outer Circle Policy Unit, 4 Cambridge Terrace, NW1, 1979.) £1.50. [116

Health and Safety Executive. Publication Catalogue '79. Pp. 107. (London: HMSO, 1979.) £1 net. [136

Handbook of British Medical Research Charities. Pp. 35. (London: The Association of Medical Research Charities, c/o British Heart Foundation, 57 Gloucester Place, W1, 1979.) [146

The Rowett Research Institute. Annual Report of Studies in Animal Nutrition and Allied Sciences, Vol. 34, 1978. Pp. 131. (Bucksburn, Aberdeen. The Rowett Research Institute, 1979.) £2.50. [156

Propolis: a Review by E.L. Ghisalberti. (*Bee World* 60(2), 1979). Pp. 26. (Gerrards Cross, Bucks.: International Bee Research Association, Hill House, 1979.) 60p or $1.40, plus 10p or $0.25 p&p. [196

The Radiochemical Centre. Technical Bulletin 79/4: Radiolabelled Neurochemicals for Receptor Studies. Pp. 8. (Amersham: The Radiochemical Centre, 1979.) [196

International Conference on Recombination in Semiconductors. (*Solid-State Electronics*:an International Journal, Vol. 21, Nos. 11/12, November/December 1978.) Pp. vi + 1273-1612. (Oxford and New York: Pergamon Press, 1978.) £20. [196

Proceedings of The 1978 Canadian Reliability Symposium, October 19-20, 1978, Ottawa, Ontario, Canada. Chairman: Nicholas Balke. (*Microelectronics and Reliability*: An International Journal and World Abstracting Service, Vol. 19, Nos. 1/2, 1979.) Pp. 1-168. (Oxford and New York: Pergamon Press, 1979.) £20. [196

Catalogue of the Ocean Bottom Deposits Collection in the British Museum (Natural History). Part 1: Atlantic and Arctic Oceans including the European Seas. By H. A. Buckley, C. J. Elliott, Nancy M. Graham, L. R. Johnson, D. R. C. Kempe and D. B. Williams. On Microfiche. Pp. 12. (London: British Museum (Natural History), 1979.) £12. [206

Countryside Commission. Recreational Paths. Pp. 24. (Cheltenham: Countryside Commission, John Dower House, Crescent Place, 1979.) *gratis*. [206

Cancer Research Campaign, (British Empire Cancer Campaign for Research). 56th Annual Report, 1978. Pp. 171. (London: Cancer Research Campaign, 2 Carlton House Terrace, SW1, 1979.) [206

Grassland Research Institute. Annual Report 1978. Pp. xii + 133. (Hurley, Maidenhead: The Grassland Research Institute, 1979.) £2.50 [216

Health and Safety Executive. Safety During Semicontinuous and Continuous Casting of Copper and Copper-Based Alloys. Pp. 18. 80p net. Molton Metal and Water Explosions. Pp. 24. £1 net. (Joint Standing Committee on Health, Safety and Welfare in Foundries.) (London: HMSO, 1979.) [216

— July

Royal Observatory, Edinburgh. Annual Report, 1977-1978. Pp. 54. (Edinburgh: Royal Observatory, 1979.) [97

Annual Report of the Health Service Commissioner 1978-79. Pp. 33. (London: HMSO, 1979.) 80p net. [97

United Kingdom Atomic Energy Authority. Radioactive Fallout in Air and Rain: Results to end of 1978. By R.S. Cambray, Miss E.M.R. Fisher, K. Playford, J.D. Eakins and D.H. Peirson. Pp. 43. (London: HMSO, 1979.) £1.50 net. [97

The Macaulay Institute for Soil Research. 1977-1978 Annual Report, No. 48. Pp. 139. (Craigiebuckler, Aberdeen: The Macaulay Institute for Soil Research, 1979.) [97

John Innes Institute. Sixty Ninth Annual Report, 1978. Pp. 151. (Norwich: John Innes Institute, 1979.) £3. [97

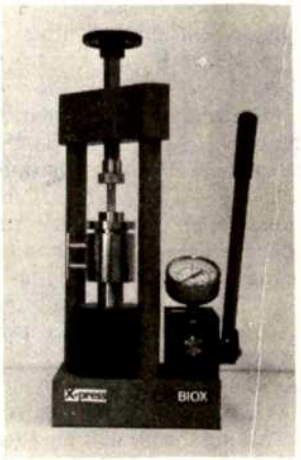
List of University Institutions in the Commonwealth. 23rd edition. Pp.37. (London: The Association of Commonwealth Universities, 1979.) [97

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The British Industrial Biological Research Association. Annual Report, 1978. Pp.35. (Carshalton, Surrey: BIBRA, BIBRA, 1979.) [97

newly on the market

Conversion master. The Decimo Goldfinger conversion master gives instant conversion of measures of length, area, volume and temperature into and from their metric equivalents. This new liquid crystal display model, which has all the standard calculator functions, including full 'live' memory, comes complete with its own wallet.
Circle No. 84 on Reader Enquiry Card.



The Life Science hydraulic press

Hydraulic press for cell disintegration. Life Science Laboratories have introduced a 16-ton hydraulic press for use with the Biox X-Press cell disintegration equipment. This unit has been modified for use with the 5 and 25 ml X-Press models. The X-Press system operates at -20°C and gurantees a high percentage breakdown of even the toughest cell walls. The low temperature and method of disruption protect the cell contents from damage and deterioration, while the compact, lightweight design of the press enables the equipment to be used anywhere.
Circle No. 85 on Reader Enquiry Card.

Protein Assay. Bio-Rad Laboratories have introduced the protein assay kit II, which is a 5-min, single reagent, dye-binding technique that is easily automated and has none of the interference characteristics inherent in the Lowry and Biuret tests. The kit contains bovine plasma albumin as a standard reference protein. Protein assay kit I has not changed and uses bovine γ globulin as a protein reference. The kits are available complete or as separate component reagent or standards.
Circle No. 86 on Reader Enquiry Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Enquiry Card bound inside the cover.

Blood separation. Histopaque from Sigma London is a new cost-saving multiple pack (size 12 × 100 ml) of Histopaque-1077. Histopaque-1077 is an inexpensive reagent that readily effects the separation of mononuclear leukocytes from whole blood without significant cell damage. It is packed in 100-ml bottles. The reagent is quite stable and may be stored in a refrigerator for at least 12 months without deterioration.
Circle No. 87 on Reader Enquiry Card.

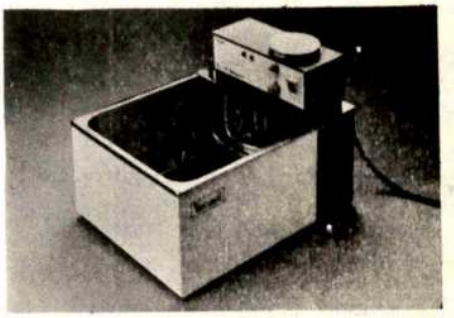
Microscope slide engraver. Temtool have introduced a diamond engraver for marking glass. The engraver is powered by a 12-V mains transformer and is automatically switched on when inclined to a writing position. A rotating diamond tip is held in a pen-like drive unit made from aluminium — all electrical and moving parts are sealed from solvent attack.
Circle No. 88 on Reader Enquiry Card.

Blood storage refrigerator. Forma Scientific has introduced a new line of giant capacity blood storage refrigerators designed for blood bank use. The 48 cu. ft blood bank unit protects up to 660 plastic bags of whole blood at a precise +4°C operating temperature. The exclusive Forma 'ringback' alarm is designed with normal and standby modes to monitor normal operation, pulldown and recovery. All models feature stainless steel roll-out drawers, automatic defrost and internal condensate removal and a pressure-writing temperature recorder. Standard dimensions are 54.75″ wide × 35.5″ deep × 83.125″ high.
Circle No. 89 on Reader Enquiry Card.

Automated Stat/routine analyser. Beckman. The Astra 8 Analyzer is a free-standing, computer-controlled chemistry analyser that accommodates eight analytical modules. In a common six-module configuration, the system gives glucose, BUN, creatinine, sodium, potassium, chloride and CO_2 determinations, plus calculated osmolality, BUN/creatinine ratio and anion gap values. All the necessary controls are clearly identified for easy instrument operation. Any or all of the individual tests for each patient sample or up to two trays — 80 samples — can be selected at one time for walk-away operation. Up to 80 samples can be run per hour by selecting individual tests for each sample. Throughput for routine, or run-all, determinations is typically greater than 60 samples per hour.
Circle No. 90 on Reader Enquiry Card.

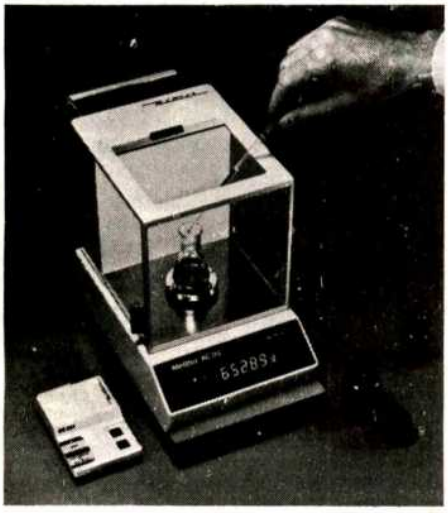
Fibre optic light detector. Life Science Laboratories have recently added a fibre optic light detector to their QSM 2500 Quanta spectrometer. The QSM is a portable instrument capable of scanning the visible wavelengths and recording the number of quantum units per m^2 per s per nm. The flexible fibre optic head enables measurement in a restricted space. The complete instrument is battery powered and a new optional field housing makes carrying easy and ensures protection from mud and moisture. Further options include a mains power supply, an underwater housing for marine applications and an integrator module capable of measuring the total energy falling over all or part of the spectrum.
Circle No. 91 on Reader Enquiry Card.

Constant temperature baths. Techne. Two new series of constant temperature baths are now available from Techne. The TE Series is the standard choice for the laboratory, while the TU Series is the ultimate for very fine temperature control. They cover temperature ranges from -40°C to +180°C. The TE Series is based on the recently introduced Tempette thermoregulator. The baths have a temperature stability of ± 0.02°C, offer fine control and are easy to use. The TU Series is based on the Tempunit thermoregulator — features include three preset controls and temperature stability of ± 0.005°C.
Circle No. 92 on Reader Enquiry Card.


The Techne EU Series

Luminometer. The Packard Instrument Company announces the introduction of the Pico-Lite, a microprocesor-controlled luminometer which measures light produced in bio- and chemiluminescent reactions. The Pico-Lite determines ATP by using the luciferin–luciferase system of the firefly. The instrument can be operated at temperatures above or below ambient and it has a detector system that can store up to six samples.
Circle No. 93 on Reader Enquiry Card.

Top-loading balance. Mettler. The AC88 top-loading balance from Mettler is a balance capable of performing complicated analytical weighing by the simple touch of a single control bar, also featuring the Mettler Deltarange, which gives an extremely accurate fine range. This additional facility allows up to 8 g to be weighed to 0.1 mg anywhere within the overall range of 80 g. If the fine range is exceeded during weighing the balance can be tared by pressing the control bar, which brings the Deltarange back into play and weighing starts again. The AC88 is suitable for individual as well as serial weighings and as a prescription balance.
Circle No. 94 on Reader Enquiry Card.



The Mettler AC88

Automated gel permeation chromatography system. An automated data reduction system is available from Perkin-Elmer. Offered as a complete hardware and software package or separately, the gel permeation chromatography system includes: Series 1 LC pump, LC-100 column oven, a selection of GPC columns, sigma 10 data station, gel permeation chromatography software and complete user instructions. The new system automates manual procedures associated with gel permeation chromatography, and assures instant, precise results.
Circle No. 95 on Reader Enquiry Card.

Analytical balance. The Torsion Balance Company has introduced the Torbal SEA-1, a compact analytical balance specifically designed for university and school laboratory use. It features 160-g weighing capacity, 0.1-1 mg accuracy, electronic null indication and full digital display to 159.9999 g. The new balance contains several design and control features that make it especially suited for use in educational applications. Unknown weight quantities can be determined simply by turning control knobs until the needle of the null indicator rests at zero. The weight — shown to 4 decimal places — is displayed in digital form. No vernier or micrometer adjustments are required.
Circle No. 96 on Reader Enquiry Card.

Sound power calculator. B & K Laboratories have announced a new sound power calculator for the determination of sound power levels to the most rigorous national and international standards. The 7507, as it is known, averages sound pressure levels over a selected time interval and adds to them a room correction term to yield the sound power level. The introduction of the adjustable room correction term means that measurements can be performed in both reverberant and anechoic environments. The sound pressure and sound power levels are indicated on the digital display as A-weighted levels or as octave or third-octave band levels over the frequency range of 100-10 kHz, and if required these results can be read out via the digital or analogue output. The 7507 has built-in interface for IEC standard bus, allowing output in either 'addressable' or 'talk only' modes.
Circle No. 97 on Reader Enquiry Card.

Adjustable flatness polishing plates. Logitech have introduced a range of lapping and polishing plates incorporating a wholly new design feature which allows plate curvature to be adjusted by simply turning a screw. The Logitech plates substantially reduce initial conditioning time and allow subsequent curvature modifications to be performed in seconds. The plates are available in a variety of materials including cast iron, tin, lead, solder and wax. They are available in 200 mm and 300 mm diameter sizes to fit Logitech PM2, PM2A and LP30 machines.
Circle No. 98 on Reader Enquiry Card.

Microprotein Rapid Stat Kit. Pierce. The new Pierce Microprotein Rapid Stat Kit is the first commercially available test for the accurate determination of low levels of protein in biological fluids. The reagent uses the highly protein-sensitive dye Coomassie brilliant blue (CBB) G250. The dye binds to the protein causing a change in its optical properties. The dye–protein complex absorbs strongly at 610 nm, where it is colorimetrically evaluated. The CBB G250 will bind with almost the same affinity to the wide variety of proteins found in serum, CSF or urine. Advantages of the kit include no precipitating or centrifugation needed; single, stable colorimetric reagent; quantitative results obtained in 20 min at 37° C; can measure undiluted CSF protein to 100 mg dl^{-1} and undiluted urine protein to 10 mg dl^{-1}.
Circle No. 99 on Reader Enquiry Card.

Disposable dust respirator. Racal Safety. Dustopper, from Racal, is a disposable dust respirator which can be worn with comfort by workers in dusty environments. The respirator can be folded flat and put in a pocket when not in use, and is easily fitted over a wearer's mouth and nose.
Circle No. 100 on Reader Enquiry Card.

Dispersion-consolidated platinum. Degussa. The high strength values of the dispersion-consolidated platinum available from Degussa remain unchanged at temperatures of over 1,000°C, and allow considerable savings in comparison with pure platinum because of the longer service life of the components or the reduction of weight while retaining dimensional stability. The high thermal resistance and creep strength of dispersion-consolidated platinum at high temperatures are due to the small amounts of finely dispersed zirconium oxide that are added to the metal to stabilise the platinum matrix. The ductility of the material allows hot or cold shaping by conventional methods. Even machining of the material, which has the same corrosion behaviour as pure platinum, presents no difficulties.
Circle No. 101 on Reader Enquiry Card.

Bichromatic analyser. The Abbott VP is a fast and easy to use automated chemistry system featuring advanced microprocessor technology. Procedures are automatically programmed with capability for easy operator changes or reprogramming of additional user defined methods. Maximum throughput is 465 substrate tests h^{-1} or 310 rate tests h^{-1}. Average instrument analysis time is rapid with an average of 5.5 min for 30 samples of an individual test. Operator involvement is minimal, dispense system washing and priming are automatic, test changeover rapid. Average sample requirement is less than 10 μl per test with a reagent volume of only 250 μl per test.
Circle No. 102 on Reader Enquiry Card.



The Abbott VP bichromatic analyser

X-Ray spectroscopy system. Tracor Northern. The TN-2000 X-ray spectroscopy system from Tracor Northern performs quantitative analysis for elements $z = 10$ to 99. Programmable operation is offered with a number of field-proven data reduction and analysis routines. The system is equipped with an LSI-11/2 microprocessor and a PDP-11 type miniprocessor can be substituted for greater CPU power. Available options for system upgrade include floppy disc, mag tape, printers, plotters and teletype.
Circle No. 103 on Reader Enquiry Card.

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APPOINTMENTS VACANT

INSTITUTE OF CANCER RESEARCH

A GRADUATE

who has had several years experience of computing or completed a project with extensive computer usage is required to work on real time data processing on a PDP 11/70 using MUMPS.

The post would be for 2½ years in the first instance and suitable for someone completing a higher degree this year. It would be established on the Institute's Scientific Scale II in the range £4,261 to £5,591 p.a. plus London Allowance of £502 p.a. according to age and experience.

The successful applicant would work in close co-operation with the staff of the Royal Marsden Hospital both at Fulham Road and at Sutton, Surrey.

Applications in duplicate with the names of two referees should be sent to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 300/G/12. 2856(A)

THE MEDICAL COLLEGE OF WISCONSIN, INC.

BIOCHEMIST OR CELL BIOLOGIST

I am looking for 2 imaginative scientists with interest and enthusiasm to study interaction of interferon with cell membranes. This project is funded by a three-year grant from NIH and provides for two positions, one at a postdoctoral level (appr. $13,000 per annum), the other at a lower level, which will pay somewhat less. Thus, persons with PhD, MD, MSc or equivalent training or experience will be considered. The College provides fringe benefits and the atmosphere in a diversified biochemistry department is stimulating and cordial. We recently moved into brand-new and well-equipped facilities.

Please send enquiries to Dr Helmut Ankel, Department of Biochemistry, Medical College of Wisconsin, PO Box 26509, Milwaukee, Wisconsin 53226. W288(A)

UNIVERSITY OF LONDON KING'S COLLEGE

DEPARTMENT OF ANATOMY

Applications are invited for an

MRC POSTDOCTORAL RESEARCH ASSISTANT

in

DEVELOPMENTAL NEUROBIOLOGY

to work on the development of limb innervation in the chick embryo. Experience in electrophysiology and/or developmental biology would be an advantage.

The post will be available from 1st October 1979 for up to 3 years, salary in the range £4,333 to £4,910 p.a. plus £502 p.a. London Allowance.

Applications, together with c.v. and names of two referees, to: Dr J.H. Lewis, Department of Anatomy, King's College, Strand, London WC2R 2LS as soon as possible, quoting 191689/ 2858(A)

UNIVERSITY OF LEICESTER

DEPARTMENT OF GENETICS

POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for a post-doctoral research appointment supported by the Medical Research Council to study plasmid controlled iron-uptake in *Escherichia coli* as part of a group working on virulence determinants of bacterial strains pathogenic for man. The position is available from 1st October 1979, and is for up to 3 years. The salary will be on the Research Support Scale 1A, plus superannuation.

Applications, with curriculum vitae, and the names of two referees, should be addressed to Dr P.H. Williams, Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH. 2879(A)

THE AUSTRALIAN NATIONAL UNIVERSITY

Research School of Biological Sciences

DEPARTMENT OF POPULATION BIOLOGY

RESEARCH FELLOW

Applications are invited from suitably qualified persons for appointment to the above-mentioned position.

The Department is expanding the area of molecular cyto-genetics, specifically genome structure and evolution in eukaryotes. Current interests encompass genetic and molecular studies on DROSOPHILA and rodent genomes, with special emphasis on highly repeated DNA. Applicants should have postdoctoral experience, be familiar with chromosome manipulation in DROSOPHILA and have a desire to develop a programme which includes molecular and genetic approaches to eukaryotic genome structure.

Closing date 14 September 1979.

Salary will be in accordance with qualifications and experience within the range $A16,291 to $21,265 per annum. Present exchange rates are $A1 = 50p = $US1.12.

Term of appointment: three years in the first instance with the possibility of extension to five years.

Other conditions: reasonable appointment expenses are paid. Superannuation benefits are available for applicants who are eligible to contribute. Assistance with finding accommodation is provided for an appointee from outside Canberra. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should first obtain further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 2837(A)

UNIVERSITY OF AUCKLAND

New Zealand

GEOGRAPHY TEMPORARY FULL-TIME LECTURESHIP

Closing date 28 September 1979.

Applications are invited from specialists in any branch of physical geography, although an interest in instrumentation, laboratory and field techniques would be an advantage. The appointment is available for three years from 1 February, 1980 to 31 January 1983.

GEOTHERMAL INSTITUTE SENIOR LECTURESHIP/ LECTURESHIP

Closing date 14 September 1979.

Applications are invited from Professional Engineers. Applicants must have a strong academic background in Chemical, Civil or Mechanical Engineering, or in Engineering Science, knowledge and experience of one or more aspects of geothermal technology; and be prepared to lecture on those aspects of geothermal technology in which they have specialist knowledge.

Commencing salaries in accordance with qualifications and experience will be established within the range for Lecturers or Senior Lecturers, at present NZ$11,529 to $16,780 per annum. At present all salaries are supplemented by a General Wage Order of NZ$365 per annum. Conditions of Appointment and Method of Application are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications, in accordance with 'Method of Application', should be forwarded as soon as possible but not later than the closing dates stated. 2839(A)

HEAD OF IMMUNOLOGY SECTION

Applications are invited for the post of Scientific Head of a new Section of Immunology in the Institute. The main functions of the Institute are the standardization and statutory control of biological substances used in human medicine, work which is well supported by research. It has been decided to form this new Section to assume responsibility for standardization, control and research in immunology, and to provide immunological support to the other Institute departments. The Section will also incorporate the Institute's existing work in the field of allergens.

The post calls for the ability to undertake independent and collaborative research and to lead a small group of staff. Applicants should be experienced in laboratory immunology and research.

Salary will depend on experience and age and is likely to be in the Senior or Special Appointments Grades.

Further details of the post are available from the Personnel Officer, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3. Telephone 01-435 2232 Extension 200, quoting reference IM/208.

NIBSC

National Institute for Biological Standards and Control

2822(A)

Ministry of Agriculture, Fisheries and Food, Food Science Division

CHEMIST/BIOCHEMIST

London

. . . to join a multi-disciplinary team which provides a wide range of scientific advice to those responsible for legislation on all aspects of the manufacture, composition and use of food additives. The post involves work on colouring matters, clouding agents and enzymes used in food preparation, and includes close liaison with industry and other Government Departments which have responsibilities for food composition and safety.

The successful candidate will also participate in the Scientific Secretariat of the UK Food Additives and Contaminants Committee and assist in the implementation of the Committee's recommendations by revised or new legislation. The work includes preparing papers and briefs for use at international meetings and, after training, attending internal meetings especially in relation to the negotiations of EEC Directives on food additives. No laboratory work is involved with this post.

Candidates (normally aged under 32) should have a good honours degree in chemistry, biochemistry or a closely related science and should have at least 4 years' postgraduate experience. A working knowledge of food technology or the chemical manufacturing industry would be an advantage. Appointment as Senior Scientific Officer. *Ref SB/39/AE.*

CHEMIST

London

. . . to undertake the chemical analysis of such foods as flour, dried skim-milk, sugar, fat and biscuits both initially and during storage. Tests include moisture, protein, reducing sugars, fat content and fat rancidity parameters. An important aspect of the work will be the introduction of modern instrumental techniques and the development and application of new methods.

Candidates (normally aged under 27) should have a degree or equivalent in chemistry or food science. An interest in analysis would be an advantage. Appointment as Scientific Officer. *Ref SB/40/AE.*

CHEMIST

Norwich

. . . to join a team studying both potentially hazardous and nutritionally important trace elements in food. Work includes special surveys, analytical method evaluation and development, collaborative trials and metal speciation studies in foods. Atomic absorption and plasma emission spectrometry techniques are used extensively together with polarography, anodic stripping voltametry and gas and high pressure liquid-chromatography.

Candidates (normally aged under 27) should have an honours degree in chemistry. Some analytical laboratory experience would be an advantage. Appointment as Scientific Officer. *Ref SB/41/AE.*

Salary: Senior Scientific Officer £6,400 to £8,385; Scientific Officer £3,810 to £5,605; (to become £6,855 to £9,225 and £4,115 to £6,010 respectively from 1.1.80). Salary £524 less in Norwich. Starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

These posts have been exempted from the Government's ban on recruitment

For further details and an application form (to be returned by 3 September 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 6k8551 (answering service operates outside office hours). *Please quote appropriate reference.* 2878(A)

ALL THESE POSTS ARE OPEN TO MEN AND WOMEN.

Science Group
CIVIL SERVICE

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university college o swansea

SENIOR RESEARCH ASSISTANT/ RESEARCH ASSISTANT

Applications are invited from suitab qualified candidates with experience biochemistry and microbial genetics for tl vacancies of **Senior Research Assistant a Research Assistant** in the **Department Genetics.** The successful candidates will jo an SRC funded project on DNA repa activity in yeast cells. Applications for tl Senior Research Assistant should have a Ph degree or be about to submit for such degree.

The appointments will be for two yea from 1 October, 1979 and will be on tl following salary scales.

Senior Research Assistant: Commenci salary up to £4,910 per annum plu USS/USDPS benefits.

Research Assistant: Commencing sala £3,775 per annum plus USS/USDF benefits.

Informal enquiries may be made to Dr J Parry in the Department of Genetics, b further particulars and application forn (two copies) may be obtained from tl Personnel Officer, University College Swansea, Singleton Park, Swansea, SA 8PP, to whom they should be returned l *1 September, 1979.* 2871(A)

UNIVERSITY OF SOUTHAMPTON

LECTURER IN CRYOGENICS

Applications are invited for the post

LECTURER

in the newly-formed Cryogenics Grou in the Faculty of Engineering ar Applied Science.

Candidates should have an acti research interest in the application low temperatures and wide experien with cryogenic fluids and associate instrumentation. The perso appointed will be expected to intera effectively with the group and play full part in the postgraduate trainir activities.

Salary scale:- £4,333 to £8,992 p annum (under review). The initi salary will depend on qualificatio and experience.

Further particulars may be obtaine form D.A.S. Copland, Th University, Southampton SO9 5NH whom applications (7 copies fro U.K. applicants) should be sent n later than 17 September 1979. Plea quote reference 215/A./Na.2876(A)

THE UNIVERSITY OF MELBOURNE

CHAIR OF VETERINARY BIOLOGY

Applications are invited for appointment to the Chair of Veterinary Biology. The Chair has been established to give academic leadership in the Department of Veterinary Preclinical Sciences and the University will consider applications from persons appropriately qualified in Veterinary Anatomy, Physiology or Pharmacology.

Salary: $A34,119 per annum.

Further information, including details of application procedure, superannuation, travel and removal expenses, housing assistance, and conditions of appointment is available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on *26 October 1979.* 2860(A)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF OBSTETRICS & GYNAECOLOGY

Applications are invited for the post of

RESEARCH ASSISTANT

to join a group working on the renin-angiotensin system in pregnancy. Candidates should hold a good honours degree in Biochemistry, Physiology or a related subject. The successful candidate will be expected to register for a PhD degree.

The appointment is for 3 years at an initial salary of £3,775 (£4,333 if 26 or over).

Applications, with curriculum vitae and names of three referees, should be sent by 3rd September 1979 to Dr F. Broughton Pipkin, Department of Obstetrics and Gynaecology, City Hospital, Hucknall Road, Nottingham. 2855(A)

UNIVERSITY OF OTTAWA

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANTS

Two recent PhD graduates required to join research group working on plant protein utilization. An enzymologist with experience preferably in cereal chemistry or plant physiology is needed for characterization of lipase in high-protein oats during maturation and storage. A microchemist experienced in stain development is needed to study localization of phytate, glucosinolates, etc. in rapeseed. Research involves collaboration with the food industry and the Food Research Institute of Agriculture Canada. Salary minimum $12,200.

Please apply to Dr I Altosaar, Biochemistry Dept, University of Ottawa, 40 Somerset Street East, Ottawa, Ontario K1N 6N5 Canada. W287(A)

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF ZOOLOGY

Applications are invited for the post

POSTDOCTORAL RESEARCH ASSISTANT

(Molecular Biology)

to work in collaboration wi Dr J Sommerville on an investigation of t packaging and processing of RNA tra scripts in eukaryote cells. Applicants shou have a training in biochemistry or micr biology. Experience in nucleic acid biochei istry or the electron microscopy of nucle acids would be an advantage. The appoir ment is for up to 3 years, starting date arrangements, and is supported by the SR Starting salary within range 1A £4,333 £4,910 (under review), plus FSSU/US Informal enquiries may be made Dr J Sommerville (Tel: St Andrews 72823

Applications (two copies preferably typescript), with the names of two refere should be lodged by **12th September 19** with the Establishments Officer, Tl University, College Gate, St Andrews, Fi KY16 9AJ. 2832(A)

British Museum (Natural History)

Editorial Assistant



The Museum is replacing the existing exhibition by a completely new scheme designed to present an integrated view of modern biology. The Editorial Assistant, as a member of a team, will collaborate with the biologists and designers who are devising the exhibits to ensure that all exhibition labels, texts, commentaries and associated publications are written in simple attractive everyday language.

Candidates should normally be aged under 27 and have a degree or equivalent in a biological science. They should also have a sound biological background. A genuine interest in communicating scientific ideas to others would be advantageous.

Appointment will be as Scientific Officer. Starting salary (according to qualifications and experience) within the range £3,810 to £5,605 (to become £4,115 to £6,010 from 1.1.80).

For further information and an application form (to be returned by 4 September 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). **Please quote ref: SB/59/DK.** 2844(A)

UNIVERSITY OF BIRMINGHAM

HEMICAL ENGINEERING RESEARCH FELLOW

ıplications are invited for a Research llow in Biological fouling film formation d removal, to be a member of a team 'estigating the biological fouling of heat :hanger surfaces, for a period of two ars.

This involves the mechanisms of laying wn, adhesion and removal of biological uling films on surfaces, and the crobiology of the deposits. The Fellow ll also act as an advisor on microbiologist a biochemist/biochemical engineer with sound understanding of the ndamentals of microbiology.

The Fellow will be based at AERE ırwell, where the Heat Transfer and uid Flow Service is sponsoring an overall oject on the fouling of heat exchanger ınt. The appointment is subject to the rmal security arrangements presently in rce at Harwell.

Salary will be in the RF1A range £4,333 £7,521, plus superannuation. Maximum ırting salary will be £5,777. Starting date as soon as can be arranged.

Further particulars are available from e Assistant Registrar, Science and ıgineering Registry, University of rmingham, PO Box 363, Birmingham 5 2TT, to whom applications including ll curriculum vitae (3 copies), and naming ree referees, should be sent by Friday th August 1979. Please quote ref NT9.

2820(A)

UNIVERSITY OF HEIDELBERG

EPARTMENT OF NEUROBIOLOGY

research worker with experience in ctron microscopy, preferentially of the rvous system, is required for supervision the newly established electron croscopy unit in the department. ɔplicants should be interested in velopmental neurobiology and willing to rticipate in ongoing immunological, cell ological and electrophysiological ɔjects. The level of the position is flexible pending on background and alifications.

Applications with curriculum vitae and mes and addresses of three referees ɔuld be sent to Dr M Schachner, Dept of :urobiology, Heidelberg University, Im :uenheimer Feld 347, D-6900 :idelberg, West Germany. W277(A)

THE QUEEN'S UNIVERSITY OF BELFAST

RESEARCH ASSISTANT

MARINE BIOLOGY

Research Assistant required to continue a biological study of the shore in the vicinity of the new oil-fired power station at Kilroot commissioned by the Northern Ireland Electricity Service. The post to commence as soon as possible is tenable for approximately three years. Candidates must have a degree or equivalent qualification in an appropriate biological subject. Commencing salary in the range £3,690 to £4,233 per annum (under review).

Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland, to whom application should be made enclosing a full curriculum vitae and the names and addresses of two referees. Closing date 15th August, 1979. 2825(A)

UNIVERSITY OF EDINBURGH DEPARTMENT OF PATHOLOGY

RESEARCH FELLOW IN RESPIRATORY PATHOLOGY

A post-doctoral Research Fellow with a biological background is required to work on the structural and functional abnormalities of the lung in chronic bronchitis. Previous experience of histology and quantitation morphology desirable.

Salary from £4,232 pa. Starting date — October 1st, 1979.

For further details contact Dr D Lamb, Department of Pathology, University of Edinburgh, Teviot Place, Edinburgh, EH8 9AG (031 667 1011 ext 2262/2275). The closing date for applications is 28th August, 1979. Please quote reference 5044.

2834(A)

SCIENTIFIC/MEDICAL EDITOR

Medical foundation in central London needs an editor to prepare the proceedings of biomedical conferences for publication. Previous experience of manuscript editing desirable. A good honours degree in physiology/biochemistry/zoology (or a combination of these) is essential, as is a flair for language, an eye for relevant detail and the ability to deal tactfully with authors. Pleasant working conditions, with own office, free lunch, four weeks' holiday. Salary in scale £4,370 to £6,141. Age 25-35.

Please write, with full c.v., to
Senior Editor, Ciba Foundation, 41 Portland Place, London W1N 4BN

2863(A)

LUDWIG INSTITUTE FOR CANCER RESEARCH (LONDON BRANCH)

(in association with the Royal Marsden Hospital and the Institute of Cancer Research, Sutton, Surrey)

RESEARCH OFFICER

The Ludwig Institute for Cancer Research (established in Zurich in 1971) maintains branches located in England, Switzerland, Australia and Belgium. The London Branch is located at Sutton, Surrey and, in association with the Royal Marsden Hospital and Institute of Cancer Research, is engaged in a research programme focussed particularly on aspects of breast disease, both clinical and fundamental.

The Unit of Molecular Biology, now under active formation, will undertake basic research into breast cancer and requires a RESEARCH OFFICER who will be involved in the maintenance of cultured cell lines and in their biochemical characterisation.

Candidates for this appointment should have had two years or more post qualification relevant experience of tissue culture and biochemical techniques to qualify for appointment within the salary scale £3,582 to £4,902 plus LW. Applications from less qualified candidates who nevertheless possess suitable experience will also be considered for appointment within the Technician grade £3,261 to £4,680.

Apply with full particulars and names of two referees to The Secretary, London Branch, Ludwig Institute for Cancer Research, Clifton Avenue, Sutton, Surrey SM2 5PX.

2809(A)

Vacancies in Drug Metabolism

The following vacancies exist in the Department of Drug Metabolism and Pharmacokinetics at the Hoechst Pharmaceuticals Research Laboratories in Milton Keynes.

Senior Scientist

The successful candidate will lead a small team of scientific and technical staff investigating the metabolic fate of candidate drug compounds in animals and man.

The work for which a Ph.D. in Biochemistry, Pharmacology, Chemistry or an associated discipline is essential, would require a scientist with several years experience in this field, preferably in industry.

Scientist

The position would be ideally suited to a Ph.D. who has just graduated in one of the above mentioned disciplines, or Graduate with some experience within the area of drug metabolism.

Technician

A vacancy also exists for a Technician who would assist a Ph.D. Scientist in metabolic experiments. Minimum qualifications would be HNC, 'A' levels or equivalent. Further education would be encouraged. Graduates would also be considered for the position.

All positions carry first-rate salaries. Benefits include at least four weeks holiday a year, flexible working hours, generous pension scheme and private health scheme. Relocation expenses would be paid where appropriate.

Please write or telephone for an application form to:
Sheila Matthews, Personnel Department, Hoechst UK Limited, Walton Manor, Walton, Milton Keynes, Bucks.
Tel: Pineham 5068

Hoechst

2813(A)

THE OPEN UNIVERSITY

FACULTY OF SCIENCE

POST-DOCTORAL RESEARCH FELLOW IN ISOTOPE GEOLOGY (TEMPORARY)

Applications are invited for the above pos from candidates with an honours degree, doctorate, and several years experience i operating mass spectrometers, preferably i the geological sciences. In cases of prove expertise applications will be considere from candidates without a higher degree The Department of Earth Sciences ha recently purchased the latest VG solid sourc mass spectrometer (Isomass 54E) which wi be commissioned shortly when work on th isotope laboratories is complete. Th successful applicant will work closely with D C J Hawkesworth but will be encouraged t initiate new projects, develop techniques an be responsible for the day to day running o the isotope laboratories.

Appointment will be from the earlies convenient date for a period of three years i the first instance, on the salary scale £4,23 to £7,145 pa.

Further information can be obtained fron The Secretary, Department of Eart Sciences, Open University, telephone 090 63012.

Application forms and further particular are available, by postcard request please from The Recruitment Office (JD3915/1) The Open University, PO Box 75, Walto Hall, Milton Keynes, MK7 6AL or telephon Milton Keynes 63404: there is a 24 hou answering service on 63868.

Closing date for applications: 23rd August 2868(A)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF MICROBIOLOGY

Applications are invited for a post of **RESEARCH FELLOW** in the above Department. Candidate should already possess, or expect to b awarded, a PhD degree. Th appointment will be made for a fixe period of three years with effect from January 1980.

The successful applicant will work under the direction of Dr P.W. Taylo and will take part in a research projec on the biosynthesis of surface antigens associated with the virulence o *Escherichia coli*. The work is financed by the M.R.C. An individual wit experience of continuous culture and/or biochemical techniques for the analysis of Gram-negative cell surface components would be preferred.

Preliminary enquiries may b directed to Dr P.W. Taylor Department of Microbiology, The University, Leeds LS2 9JT (Telephone (0532) 36171 ext. 145).

Starting salary in the range £4,333 t £4,910 on the IA scale for Research and Analogous Staff (£4,333 to £7,521 (under review).

Curiculum vitae and the names o three referees should be sent to the Registrar, The University, Leeds LS2 9JT, quoting reference numbe 96/14/D. Closing date for applications 1 October 1979. 2877(A)

TECHNICIAN/ RESEARCH OFFICER

required by our Medical Oncology Unit at S Bartholomew's Hospital to assit wit cytogenic studies on human leukaemia an lymphoma. Experience of tissue culture an banding techniques an advantage Degree/HNC. Salary range £3,650 to £5,256

For further information and applicatio form telephone or write to Miss S M Hurley Imperial Cancer Research Fund, Lincoln' Inn Fields, WC2 on 242 0200 ext 305, quotin reference 348/79. 2866(A)

PROFESSOR AND CHAIRPERSON, Microbiology. A major state university seeks applicants for head of its Department of Bacteriology and Immunology in the School of Medicine. Selection criteria will include evidence of: leadership ability; broad interests in medical and paramedical teaching at graduate and undergraduate levels; scientific achievement; state/national/international endeavours in the field; and compatability with the philosophy of a well-established, dynamic department. Interested parties should submit enquiries or letters of application with curriculum vitae and references to Wallace A. Clyde Jr, MD, Search Committee Chairperson, Department of Paediatrics, 535 Burnett-Womack Building 229-H, University of North Carolina, Chapel Hill, North Carolina 27514. (An affirmative action/equal opportunity employer). W290(A)

To place your advertisement in these pages Phone: 01-831-6901

University of Riyadh, Saudi Arabia

Medical Faculty

Applications are invited from suitably qualified men and women to fill posts as Professors, Associate Professors, Assistant Professors, Lecturers and Demonstrators in the Preclinical and Clinical Subjects and Related Studies as indicated below for the academic year 1979/1980. Applicants should have higher qualifications and teaching experience at University level.

Anatomy	**Associate Professors; Assistant Professors; Demonstrators**
Physiology	**Professor; Associate Professors; Assistant Professors; Lecturers**
Biochemistry	**Professors; Associate Professors; Assistant Professors; Lecturers; Demonstrators**

Salary Scales: **Monthly in Saudi Ryals (SR7·7=£1, approx.)**

	Basic monthly salary	Annual Increment (Monthly)	Maximum monthly salary
Professors:	**6 595**	**400**	**8 995**
Associate Professors:	**5 835**	**350**	**7 585**
Assistant Professors:	**4 695**	**315**	**7 215**
Lecturers:	**3 385**	**300**	**5 685**
Demonstrators:	**2 785**	**250**	**4 780**

(Associate Professor and Assistant Professor are equivalent to Senior Lecturer and Lecturer respectively in the U.K.)

Consideration will be given to increases of up to 100% on the monthly salaries quoted above for candidates who have been or are holders of academic posts equivalent to those for which they are applying or who have been and are engaged in teaching at University level.

Appointments are normally for one year and renewable.

Clinical Allowance – For those engaged in clinical care of patients in hospital there is an allowance at the rate of 80% of the basic salary. For medically qualified staff not engaged in clinical care of patients there is a *Professional Allowance* at the rate of 30% of basic salary.

Free furnished accommodation or if not available a housing allowance and installation grant. Terminal gratuity, local transport allowance and annual leave passages for incumbent and up to three dependents are paid. There is no income tax in Saudi Arabia and money is freely convertible and transferable.

Application with a comprehensive c.v. and names and addresses of two referees to be sent to: Dr. Hassan Kamel M.R.C.P., The Dean, Medical Faculty, P.O. Box 2925, Riyadh, and at the same time four copies of application and c.v. with names and addresses of referees to be sent to Dr. A.T.G. Johnston, c/o London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, who will send further details on request. **Closing date – 23rd August, 1979. There are no special application forms.**

2836(A)

THE UNIVERSITY OF PAPUA NEW GUINEA

plications are invited for the post of

SENIOR TECHNICAL OFFICER

in the

DEPARTMENT OF BIOLOGY

e appointee will take charge of the partments Teaching Museum which rently has in excess of 18,000 specimens. sponsibilities include the care and velopment of the reference and teaching lection; preserving; mounting and play of biological specimens, as well as wing, modelling and photography. The ointee will also be responsible for some the day to day running of courses. cial emphasis is given on the ability of ervising and training junior technical ff. Applicants should have relevant loma or certificate and several years erience in an educational institution. ary scale: K10,165 pa (£1 sterling £1.61). Family passages; baggage wance; 3 year appointment; 24% tuity of which 2% is taxable; rent-free ommodation; overseas leave; education wances; medical aid scheme.

Detailed applications (2 copies) with riculum vitae and naming 3 referees to sent direct to the Secretary, PO Box 0, University PO, Papua New Guinea 10 September 1979. Applicants resident he UK should also send one copy to the er University Council, 90/91 Tottenham urt Road, London W1P 0DT. Further ails may be obtained from either ress. 2854(A)

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF CHEMISTRY

A RESEARCH ASSISTANT with a background in Chemical Physics or Molecular Biology is required for work in the Regional Workshop of the National Foundation for Cancer Research (NFCR), located in the Chemistry Department at St Andrews.

The research project involves the investigation of the mechanism of carcinogenesis at the molecular level, and will involve both theoretical quantum chemical computations and/or experimental investigations using ESR and other spectroscopic techniques.

Applications are invited from graduates with a good Honours degree in Chemistry or Biochemistry, and preference will be given to those candidates with a strong background in quantum chemistry and an interest in molecular biology. Candidates should preferably have some experience with computer programming.

The appointment is initially for 1 year, from October 1979, at a starting salary of £3,775 per annum (under review).

Applications, with the names of two referees, should be sent as soon as possible, to the Regional Director, Dr C Thomson, Regional Workshop of the NFCR, Department of Chemistry, North Haugh, St Andrews, Fife, KY16 9ST from whom further details may be obtained.

2823(A)

DEAKIN UNIVERSITY GEELONG VICTORIA AUSTRALIA

Lecturer in Microbiology

Applications are invited from persons holding a PhD degree in Microbiology, or a closely related subject, for the post of Lecturer in Microbiology at Deakin University, Geelong, Australia.

Research interest in the microbiology of foods and/or the human environment would be particularly appropriate.

The appointment will be for an initial term of three years with review for continuing tenure after two years service.

Additional information concerning this post is available from the Association of Commonwealth Universities at the address below.

Salary: $A16,291 to $A21,401 per annum.

Applications should quote reference number 79/1/04 and include a full curriculum vitae, the names and addresses of three referees and a recent photograph and should be sent to reach the undersigned by 7 September 1979. Applicants resident in North America, Europe or Africa should send a copy of their application to the Association of Commonwealth Universities (Appointments), 36 Gordon Square, London WC1H 0PF, United Kingdom.

A. L. Pritchard, University Secretary, Deakin University, Victoria 3217, Australia. **2843(A)**

BIOGEN S.A.

a young company engaged in basic and applied research in the field of molecular biology and microbiology, and with the objective of utilizing the potential of genetic engineering for the production of substances of commercial and medical interest in such areas as health care, agriculture and energy, is inviting applications from

SENIOR AND JUNIOR SCIENTISTS

with experience in molecular biology, nucleic acid biochemistry and microbiology,

for its **Research Laboratory in Geneva,**

Switzerland.

Excellent working conditions, in close contact with leading scientists from Europe and the US.

Salary scale: Group Leaders = SFr 60-85,000, Research Associates = SFr 35-55,000.

Applicants with real interest in both basic research and its applications should send their curriculum vitae (with a description of previous experience and a list of publications) to BIOGEN Laboratory, 3 Route de Troinex, 1227 Geneva, Switzerland. W248(A)

THE QUEEN'S UNIVERSITY OF BELFAST

DEPARTMENT OF AGRICULTURAL AND FOOD CHEMISTRY

RESEARCH SCIENTIST

A challenging opportunity exists to study the mass culture o microalgae in the liquid phase o farm wastes under temperate climatic conditions. The three year project will be based or extensive laboratory and controlled environment work and will involve the establishmen and operation of a pilot plant and the evaluation of its performance with emphasis on energy balance and nutrient recovery in the alga biomass.

Candidates should have sound biological background an engineering aptitude. A PhD i bio-engineering/bio-technolog would be preferred. The startin salary is £4,233 pa + USS. Apply giving details of experience et and the names and addresses o two referees to Personnel Officer The Queen's University o Belfast, University Road, Belfas BT7 1NN, UK. Closing dat August 31, 1979. 2824(A)

UNIVERSITY OF SURREY

DEPARTMENT OF PHYSICS

Applications are invited for a Science Research Council supported

POST DOCTORAL RESEARCH FELLOW

to work with Dr R C Johnson on the theory of nuclear reactions involving heavy and light ions. Familiarity with techniques for antisymmetrization effects in nuclear collisions would be an advantage. The position is for one year.

The salary will be £6,066 per annum, on the Research and Analogous Range 1A Scale.

Applications giving the names and addresses of two referees should be sent to the Deputy Secretary (Personnel), University of Surrey, Guildford, Surrey, GU2 5XH not later than 24th August, 1979. 2803(A)

UNIVERSITY OF GLASGOW

DEPARTMENT OF DERMATOLOGY

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the post of Postdoctoral Research Fellow to work on the problem of suppressor and helper cell function in cutaneous malignancy. This post is funded by the Cancer Research Campaign. Background experience of immunological techniques is required. The appointment is for one year commencing at any time after September 1st, 1979. The salary will be commensurate with age and experience to a maximum of £6,350 (scale 1A).

Applications including a full curriculum vitae with the names of 2 referees should be made by August 27th, 1979, to Professor R M MacKie, University Dept of Dermatology, Anderson College Building, 56 Dumbarton Road, Glasgow G11 6NU. 2831(A)

NORTH NOTTINGHAM TEACHING DISTRICT

RESEARCH APPOINTMENT IN BIOMEDICAL STATISTICS

The Renal Unit at Nottingham City Hospital, in collaboration with Professor Adrian Smith, Professor of Mathematical Statistics, The University of Nottingham, wish to appoint a graduate in the mathematical or biological sciences with an interest in medical applications of statistics and computer programming.

The Post will provide varied experience in the analysis of medical data which would be relevant to a variety of potential careers. A suitable graduate would be encouraged to register for a higher degree.

Further information and application forms from the Personnel Department, Valebrook House, Sherwood Hospital, Hucknall Road, Nottingham. Tel: 625459 anytime. 2835(A)

GREENWICH HEALTH DISTRICT GREENWICH DISTRICT HOSPITAL

Vanbrugh Hill, Greenwich SE

RESEARCH ASSISTANT BIOCHEMIST

Applications are invited from rec graduates in Biochemistry to undertak investigation into the effects of ethanol amino acid metabolism in humans animals. The position is for one year in first instance but may be extended to f the basis for a PhD. Salary £3,214 to £3, per annum plus £354 London Weightir

Applications to Dr A D Thoms Consultant Physician, Greenwich Dist Hospital, London SE10 9HE. Applic may visit the department by arrangem Telephone 858 8141. Closing date applications — two weeks after insert date. 2852(A

THE MAX-PLANCK-INSTITUTE of BIOCHEMISTRY

DEPARTMENT OF STRUCTURE RESEARCH I

EXPERIMENTAL PHYSICIST

A vacancy exists for one postdoctoral research scientist. The applicant should have experience in electronics and high vacuum technology (and possibly in electron microscopy). He will be engaged in the development of methods for three-dimensional electron microscopy.

Enquiries and applications should be sent to **Professor Dr. W. Hoppe, Max-Planck-Institut für Biochemie, 8033 Martinsried/Munich, F.R.G.** W281(A)

UNIVERSITY OF SURREY

DEPARTMENT OF ELECTRONIC AND ELECTRICAL ENGINEERING

POSTDOCTORAL RESEARCH FELLOW

required for a one year appointment to work on the ion implantation of evaporated silicon thin films. Applicants should have experience of ion beam technology.

Salary according to experience will be £6,932 per annum on the Research and Analogous Staff Range 1A Scale.

Applications, consisting of a curriculum vitae and names and addresses of two referees, should be sent to Professor K G Stephens, Department of Electronic and Electrical Engineering, University of Surrey, Guildford, Surrey GU2 5XH by 24th August, 1979. 2806(A)

THE ROYAL SOCIETY

The Royal Society requires sci graduate (up to 25 years old) to assist i editorial department, mainly on mathematical & physical journals. Ger aptitude for scientific editorial v required; sound grounding in mathem essential. Progressive salary scale superannuation scheme. Commen salary up to £4,464 per annum (subje revision w.e.f. 1 October, 1979) depen on age and experience.

Applications with names, etc. of referees to the Executive Secretar Carlton House Terrace, London SV 5AG by 31 August, marking the enve 'Personal; W.G.E.'. 2865(A

ASSISTANT SCIENTIFIC SECRETARY

Cancer Research Campaign

(British Empire Cancer Campaign for Research)

Due to the growth in both the extent and scope of its scientific activities the Cancer Research Campaign, one of the largest medical charities with an annual turnover in excess of £7 million, is expanding its Scientific Department and requires an Assistant Scientific Secretary.

The Scientific Department is responsible for the administration of the Campaign's scientific (as distinct from fund-raising) endeavours and the Assistant Scientific Secretary will help the Scientific Secretary and his staff in most aspects of their work, particularly in the processing of grant applications and the subsequent administration of those that are successful: at present the Campaign is supporting over 450 projects in 80 centres. A capable and enthusiastic individual will eventually have the opportunity to undertake most facets of the administration of scientific research.

The successful candidate is likely to be aged between 28-38 with some experience of biomedical research at a postdoctoral level and with a knowledge of science and medicine in the broadest terms, as well as an interest in a career in scientific administration. The appointment will be at the level of Senior Scientific Officer with a salary scale (currently under review) of £5,678 to £7,422 (with superannuation provision), and subsequent to satisfactory completion of a two-year probationary period can be permanent.

Applications by letter, accompanied by a curriculum vitae and the names of three referees, should be sent *marked "Personal"* and not later than 14 September 1979 to:-

The Administrative Officer
Cancer Research Campaign
2 Carlton House Terrace,
London SW1Y 5AR Tel 01-930 8972

2779(A)

THE QUEEN'S UNIVERSITY OF BELFAST

POSTDOCTORAL RESEARCH ASSISTANT

DEPARTMENT OF APPLIED MATHEMATICS AND THEORETICAL PHYSICS

Required to work on the theory of the interaction of intense laser light with atomic systems (particularly multiphoton processes) with Dr S Swain. The basic requirement is a good working knowledge of quantum mechanics; experience in quantum optics would be an advantage but is not essential. The appointment supported by SRC is for three years from 1st October, 1979 or as soon as possible thereafter, with starting salary (under review) up to £4,776 with USS benefits.

Curriculum vitae and the names and addresses of two referees should be sent as soon as possible to the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. 2848(A)

DERBYSHIRE AREA HEALTH AUTHORITY

CENTRAL DERBYSHIRE DISTRICT

DERBY CITY HOSPITAL
Uttoxeter Road,
Derby DE3 3NE

SCIENTIFIC OFFICER

Required to extend established research programme related to immunological assessment of patients with malignant disease. Techniques involved include lymphocyte culture, sub-typing and cell freezing. Candidates should possess a degree or equivalent in biological science.

Salary scale £2,991 to £4,899 (entry point dependent on experience/qualifications).

Application forms from Sector Administrator. Ref. No. 2639.

2846(A)

KINGSTON POLYTECHNIC

SENIOR TECHNICIAN

SCHOOL OF CHEMICAL and PHYSICAL SCIENCES

Required to be responsible for a Life Science Laboratory, which is used for undergraduate teaching, research and project work and duties will include microbiology, plant studies and tissue culture work plus experimental development and investigational work.

Important post requiring someone with initiative and enthusiasm.

Salary T3/4 £4,053 to £4,953 (under review).

Details and application forms from Assistant Registrar (Personnel), Kingston Polytechnic, Penrhyn Road, Kingston upon Thames 01-549 1366. 2874(A)

UNIVERSITY OF QUEENSLAND
Australia

LECTURER IN PHYSIOLOGY

Graduates in Science, Medicine, Dentistry or Veterinary Science. Required — research experience in endocrinology, andrology, renal-cardiovascular, foetal or female reproductive physiology. 1.9.79.

Salary: $A15,786 or $A20,737 pa. Other benefits: opportunities to undertake programs of special studies, superannuation, housing assistance, travelling and removal expenses.

Additional information and application forms are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 2815(A)

Molecular Genetics of Yeast

A Molecular Biologist/Geneticist is required to join an established group involved in the genetic modification of brewing yeasts (saccharomyces cerevisiae and saccharomyces uvarum). The person appointed will work for Britain's largest brewing group, in the research department, located at Burton on Trent, Staffordshire, which undertakes projects on behalf of the companies within Bass Limited.

The successful applicant (male/female) will contribute to the development of genetic systems for brewing yeasts, and in particular to the establishment of recombinant DNA techniques as a method for constructing organisms with specified biochemical or physiological properties. He/she will possess a Ph.D. in a relevant field of biochemistry, genetics or molecular biology, possibly with some post-doctoral experience and will already be conversant with the techniques of recombinant DNA work. The person appointed will be encouraged to maintain and extend liaison with appropriate academic laboratories, and to publish results where appropriate.

Applicants for the position should possess the ability to achieve rapid progress towards agreed objectives.

Salary will be commensurate with age and experience. The company operates a generous life assurance and pension scheme and provides comprehensive recreational facilities.

Detailed applications, including a curriculum vitae and the names of two referees should be sent as soon as possible to:-

Staff Personnel Officer,
Bass Trade Development Limited,
Burton on Trent,
Staffordshire DE14 1JZ.

Bass Limited

2853(A)

CERRO TOLOLO INTER-AMERICAN OBSERVATORY
LA SERENA, CHILE

May have an opening on 1 October, 1979 for an Experimental Physicist or Instrumental Astronomer for the Observatory's Infrared Program. The applicant should have a master's or PhD degree or the equivalent experience. The primary responsibility will be the development of modern infrared observational instrumentation for CTIO's telescopes. Primary place of work will be at the Observatory's headquarters in La Serena, Chile. The minimum salary will be $18,940 per year including an overseas allowance, but the actual salary is negotiable according to experience. Generous fringe benefits include the usual medical insurance coverage and retirement, annual family trips to point of origin and special duty-free importation privileges. W289(A)

COLAISTE NA HOLLSCOILE CORCAIGH
UNIVERSITY COLLEGE CORK
RESEARCH FELLOWSHIP

BIOCHEMICAL GENETIC/BACTERIAL PHYSIOLOGIST

required to work on regulatory aspects of nitrogen fixation in Rhizobium.

Minimum qualifications: First or upper second class honours degree in appropriate subject and at least two years relevant post-qualifying experience. Preference will be given to applicants with a PhD, degree. This post, funded by the National Board for Science and Technology, is tenable for up to 3 years and available from 1 October 1979. The salary range is £3,365 to £4,476 (post-doctoral £3,837 to £4,476) and starting salary will depend on experience (salary under review).

Applications, giving full particulars of qualifications and experience and names of two referees should be sent to:
Dr F. O'Gara, Department of Microbiology, University College, Cork, (Ireland).

2801(A)

LABORATORY ANALYSTS

Vacancies exist in the Chemistry Section of our Central Laboratory at Wincanton, Somerset.

The work involves the analysis of infant foods and nutritional products.

Applicants should be ONC or HNC standard and preferably have some experience in the foods or pharmaceutical industry.

Applications to the Laboratory Manager, Cow and Gate Limited, Wincanton, Somerset.

Cow & Gate

2826(A)

ILLINOIS ANALYTICAL POSTDOCTORAL

Electrohydrodynamic ionization mass spectrometry of surfactants, polymers, and biochemicals. Instrument refinement, interfacing, and characterization. One year, with possible extension. Starting immediately.

Send résumé and three references: Dr Kelsey Cook, School of Chemical Sciences, Urbana, Illinois 61801, USA. An affirmative action/equal opportunity employer. W286(A)

UNIVERSITY OF QUEENSLAND
Australia

SENIOR LECTURER/ LECTURER IN MEDICAL PARASITOLOGY

To teach in the M.B., B.S. Course and in the postgraduate Certificate in Medical Parasitology, and to undertake research. Applicants should have qualifications (preferably a registrable medical degree) and experience in medical parasitology. A pre-clinical loading of $A2,500 is payable to a medical graduate with the possibility of an additional loading dependent on the actual clinical responsibilities undertaken. *17th September, 1979.*

TEMPORARY LECTURER IN PHARMACOLOGY

A Medical, Veterinary Science, Science, Pharmacy or Dental graduate is required, and will be expected to continue research, and participate in teaching and related duties. *24th August, 1979.*

SENIOR TUTOR IN PHARMACOLOGY

Honours, B.Sc or equivalent degree, preferably in Pharmacology is required. Expected to assist in organising practical classes in this subject and to take part in research programme. *24th August, 1979.*

Salaries: Senior Lecturer: $A21,858 to $A25,477. Lecturer and Temporary Lecturer: $A16,291 to $A21,401. Senior Tutor: $A14,041 to $A16,085.

Other benefits: Lecturer and above: Superannuation, housing assistance, travelling and removal expenses and special studies programmes. Temporary Lecturer: Single fare for appointee only to Brisbane. Senior Tutor: Superannuation, housing assistance, travelling and removal expenses.

Additional information and application forms are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF. 2881(A)

BEDFORD COLLEGE
(University of London)

RESEARCH ASSISTANT

required for SRC-funded post in the Department of Chemistry to work on Field Emission of Electrons from Glass. Applicants should be graduate Chemists or Physicists and will be expected to register for a higher degree of the University of London. The appointment is from October 1979 for 3 years. Initial salary up to £4,333 pa plus £502 pa London Allowance.

Applications giving full cv details, date of birth, etc with names and addresses of two referees to Senior Assistant Secretary (Personnel), Regent's Park, London NW1 4NS as soon as possible. 2841(A)

THE AUSTRALIAN NATIONAL UNIVERSITY
FACULTY OF SCIENCE

SENIOR TUTOR OR TUTOR IN BOTANY (TWO POSTS)

Applicants should have a broad knowledge of plant sciences, with specialisation in modern and classical Genetics (Post A) or any other botanical field (Post B). They should have some teaching experience, and demonstrated ability and active interest in pursuing research.

The appointees will be expected and encouraged to conduct research. There are goopd facilities and opportunities for collaboration with other research workers in the Botany Department and in groups located nearby.

The successful applicants will be expected to take up duty by February 1980.

Closing date: 30 September, 1979.

Conditions of Appointment:

Salaries will be in accordance with qualifications and experience within the ranges: Senior Tutor $A14,041 to 16,805; Tutor $A11,579 to 13,633 per annum. Present exchange rates are $A1 — 49p — $US1.27.

Appointment as Tutor and Senior Tutor is on a yearly basis up to maximum of five years.

Reasonable appointment expenses are paid. Travel expenses for Tutors and Senior Tutors however, are paid only for persons appointed from within Australia or New Zealand. Superannuation benefits are available for applicants who are eligible to contribute. Assistance with finding accommodation is provided for an appointee from outside Canberra. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should first obtain further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 2870(A)

UCG Coláiste na hOllscoile Gaillimh

JUNIOR LECTURESHIP IN BIOCHEMISTRY

Applications are invited for the above post. Salary scale £4,852 × (10) — £6,706. Closing date for receipt of applications, 7 September 1979. Further information may be obtained from the registrar. 2827(A)

UNIVERSITY OF THE WEST INDIES
Trinidad

Applications are invited for the post of

RESEARCH FELLOW/ JUNIOR RESEARCH FELLOW (GEOLOGIST)

in the

SEISMIC RESEARCH UNIT

Applicants should preferably have experience in Volcanology. Salary scales 1979/80, Research Fellow TT$27,072 to 39,744 pa, Junior Research Fellow TT$21,960 to 24,072 pa (£1 sterling = TT$5.52). Family passages: FSSU; unfurnished accommodation if available at 10% or furnished at 12½% or housing allowance of 20% of pensionable salary; study and travel grant.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent to the Secretary, UWI, St Augustine, Trinidad as soon as possible. Applicants resident in the UK should also send one copy to the Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 2838(A)

MEDICAL RESEARCH IN LEPROSY

A medical research position is available at the Armauer Hansen Research Institute (AHRI) in Addis Ababa, Ethiopia. AHRI undertakes basic research on the etiology, pathogenesis and immunology of leprosy. The Institute was founded in 1969 and is operated by the Save the Children organizations of Norway and Sweden.

The Institute is well-equipped for research within the areas of microbiology and immunology, and is located at the All Africa Leprosy Rehabilitation and Training Centre (ALERT) which makes it possible to do clinically oriented studies on patients in ALERT's hospital. The Institute staff consists of three senior researchers (of whom one is the Institute Director), two research fellows, two laboratory engineers, two laboratory technicians, four laboratory assistants, an administrator, two secretaries and other personnel — altogether 24 people.

Applicants should have a background in microbiological and/or immunological research, preferably a medical degree, although this is not an absolute requirement. The normal contract period is at least two years.

Further information concerning this position can be obtained from Professor Morten Harboe, Institute for Experimental Medical Research, Ullevål Hospital, Oslo 1, Norway. Telephone 2/60 03 90.

Applications accompanied by a curriculum vitae, a list of publications and personal references should be sent within three weeks after the appearance of this announcement to REDD BARNA (Norwegian Save the Children), Lilletorget 1, Oslo 1, Norway. W280(A)

UNIVERSITY COLLEGE OF NORTH WALES
Bangor

RESEARCH OFFICER IN STATISTICS

Applications are invited for the ab appointment in the College's Faculty Science, to be responsible to the Dear the Faculty through the Chairman of School of Mathematics and Compu Science. The duties of the post primarily to carry out an inspection statistical packages and th documentation, and to assist in teaching of statistics and the provisior statistical advice.

Applicants should hold an honc degree in mathematics, statistics or cogr subjects, and have had appropri experience.

The appointment, to commence 1st October 1979, will be for two years, will be to the lower portion of the Resea and Analogous IA scale: £4,232 to £7, per annum.

Applications (two copies), giving det of qualifications and experience, toget with the names and addresses of th referees, should be sent to the Assist Registrar (Personnel), University Coll of North Wales, Bangor, Gwyn LL57 2DG, from whom further particu may be obtained. 2807(A

UNIVERSITY OF MALAYA

Applications are invited for the post o

LECTURER

in the following Faculties:-

Faculty of Science.

DEPARTMENT OF CHEMISTRY the field of Electron Spin Resona Spectroscopy with special referenc the solid state.

DEPARTMENT OF ZOOLOGY: the field of Animal Physiolo Candidates must possess teaching research experience in the field Vertebrate or General Physiology.

DEAN'S ESTABLISHMENT: fields required are:- Scientific meth scientific thought, logic, history science in relation to Greek scie Chinese science, Islamic science, moc science and philosophy of science.

Qualifications: Candidates sho possess:- (i) at least a Master's degree in required field; *or* (ii) PhD in the requ field.

Salary scale (Approx. Stg. Equi £3,602 x 168, £3,939 to £4,107 x 168, £4, to £4,780 x 280, £6,182 to Review P £6,462 x 336, £6,799 p.a. The commen salary for Lecturers with a PhD degree s be £4,107 p.a.

Further particulars and applicat forms are obtainable from the Associat of Commonwealth Universities (Apr 36 Gordon Square, London WC1H 0F

The closing date for the receipt applications is *8 September 1979.* 2861(A

THE UNIVERSITY OF NEW ENGLAND

Fixed-Term (Three Years) Lectureship

DEPARTMENT OF BIOCHEMISTRY AND NUTRITION

The successful applicant will be required to have a PhD Degree in any field of biochemistry. A demonstrated ability in research is essential, and particular consideration will be given to applicants with expertise in plant, food or microbial biochemistry. The appointee will be required to contribute to undergraduate teaching, as well as research in the Department.

Salary Range: $A16,291 to $A21,401 per annum.

Closing Date: 31st August, 1979.

Position No 404.

Assistance will be given with removal expenses.

Applications, including the names and addresses of three referees and quoting the position number should be sent to the **Staff Officer, University of New England, Armidale, New South Wales, 2351 Australia**, prior to the closing date.

ARMIDALE, N.S.W. 2351. 2842(A)

GERMAN CANCER RESEARCH CENTER

Heidelberg Institute for Immunology and Genetics

(Dr. G. J. Hämmerling)

Postdoctoral and staff positions available.

Research topics: T cell receptors, T-hybridomas, H-2 complex, V-gene expression in T and B cells, monoclonal antibodies against cell surface and V-region determinants, idiotypic interactions in the immune system.

Salary about 25,000-30,000 DM pa according to BAT scale.

For further details please contact German Cancer Research Center, Administration, Im Neuenheimer Feld 280, 6900 Heidelberg.

W284(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL

DEPARTMENT OF IMMUNOLOGY

RESEARCH/SENIOR RESEARCH OFFICER

Required for the remaining 2 years of an MRC funded project concerned with a clinical and experimental study of immunological function and graft versus host disease following bone marrow transplations, undertaken jointly between the Immunology and Haematology Departments at the Royal Postgraduate Medical School, Hammersmith Hospital. The post becomes vacant October 1st 1979, but an overlap of 2-4 weeks with the present holder is desirable and could be arranged.

Starting salary up to £5,777 a year. Applicants should have experience and preferably a higher degree in cellular immunology,and be familiar with tissue culture techniques.

Application forms and further particulars may be obtained from the Royal Postgradualte Medical School, Personnel Office, 150 Du Cane Road, London W12 0HS quoting ref no 21/200/N.

Closing date: 10th September 1979. 2873(A)

LEICESTER POLYTECHNIC

SCHOOL OF CHEMISTRY

SENIOR TECHNICIAN IN MASS SPECTROMETRY

Applications are invited from graduates in a relevant science for this post which will involve operating a new mass spectrometer system comprising a PYE 204 Chromatograph, a VG 16 F Mass Spectrometer and a VG Display Digispec Data System.

In addition to providing a service for the teaching and postgraduate work of the Science Faculty there will be novel and interesting duties in connection with the provision of a service to industrial users on a commercial basis; this will involve extensive liaison with industrial laboratories.

Applicants should have at least three years experience as mass spectroscopists and preferably be competent in the maintenance of the system. Salary £4,461 to £4,761 plus £312 supplement pa.

Application forms available from Staffing Officer, Leicester Polytechnic, PO Box 143, Leicester LE1 9BH. Tel (0533) 551551 ext 2303. 2818(A)

UNIVERSITY OF EAST ANGLIA

Norwich

LECTURER IN CHEMICAL PHYSICS

in the School of Chemical Sciences, tenable from October 1979, for a period — probably 4 years — during which Professor N Sheppard, FRS, is in receipt of an SRC Senior Fellowship. Preference will be given to candidates with experience in the field of photochemistry or reaction kinetics. Salary will initially be within the range £4,333 to £6,066 on the scale £4,333 to £8,992 (under review) plus FSSU/USS benefits.

Applications (one copy only) which should contain a full curriculum vitae including exact date of birth, together with the names and addresses of three persons to whom reference may be made, should be lodged with the Establishment Officer, University of East Anglia, Norwich NR4 7TJ, from whom further particulars may be obtained (telephone 0603 56161 ext 2126) by not later than 31 August 1979. No forms of application are issued. In naming three referees you are requested to give only the names of those who can immediately be approached by the University. 2810(A)

UNIVERSITY OF BRISTOL

School of Chemistry

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant to continue studies on the identification of reaction products from the ozonisation of organic compounds in potable waters. Instrumentation includes HPLC, capillary GLC and computerised GC-MS. Technical assistance is available. The project is financed by the Water Research Centre and relates to the purification of drinking water by ozone.

The appointment is for one year, with the possibility of renewal for a second year. Starting salary within the range £4,232 to £5,048 per annum. (Scale subject to revision with effect from 1st October, 1979).

Please send applications, by 20th August, with details and the names and addresses of two referees, to Dr J R Maxwell, Organic Geochemistry Unit, School of Chemistry, University of Bristol, Bristol BS8 1TS. 2749(A)

UNIVERSITY COLLEGE OF NORTH WALES

Bangor

DEPARTMENT OF MARINE BIOLOGY

RESEARCH ASSISTANTS

Applications are invited for two posts in the College's Marine Science Laboratories, one in the field of Microbiology and the other in Marine Chemistry/Phytoplankton studies.

The successful applicants will have first degrees in appropriate subjects, and will be required to participate in a research project which will include work at sea.

The appointments will be until 30th June 1980 in the first instance, and will be to the Research and Analogous IB scale. Starting salary will be within the range — £3,689 to £4,232 per annum.

Applications (two copies), giving details of qualifications and experience, together with the names and addresses of two referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, by not later than 20th August, 1979. 2804(A)

UNIVERSITY OF MANCHESTER

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSOCIATE AND POSTGRADUATE RESEARCH ASSISTANT

Applications are invited for these posts, which are associated with a project, directed by Professor C.I. Pogson, concerning the regulation of tryptophan hydroxylase activity in brain. Postdoctoral applicants should hold (or anticipate) a PhD in Biochemistry, Neurochemistry or a related discipline; some enzymological experience will be an advantage.

Candidates for the Research Assistantship should have a first degree in Biochemistry. Both posts are tenable for three years from October 1st.

Initial salary ranges: Research Associate, £4,333 p.a. to £4,910 p.a. Research Assistant £3,775 p.a. to £4,333 p.a. Superannuation.

Further particulars and application forms (returnable by August 31st) from the Registrar, University of Manchester, M13 9PL. Quote ref: 186/79/N. 2857(A)

UNIVERSITY COLLEGE OF NORTH WALES

Bangor

DEMONSTRATOR AGRICULTURE

Applications are invited for the above post in the College's Department of Agriculture, with particular responsibilities in Grassland Production. The person appointed will assist in the teaching of undergraduates and will be expected to participate in research on the efficiency of utilisation of grassland, for which it may be possible to register for a higher degree.

Applicants should have recently graduated in Agriculture or a related discipline. Some postgraduate experience in grassland agronomy would be an advantage although not essential.

The appointment, to commence on 1st October 1979, will be within the range: £3,689 to £4,232 per annum, and will be for one year in the first instance, with the possibility of renewal subsequently.

Applications (two copies), giving details of qualifications and experience, together with the names and addresses of three referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, from whom further particulars may be obtained. Closing date 20th August, 1979. 2805(A)

JAMES COOK UNIVERSITY OF NORTH QUEENSLAND

SCHOOL OF BIOLOGICAL SCIENCES

Applications are invited from suitably qualified candidates for enrolment in research doctorate and master degrees within the School of Biological Sciences. The School which is made up of the Departments of Botany, Marine Biology and Zoology is ideally situated for tropical studies. Research currently being carried out includes:

Botany: Plant physiology, taxonomy and ecology, systematics, ecology and physiology of marine and freshwater algae, taxonomy and ecology of fungi.

Marine Biology: Coral reef, estuary, mangrove and inshore ecology, community structure and trophodynamics of plankton and benthos, functional morphology, behaviour and sensory physiology of marine invertebrates, bioaccumulation of metals and pollution.

Zoology: Fisheries and marine mammal biology, estuarine and intertidal ecology, sensory and physiology, invertebrate larval studies, taxonomy of marine animals.

Further details and application forms may be obtained from the Registrar, James Cook University of North Queensland, Post Office, James Cook University, Queensland 4811, Australia. 2816(A)

COLAISTE NA HOLLSCOILE CORCAIGH
UNIVERSITY COLLEGE CORK
RESEARCH ASSISTANT

A Research Assistant is required for 1 October 1979 with postgraduate experience in enzyme purification and characterisation to work on oxygen and hydrogen peroxide metabolism in lactic acid bacteria.

Candidates should preferably have a PhD in microbiology/biochemistry. The appointment is funded by the National Board for Science and Technology of Ireland. Starting salary is £3,837 for a post-doctoral worker (salary under review). The post is renewable for a second year.

Curriculum vitae with names of two referees should be sent to:
Dr S. Condon, Department of Microbiology, University College, Cork, (Ireland).
Closing date for receipt of applications: Friday, 31st August 1979. 2802(A)

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

VACANCIES
RESEARCH FELLOWS

Post-doctoral research positions are available in the Department of Structural Biology. Applicants should be familiar with the techniques used in one or more of the following areas: Mammalian physiology; Electron Microscopy; Histochemistry.

Appointments will be for three years commencing as soon as possible. Details from the Establishment Officer, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. 2840(A)

POSTDOCTORAL POSITIONS IN ADRENERGIC RECEPTOR RESEARCH

Two positions available in September 1, 1979. The first position will involve an investigation into the relationship between PMN ß-receptor concentrations, glucocorticoids and asthma. The second position will involve the large scale purification of cardiac ß-adrenergic receptors.

Applicants should send curriculum vitae and names of three references to:
Dr J. Craig Venter, Department of Pharmacology and Therapeutics, School of Medicine, State University of New York at Buffalo, 127 Farber Hall, Buffalo, NY 14214. An Equal Opportunity/Affirmative Action Employer. W282(A)

DIRECTOR OF RESEARCH COMPUTER FACILITY AND ASSISTANT PROFESSOR OF BIOLOGICAL CHEMISTRY

Responsibility for operation and further development of Research Computer Facility serving multiple users; opportunity to develop new programs; graduate level teaching and research; PhD with relevant experience required. Position available November 1, 1979.

Applications and curriculum vitae are to be sent to Dr Eugene A Davidson, Dept of Biological Chemistry, Box C, The Milton S Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033 USA. Applications will be received until October 31, 1979. An Equal Opportunity/Affirmative Action Employer. W278(A)

RESEARCH CENTRE
located in
NANCY — France
seeks
PATHOLOGIST

to take charge of the haematology and histopathology laboratories in its Industrial Toxicology Department.

Applicants should have a sound knowledge of anatomy and histology and five years' experience in the field. Duties include: study of sections; taking part in general toxicology and industrial cancer research; leading a team of seven people.

Fluent knowledge of French required.

Gross annual starting salary between 108,000 Frs. and 125,000 Frs.

Candidates should send C.V. with photograph and list of works and publications to Mr Saugier, Personnel Manager, INRS, 30 rue Olivier-Noyer, F—75680 Paris, Cedex 14, France. W285(A)

KING'S COLLEGE LONDON
DEPARTMENT OF PHYSICS

LABORATORY TECHNICIAN GRADE 5

Responsibilities include developing, constructing and modifying apparatus for teaching requirements and student projects etc, maintenance, servicing and repairing of equipment and the preparation of graphs, blueprints and engineering drawings. The post also involves the training of juniors and some supervision of other technical staff. Applicants should be suitably qualified and have 7-9 years relevant experience. Salary on scale £3,998 to £4,580 pa inclusive (under review). Five weeks annual holiday. Contributory pension scheme. Interest-free loans for annual rail season tickets.

Apply in writing with full details to The Head Clerk (Ref: 217831/N), King's College London, Strand, WC2R 2LS. 2814(A)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the post of Postdoctoral Senior Research Assistant in the Department of Biochemistry. The post is supported by the SRC for two years from 1st October 1979, at an initial salary of either £4,232 or £4,505 per annum.

The field of study is 'The control of chromatic adaptation in Cyanobacteria'. Applications are invited from biochemists and microbiologists. Experience on the isolation and biochemistry of RNA an advantage.

Applications, together with the names of three referees, should be received not later than 24th August, 1979, by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote ref RV/733/N. 2829(A)

TECHNICIAN/ RESEARCH OFFICER
required by our
VIRAL ONCOLOGY

laboratory to work in close collaboration with a member of the scientific staff on research into the genetics of avian and murine oncogenic viruses. HNC/degree. Experience in tissue culture, virological and biochemical techniques an advantage, but training in specific procedures will be given. Salary range £3,615 to £5,256. For further information and application form write or telephone Miss S M Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, WC2 on 242 0200 ext 305, quoting reference 340/79. 2847(A)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE AND ST MARY'S HOSPITAL MEDICAL SCHOOL

Applications are invited for a postdoctoral Research Assistant to work on experimental neuropathies employing freeze-fracture techniques. The post will be held jointly between the Department of Neurological Science at the Royal Free Hospital, Hampstead, and the Department of Anatomy, St. Mary's Hospital Medical School.

Applicants, who should have previous experience in electron microscopy, are requested to send a curriculum vitae and the names of two referees by 24 August, 1979 to Professor P.K. Thomas, Royal Free Hospital, Pond Street, London, NW3 2QC, from whom further particulars may be obtained. 2851(A)

KING'S COLLEGE LONDON

TEACHING TECHNICIAN GRADE 2B

Required to run the teaching laboratories of the Biophysics Department. An interesting post concerned with a wide range of courses. Other duties include helping with the animal unit and assisting academic staff with research.

Minimum qualification ONC or equivalent. Salary on scale £3,281 p.a. rising to £3,662 p.a. inclusive (under review). 5 weeks annual holiday. Contributory pension scheme. Interest-free loans for annual rail season tickets. Day release available for further relevant qualifications.

Apply in writing with full details to: The Head Clerk (Ref: 217832), King's College London, Strand, WC2R 2LS. 2859(A)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

CHEMIST/BIOCHEMIST

Applications are invited for the post of Senior Research Assistant, supported by the Wellcome Trust, for studies on the relationship between the metabolism and toxicology of synthetic steroids. Initial salary will be £4,232 pa and the project is financed for three years.

Applications, together with the names of three referees should be received not later than 21st August, 1979, by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote ref RV/730/N. 2828(A)

MAX-PLANCK-INSTITUT FÜR GENETIK
Berlin-Dahlem

A position is available for a

POSTDOCTORAL FELLOW

to work on the interactions between ribosomal proteins or between ribosomal RNA and proteins. This work will involve both biochemical and biophysical techniques. The position will be funded by the DFG until the end of 1981 at a salary of approximately 40,000 DM/year (BAT IIa).

Applications, including a C.V. and the names of at least two persons from whom references can be obtained, should be sent to Dr Jan Dijk, Max-Planck-Institut für Molekular Genetik, Abt. Wittmann, Ihnestrasse 63-73, D-1000 Berlin 33, West Germany. W283(A)

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

TECHNICIAN (grade 4)

required for research project in the Department of Tropical Paediatrics. Applicants should possess HNC or equivalent qualification and have experience of alectrophoretic spectrophotometric and immunometric (ELISA) techniques. The appointment will be for 3 years.

Applications in writing to The Department of Tropical Paediatrics School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, by 31st August 1979. 2867(A)

UNIVERSITY OF KEELE
DEPARTMENT OF BIOLOGICAL SCIENCES

DEMONSTRATOR

Applications invited for post of Demonstrator initially for one year; salary £3,775 per annum, together with membership of University Superannuation Scheme. Candidates should have a good degree in a biological science with some research, and ideally teaching experience. Candidates with interests in parasitology given preference, but other areas not excluded.

Further particulars and application forms from the Registrar, The University, Keele, Staffs, ST5 5BG to whom completed forms should be returned before 31st August, 1979. 2812(A)

UNIVERSITY OF BRISTOL
DEPARTMENT OF PHYSIOLOGY

TECHNICIAN GRADE 4

required for a research laboratory concerned with mammalian neurophysiology. Applicants should be over 21 and have an ONC or equivalent qualification. They should have experience in either electronics or general laboratory work. The salary will be in the range of £2,955 to £3,402 per annum (scale under review).

Applications consisting of a brief curriculum vitae and the names of two referees should be sent to Dr Sheila Carter, Department of Physiology, The Medical School, University Walk, Bristol BS8 1TD as soon as possible. 2833(A)

QUEEN ELIZABETH MEDICAL CENTRE
Central Birmingham Health District
Edgbaston, Birmingham 15

BASIC GRADE BIO-CHEMIST

Applicants are invited for a new post in the Clinical Chemistry Department of a large teaching hospital. It will offer wide routine experience in a department recognised for training for MCB/MRC Path, and opportunities for development and research work. The Queen Elizabeth Medical Centre is pleasantly situated three miles from Birmingham City Centre and has excellent staff facilities. Temporary accommodation may be available.

Details of the post and application forms are available from the Sector Personnel Officer, Queen Elizabeth Hospital, Birmingham B15 2TH or telephone 021 472 1311 ext 165. Candidates may visit the department by arrangement with Dr A M Bold, on extension 139. Interviews will be held during the second week in September. 2817(A)

ASSISTANT PROFESSOR

Applications are invited for the position of assistant professor in the Department of Microbiology at the University of British Columbia. Of special interest are persons able to establish a research program in yeast or fungal molecular genetics. The position is available immediately. Please send full curriculum vitae and the names of three referees to Dr J J R Campbell, Professor and Head, Department of Microbiology, University of British Columbia, Vancouver BC V6T 1W5. W276(A)

FELLOWSHIPS

THE UNIVERSITY OF WESTERN AUSTRALIA
Perth

UNIVERSITY RESEARCH FELLOWSHIPS (POST-DOCTORAL)

Not less than two Research Fellowships will be offered, to be taken up in 1980. Appointment will be for one year in the first instance with the possibility of renewal for a second year; Fellowships could possibly be renewed also for a third year, but in competition with any new applications. The Fellowships will be enable in the following academic departments for work in the broad areas stated below:

Anatomy and Human Biology: Analysis of synapses in the central nervous system with emphasis on their functional state.

Biochemistry: Hormonal control of cytochrome synthesis in mitochondria of developing rat ovary.

Botany: DNA repeated sequences in native plant groups.

Physical and Inorganic Chemistry: Dynamic stereochemistry.

The Fellowships are intended primarily for PhD graduates (normally from other universities), or those with equivalent qualifications, who by publication and in other ways have demonstrated significant research capability. Salary will be within the range $A14,041 to $A16,085 pa and appointees may in certain circumstances be considered for admission to a superannuation scheme. Fellows may be given the opportunity to participate in teaching.

Applications in duplicate setting out full personal particulars, qualifications and experience, and the proposal for research during the tenure of the Fellowship should reach the Acting Staffing Officer, University of Western Australia, Nedlands 009 Western Australia by 24th September, 1979. Candidates should request three referees to write immediately to the Acting Staffing Officer. 2869(E)

RESEARCH FELLOWSHIP
Imperial Cancer Research Fund
Mill Hill Laboratories
London NW7 1AD

A post-doctoral Fellow is sought to join a group working on the control of differentiation of stalk and spore cells in Dictyostelium discoideum. The Fellow would be expected to employ genetical and biochemical techniques to analyse the cell interactions involved in differentiation in this organism.

Appointment will be for three years. Salary with entry according to qualifications and experience within range £5,823 to £7,129.

Further information from Dr J Gross (01 959 3236). Application with curriculum vitae and names of two referees, should be sent to The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, by 30th September, 1979, quoting reference 345/79. 2862(E)

UNIVERSITY OF SURREY
DEPARTMENT OF MICROBIOLOGY
POST-DOCTORAL RESEARCH FELLOWSHIP

A post-doctoral research fellow is required for a three year MRC-supported project to determine the DNA sequence of the penicillinase (B-lactamase) gene of the resistance plasmid R46. This is part of a programme of research into the structure and function of this unusual penicillinase and its relationship to other B-lactomases.

Experience in DNA sequencing is not essential, but applicants should have some experience in plasmid biology or recombinant DNA technology. The starting date will be October 1st, 1979 or as soon as possible thereafter. The commencing salary will be £4,333 per annum on Research and Analogous Range 1A.

Applications, including curriculum vitae and naming two referees, should be sent as soon as possible to Dr J W Dale, Department of Microbiology, University of Surrey, Guildford, Surrey GU25 5XH, from whom further information may be obtained. Closing date 24th August, 1979. 2808(E)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF MICROBIOLOGY
RESEARCH FELLOWSHIP

Applications are invited for the above post in an established research group concerned with molecular mechanisms in infectious diseases. The post, which is funded by the MRC until March 1984, is suitable for candidates who already hold or who expect to be awarded a PhD in the areas of cell-biology or membrane bio-chemistry. Candidates with experience in other fields will be considered. The successful applicant will be concerned with the isolation of and characterisation of those gonococcal surface components involved in the interaction with host cell receptors. Starting salary at an appropriate point on the 1A scale according to age, qualifications and experience (£4,333 to £6,066).

Informal enquiries regarding the post will be welcome and may be made through Professor P J Watt, Tel 0703 777222 ext. 3893. Applications with curriculum vitae and names of two referees to Microbiology Department, South Laboratory and Pathology Block, Southampton General Hospital, Southampton SO9 4XY. 2872(E)

MRC
Medical Research Council

Travelling Fellowships 1980-81

The Council invites applications for travelling fellowships, available for the academic year 1980-81, from suitably qualified candidates with research experience. Clinical applicants should be of at least NHS Registrar status while non-medical graduates are expected to hold a PhD/DPhil degree. The majority of these fellowships may be held in any country abroad although certain of the awards are restricted to the USA or France.

The closing date for applications is *30 Septmeber 1979.* **Application forms and further information may be obtained from Training Awards Group, Medical Research Council, 20 Park Crescent, London W1N 4AL, Telephone 01-636 5422 (Extension 240).** 2821(E)

EMBO

European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY AUTUMN 1979 AWARDS

Next deadline: August 31, 1979

EMBO long term fellowships are initially awarded for one year. Applications for a renewal for a second year and subsequently **in cases of exceptional scientific merit for a third year** are considered.

To be eligible a candidate must hold a doctors degree. Preference will be given to European and Israeli applicants wishing to work within Europe or Israel. EMBO long term fellowships are not, however, awarded for exchanges between laboratories within any one country. Applications for fellowships to be held outside Europe and Israel are considered but they have a lower priority, as do applications from non-European scientists wishing to work in Europe or Israel.

Successful applicants will be notified of their awards on October 29, 1979.

Further details and application forms may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F. R. Germany. W275(E)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF BIOCHEMISTRY
RESEARCH FELLOWSHIP

A Research Fellow is required from 1st October 1979 until 31st October 1980 for work supported by the Medical Research Council on hepatic glucokinase. The Fellow will work with Professor D G Walker and continue a project concerned with several aspects of the metabolic regulation of the enzyme.

Salary on scale starting at £4,333 (October scales) according to age and experience, plus USS: maximum starting salary £6,066.

Applications by 31st August, 1979 to Miss L R Heyes, Senate Registry, University of Birmingham, PO Box 36, Birmingham B15 2TT, from whom further particulars may be obtained. 2849(E)

UNIVERSITY OF KEELE
DEPARTMENT OF PHYSICS
RESEARCH FELLOWSHIP
Synchrotron Radiation Protein Crystallography

Applications are invited for a three year SRC supported Post Doctoral Fellowship commencing on 1st October or as soon as possible thereafter to work with Dr J. R. Helliwell on the X-ray crystallographic study of 6-phosphogluconate dehydrogenase from Bacillus stearothermophilus utilising the Synchrotron Radiation Source presently under construction at Daresbury Laboratory as well as facilities within the Department. Salary starts at point 3 of the research scale 1A £4,910 to £5,488. Experience in the field advantageous but not essential.

Further particulars and application forms from The Registrar, The University, Keele, Staffs, ST5 5BG to whom completed forms, as well as curriculum vitae and details of research experience, should be returned as soon as possible. 2850(E)

THE UNIVERSITY OF LEEDS
FACULTY OF MEDICINE
THE EMMA AND LESLIE REID FELLOWSHIP

Applications are invited for the above Fellowship tenable in the University for a fixed period of up to three years for a person qualified to prosecute research in connection with diseases of the heart or brain (or kindred diseases). A medical qualification is not essential.

Remuneration will be at an appropriate point on either the University clinical scale (£4,296 to £8,281 under review from 1 April 1979) or non-clinical Research and Analogous scale (£4,333 to £7,521 under review from 1 October 1979).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 93/3/D. Closing date for applications 12 October 1979. 2875(E)

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DEPARTMENT OF GENETICS
RESEARCH FELLOWSHIP

Applications are invited for a post-doctoral research fellowship funded by an SRC grant to Dr D A Smith. The project is the analysis of spore germination in *Bacillus subtilis* using germination mutants, conventional genetic analysis and gene manipulation procedures.

The post is tenable for three years. Salary will be on the RF1A scale £4,333 to £7,521 (as from October 1st, 1979), plus superannuation. Maximum starting salary will be £4,910.

Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham, PO Box 363, Birmingham, B15 2TT to whom applications (three copies) including full curriculum vitae and naming three referees, should be sent by Friday 24th August, 1979. Please quote ref: NL8. 2819(E)

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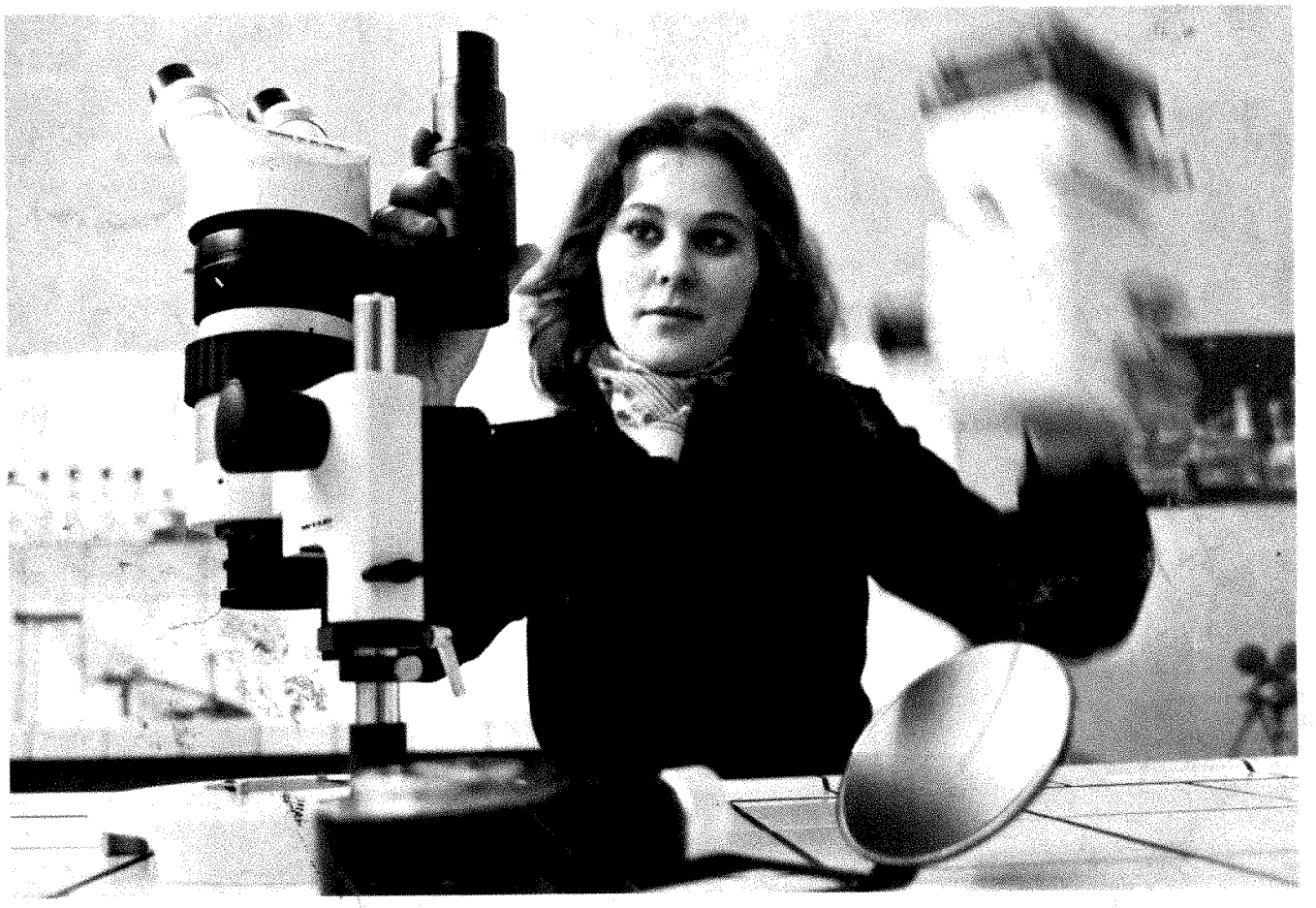
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Vol.280 No.5723 16 August 1979

nature

Volume 280 16 August 1979

Dependence or autonomy: UNCSTD's hidden agenda 525
UNCSTD: What's to be done? 14 experts comment 527
Agroforestry: new hope for subsistence farmers 533
Peer seeks to improve UK animal research law 534
In brief 535
Correspondence 536

NEWS AND VIEWS
Stops and starts in microtubules/Conformational flexibility in protein molecules/Meteoroid fragmenation in the auroral zone/Super fluid vortex waves/ The cladistic debate continued 537

REVIEW ARTICLE
Superheavy-element research — *G. Herrmann* 543

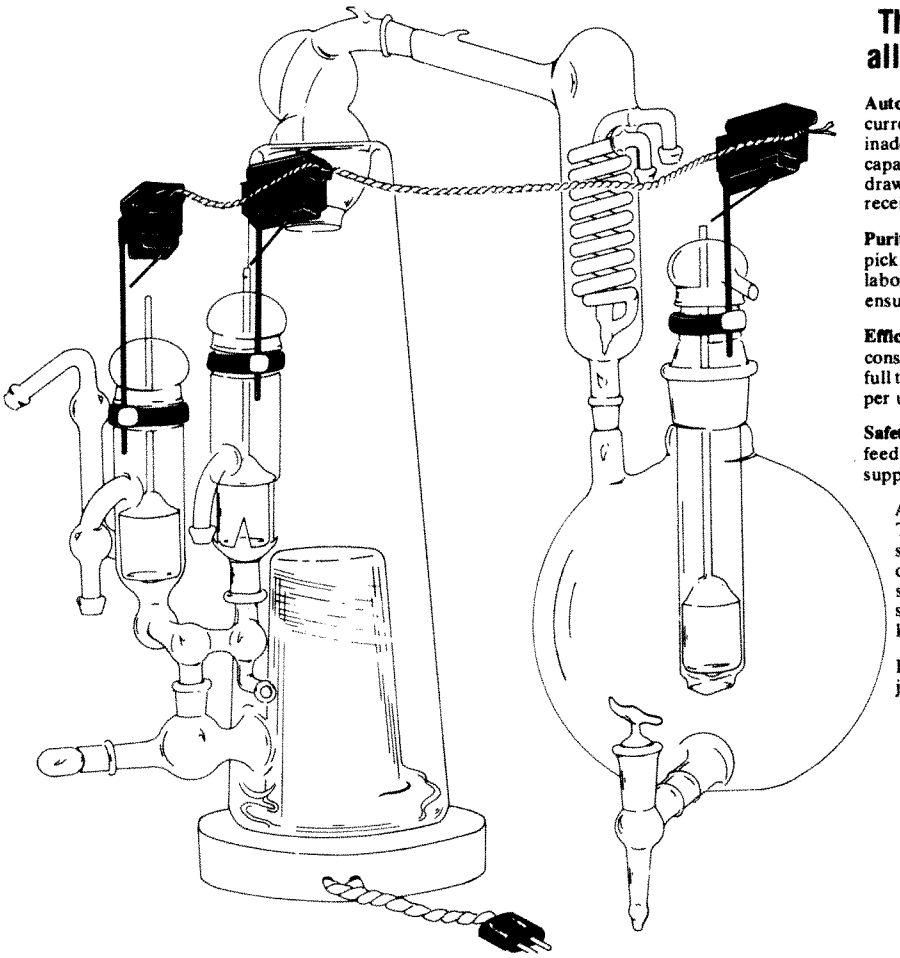
ARTICLES
Boninites, komatiites and ophiolitic basalts — *W. E. Cameron, E. G. Nisbet and V. J. Dietrich* 550
Disappearance of pink-pigmented *Globigerinoides ruber* at 120,000 yr BP in the Indian and Pacific Oceans — *P.R. Thompson, A.W.H. Bé, J.-C. Duplessy and N.J. Shackleton* 554
Temperature-dependent X-ray diffraction as a probe of protein structural dynamics — *H. Frauenfelder, G.A. Petsko and D. Tsernoglou* 558
Crystallographic studies of the dynamic properties of lysozyme — *P.J. Artymiuk, C.C.F. Blake, D.E.P. Grace, S.J. Oatley, D.C. Phillips and M.J.E. Sternberg* 563

LETTERS
Soft X-ray emission from the vicinity of MV Lyr — *K.O. Mason, S.M.Kahn and C.S. Bowyer* 568
Parity nonconservation and stellar collapse — *A. Vilenkin* 569
Solar radio spectrum extended to 10 km wavelength — *R.R. Weber* 570
Chemical modification of a titanium (IV) oxide electrode to give stable dye sensitisation without a supersensitiser — *S. Anderson, E.C. Constable, M.P. Dare-Edwards, J.B. Goodenough, A. Hamnett, K.R. Seddon and R.D. Wright* 571
Odd and even numbered hydrogen ion clusters — *D. Mathur and J.B. Hasted* 573
Processes controlling the stanol/stenol ratio in Black Sea seawater and sediments — *R.B. Gagosian, C. Lee and F. Heinzer* 574
Analysis of aliphatic and aromatic hydrocarbons in Antarctic marine sediment layers — *H.M. Platt and P.R. Mackie* 576
Ordovician conglomerates and the evolution of the Midland Valley — *C.D. Longman, B.J. Bluck and O. van Breemen* 578
Fly photoreceptor fluorescence is related to UV sensitivity — *W.S. Stark, D.G. Stavenga and B. Kruizinga* 581
Saccadic suppression by corollary discharge in the locust — *M. Zaretsky and C.H.F. Rowell* 583

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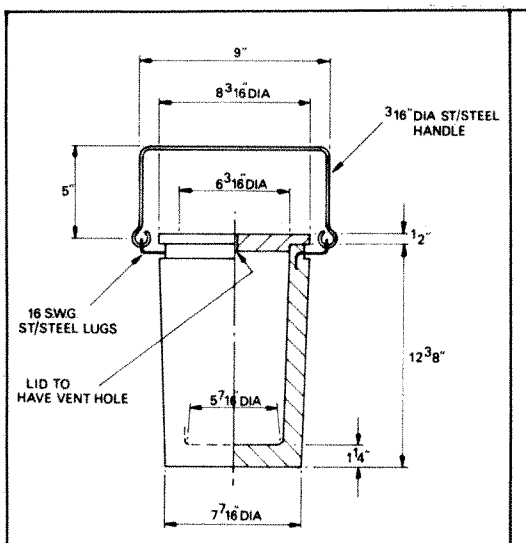
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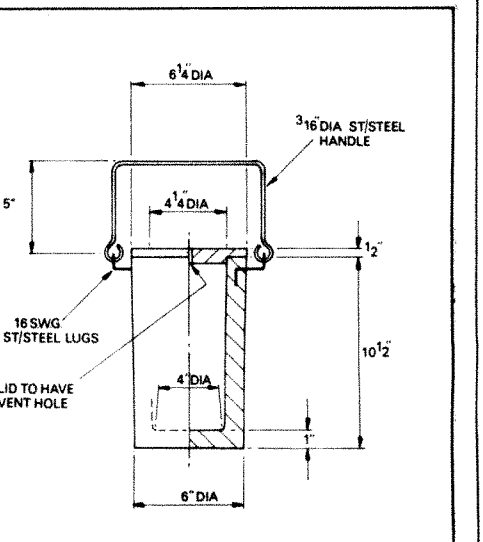
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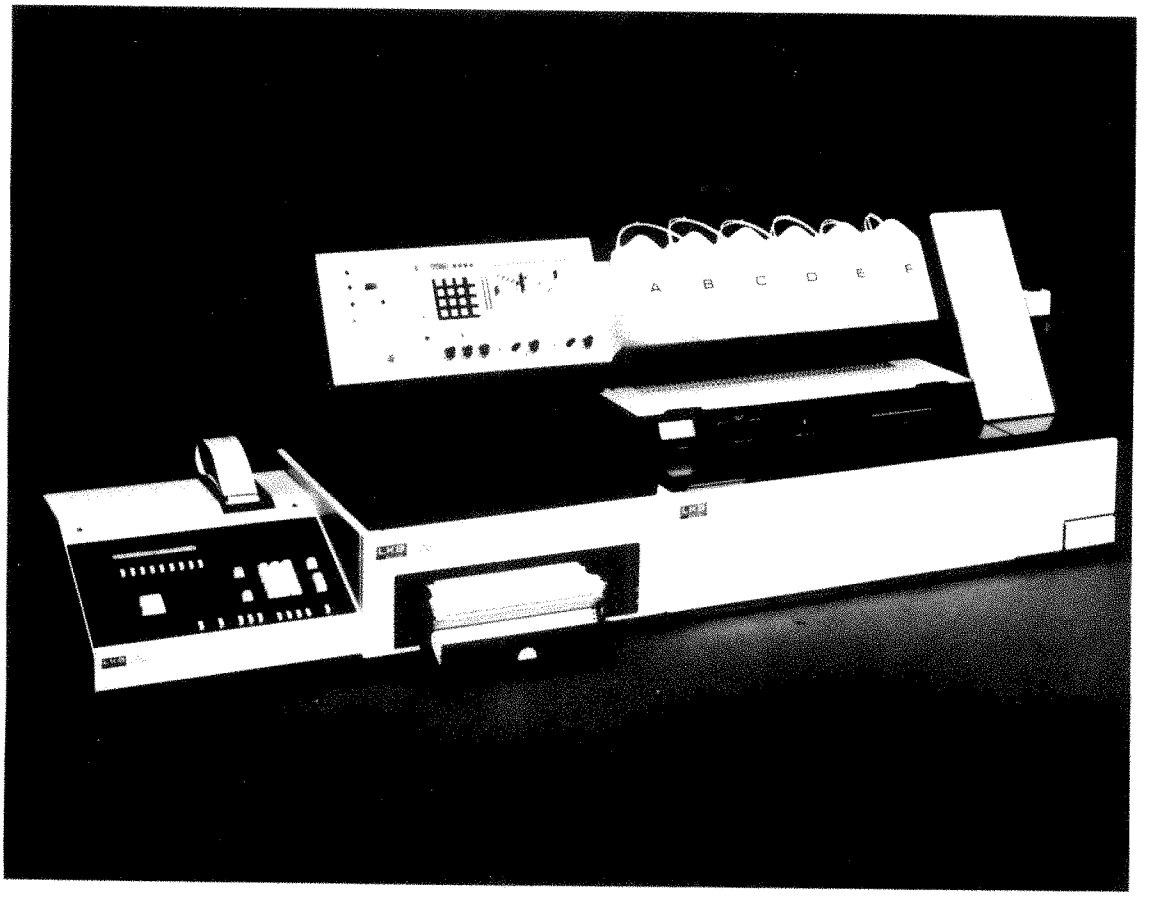


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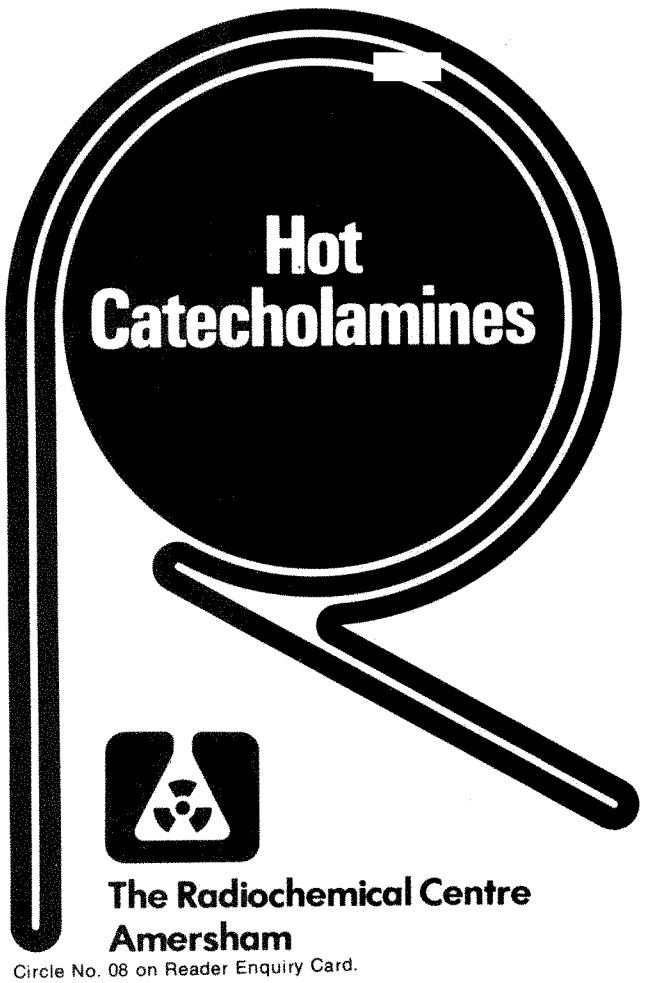
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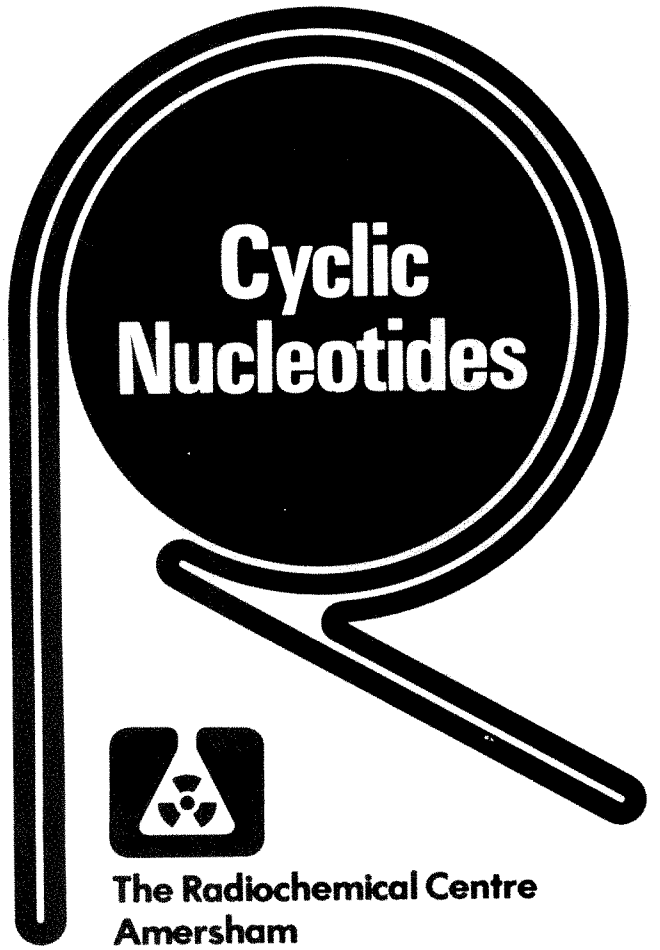
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Nuclear transplantation in teleost *Misgurnus fossilis* L.	*K.G. Gasaryan, N.M. Hung, A.A. Neyfakh and V.V. Ivanenkov*	585
Expression of genetic resistance to an oncogenic herpesvirus at the target cell level	*W.M. Gallatin and B.M. Longenecker*	587
Feedback inhibition by human primed T-helper cells	*C.J. Heijnen, F. Uytdehaag, C.H. Pot and R.E. Ballieux*	589
Does lectin–receptor complex formation produce zones of restricted mobility within the membrane?	*Y.A. Zagyansky and S. Jard*	591
(DTrp^6-Pro^9-NEt)-luteinising hormone-releasing factor inhibits follicular development in hypophysectomised rats	*S.-Y. Ying and R. Guillemin*	593
Endogenous opiates block dopamine inhibition of prolactin secretion *in vitro*	*A. Enjalbert, M. Ruberg, S. Arancibia, M. Priam and C. Kordon*	595
Electrical properties of giant mitochondria studied with a double impalement technique	*C. Bowman and H. Tedeschi*	597
Use of Panamanian sea urchins to test the molecular clock	*H.A. Lessios*	599
Placental alkaline phosphatase in Hominidae	*G.J. Doellgast and K. Benirschke*	601
Human placental alkaline phosphatase differs from that of other species	*D.J. Goldstein and H. Harris*	602
α-Globin gene organisation in blacks precludes the severe form of α-thalassaemia	*A.M. Dozy, Y.W. Kan, S.H. Embury, W.C. Mentzer, W.C. Wang, B. Lubin, J.R. Davis Jr and H.M. Koenig*	605
Phosphorylation in a simple system of lipids and chloroplast ATP synthetase driven by pulsed ionising radiation	*J.M. Gould, L.K. Patterson, E. Ling and G.D. Winget*	607
In vivo reduction of cyclopropene by *Azotobacter vinelandii* nitrogenase	*C.E. McKenna and C.W. Huang*	609
Reduction of cyclopropene as criterion of active-site homology between nitrogenase and its Fe–Mo cofactor	*C.E. McKenna, J.B. Jones, H. Eran and C.W. Huang*	611

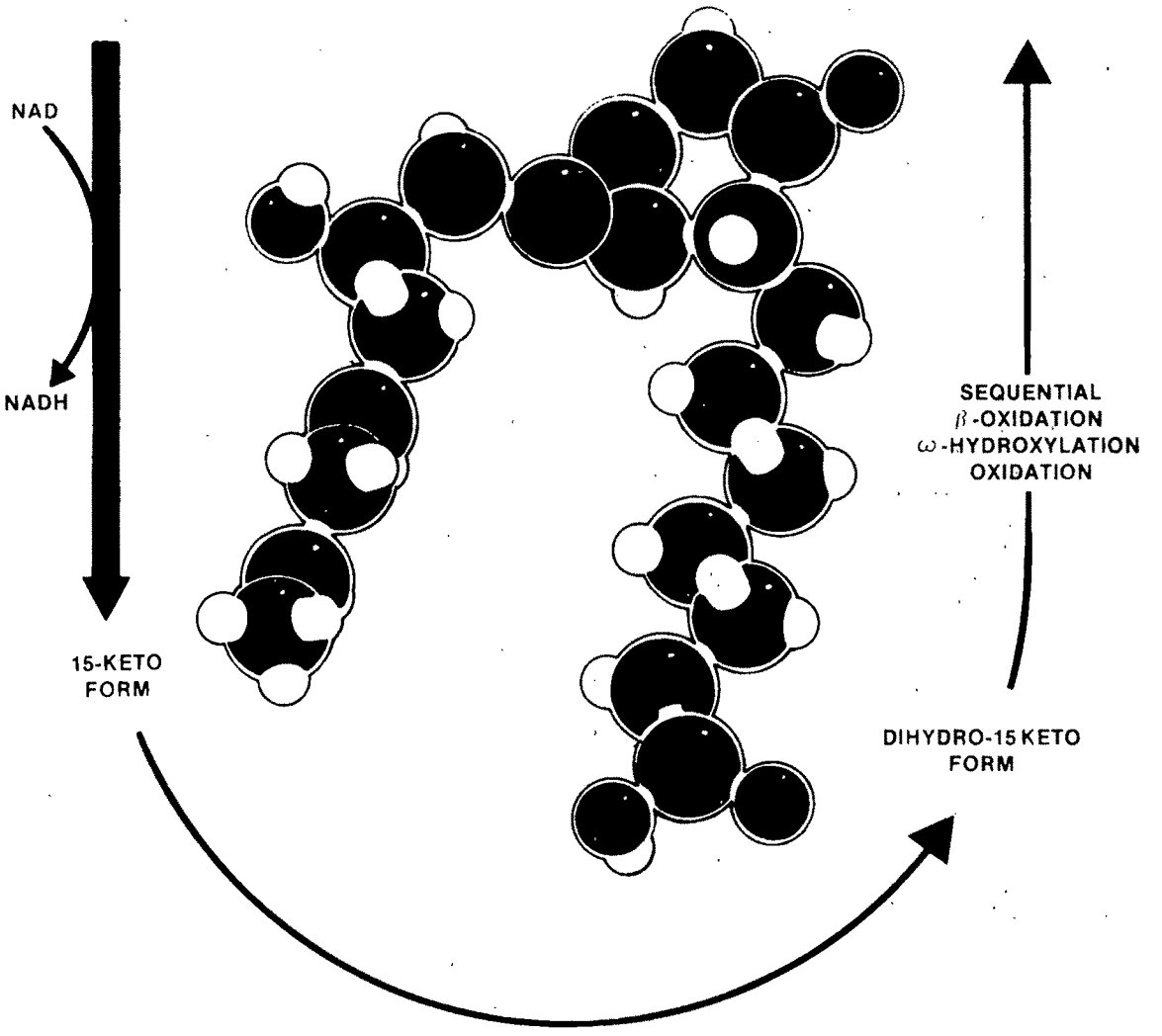
MATTERS ARISING

Evolution of dominance	*J.S. Gale and I.J. Mackay*	613
Reply	*B. Charlesworth*	613
Haemoglobin S and *P. falciparum* malaria	*H. Laser and R. Klein*	613
Reply	*G. Pavsol, D.J. Weatherall and R.J.M. Wilson*	614

BOOK REVIEWS

General Relativity: An Einstein Centenary Survey (S. W. Hawking and W. Israel, editors)	*Robert Wald*	615
The Operon (J.H. Miller and W.S. Reznikoff, editors)	*Richard Calender*	616
Sex, Hormones and Behaviour (Ciba Foundation)	*Alan F. Dixson*	617
Atomic Absorption Spectroscopy (M. Slavin)	*Allan M. Ure*	617
Neural Modelling: Electrical Signal Processing in the Nervous System (R. J. McGregor and E.R. Lewis)	*Jack Cowan*	618
Laboratory Handbook of Chromatographic and Allied Methods (O. Mikeš, editor)	*R.L.M. Synge*	618

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Dependence or autonomy: UNCSTD's hidden agenda

With science-based technology playing an increasingly dominant role in the world economy, the politicisation of next week's conference has been inevitable. The major task is to make it constructive.

WHAT hope for UNCSTD? Delegates currently heading for Vienna, where the United Nations Conference on Science and Technology for Development opens on Monday, carry with them a fat set of gloomy prognostications. A successful conference — and one of the difficulties here is that a single criterion of success is almost impossible to define — still hangs in the balance. One thing is certain, however: not only is UNCSTD the last and possibly the most important of the major UN conferences to have taken place over the past decade — it has also proved to have been one of the most controversial.

There are numerous reasons for the disputes that have frequently threatened to engulf the conference preparations. Firstly there has been the growing recognition that the task of applying science and technology to development is in practice vastly more complex than it was thought to be a decade ago, when theories of technological determinism still reigned supreme in development policy. Secondly, it has become clear that many of the obstacles to this process — such as problems in energy or resource supply, or growing structural unemployment — have been partly due to the way that science and technology have been inappropriately applied in the past. Finally, perhaps less globally significant but important in the international context, has been the extent to which the issues faced by UNCSTD impinge on the existing interests and policies of other UN agencies.

Behind all these, however, is a factor which has received considerably less attention, but which seems destined to play a no less significant — perhaps even a dominant — role in the Vienna negotiations. This is the fact that over the past decade, the global economy has come to depend increasingly on science-based technology as the prime generator of wealth. And that consequently, for both developed and developing countries alike, issues relating to the ownership and control of technology have become as important in a political sense as the use of this technology has become in economic terms.

One result is that for the developed countries the whole issue of science and technology for development has increasingly shifted from the sphere of aid to that of foreign policy. As the developing countries have clamoured for political autonomy, so the interdependence between nations has shifted from the militarily-backed relationships of colonial times to the economic domain. The desire for technological know-how, as well as the conditions under which this is created, bought and sold, have subsequently moved to the centre of these interdependent relationships. In addition, political issues surrounding the impact of technology on society have increasingly taken on an international dimension.

Nowhere have the implications of these developments been more clearly recognised than in the United States. One of the most significant recent trends in foreign policy strategy has been a shift from the aggressive military stance that became so discredited during the Vietnam era, to a style of soft-shell diplomacy emphasising economic cooperation and mutual self-interest. Science and technology, through cooperative agreements, collaborative research ventures, and academic exchanges, have become a central tool in this new aproach. Dr Henry Kissinger was one of the first to point out that problems such as energy, resources, and the uses of space and the sea ranked with questions of military security, ideology and territorial rivalry which had traditionally made up the diplomatic agenda.

The use of science and technology as a tool of diplomacy has received even greater support from the Carter administration. On the one hand it has resonated with the President's own engineering persective on social problem-solving; on the other, it has been actively supported by the US business community as an alternative to more overtly coercive forms of global involvement. Perhaps the most obvious success of this new strategy has been the use of scientific and technical agreement to finesse previously -stalled diplomatic initiatives with the People's Republic of China, a possibility opened up by the new regimes desire for access for US technology. Since then, it has been used extensively to articulate relationships with both developed (e.g. West Germany and Japan), and developing countries (particularly Mexico and Latin America), and the approach has received substantial backing from the US Congress.

To this development must be added another which has also emerged since the deliberations for UNCSTD first began, namely the developing countries' demand for the establishment of a New International Economic Order (NIEO). Both have since come to dominate the perspective from which the developed and the developing countries respectively are approaching the Vienna negotiations. And where conflicts and disagreements have arisen in the preparatory process, many can be traced back to the tension between these two goals. For what the US sees as the use of science and technology in, to use one of Dr Brzezinski's favourite phrases, "the stable management of global change", developing countries see as an attempt to reinforce US hegemony in a new guise. And the latter have also concluded that issues concerning the control of science-based technologies are a key to the restructuring of international relationships envisaged in the NIEO.

Delegates meeting in Vienna next week are not substantially divided on what needs to be done, whether at the level of meeting human needs for adequate food, energy and housing, of increasing research efforts on issues relevant to these and other developing country concerns, or of building up research capacities in the LDCs. The main source of dispute is over *how* these goals should be tackled. And here the central arguments share many of the characteristics of the debates that have taken place within the developed countries over the past few years on the relationship between science and society. Given the problems that have accompanied society's use of science in the past — the pollution of the environment, the heavy imbalance towards military research and technology, the alienation of "scientifically managed" labour and processes, and so on — can better solutions

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be found by revising the responsibilities of existing institutions? Or are the problems endemic to these institutions, and does their solution require, as radical critics would suggest, a more funadmenal restructuring of social and political relationships?

Transposed to the international arena by the UNCSTD conference, the answers being given to these questions seem clear — and frequently in conflict. The developed countries, while not minimising the difficulty of applying science and technology to development, argue that this can still be done most effectively within the existing pattern of international relations. The message they bring to Vienna is that the task requires the appropriate mix of pragmatism and private enterprise with the former intended primarily to fill the holes left by the latter. As one group of US businessmen put it in a report to the States Department, a prime need is "to support and expand the existing system which on the whole has been the source of an unprecedented advance in science, technology and economic development throughout the world."

The developing countries, at least as far as their views have been presented in the preparatory process by the Group of 77 — the "trade union of the poor" according to President Nyerere of Tanzania — see the situation differently. They argue that the political strutures which support the global economy are biased in favour of the rich, industrialised nations, and that a more equitable distribution of bargaining power is central to the NIEO. In the UNCSTD context this means not only building up their own research capacities, but easier (and cheaper) access to the technology already existing in the developed world, and a substantially greater share of control over the global research and development budget.

The developed countries have, not surprisingly, dismissed such demands as "unrealistic". For example, a central objection to the international financing system which the Group of 77 has proposed to support science and technology activities has been the political unacceptability of the developing countries laying down how much the developed countries should contribute to this fund; the latter would prefer to operate through, for example, the United Nations Development Programme, where they can exercise greater control over the size and use of their contributions. In addition, developed countries argue, with some justification, that there is little domestic support for increased aid spending at a time when foreign competition is blamed as one reason for economic recession. Nor is their much enthusiasm for the political goals of the NIEO, whose provisions, according to one US industrialist, "will be a discouraging factor affecting the final resolutions and resulting programming" of the UNCSTD meeting.

In return, there exists a deep distrust among many developing countries about the way that the developed countries conceive the task of applying science and technology to development. The predominent fear is that the types of solution offered, while dressed up as cooperative ventures, will in practice be used as Trojan horses to reproduce precisely those dependency relationships which, they feel, must be broken if a just and equitable global development is to be achieved. Internationally they feel that the continued hegemony of the industrialised nations on research matters implies a dependency which only sanctions patterns of development meeting such nations' interests. Domestically there is concern — voiced more frequently from unofficial rather than official quarters — that solutions designed primarily for their compatability with a global market economy will favour local elites already integrated into this economy, at the expense of the poor who remain marginal to it (apart from providing a source of low-cost labour power). Thus in both cases it is feared that unless sufficient endogenous control is exercised over research and development programmes, however scientifically and technologically appropriate their goals, their benefits will tend to be skewed towards the relatively rich and powerful, and against the poorest and those most in need.

Given these conflicting perspectives, it is perhaps inevitable that the issue of control has become central to virtually all current debates where science intersects with development concerns — what might be called the field of development science policy.

As far as global research projects are concerned, it is already clear that one man's pragmatism is another man's political programme. Issues such as exploring the mineral potential of the sea-bed or attempting to prevent the proliferation of nuclear weapons have become highly charged as developing countries see in proposed procedures attempts to restrict their political rights. On the transfer of technology, the broad parameters of the conflict are already widely known, with the developed countries arguing that technological know-how be respected as the private property of the inventor, and the developing countries preferring to see technology more as part of the common heritage of mankind. Even in the debate over the need for appropriate technologies, developing country scepticism has focussed less on the technologies themselves than on the motives of those who suggest their widespread dissemination.

In sum, what is being challenged at UNCSTD is the extension of the institutional framework and political assumptions which have supported the growth of Western science and technology — the so-called social relations of science — as the appropriate model for applying science to society's needs on a global basis. To the extent that these social relations of science have served the interests of the Western market economies, as well as a number of their socialist counterparts, those who see a global economy operating along the same lines argue that they are appropriate to the wider context. But to the extent that they are seen as presenting obstacles to genuine development, creating unwelcome dependencies, reinforcing inequalities in wealth and power, and condemning those most in need to the economic margins, these social relations will be challenged by critics demanding a far greater control from the grass-roots of the processes leading to both the generation and the application of scientific knowledge.

Where, then, does this leave our hopes for UNCSTD? Putting political realities aside, we could envisage a set of decisions that might include: agreement substantially to increase the proportion of the global R & D budget devoted to development-related topics, say from 5 to 25 per cent by the year 2000 (in line with the Lima target on world trade); agreement to halve the proportion of this budget spent on military research by the same date; agreement that restraints on proprietary technology be substantially reduced where these present a major obstacle to their global deployment; and so on.

Pipedreams and magic wands, however, will have little place in Vienna. At present it would be wrong to expect any quantum leaps in development strategies to emerge (even though these will be hotly supported in the NGO forum). At best we can hope for some agreement on the type of actions that developing countries should take to build up their scientific and technological infrastructures; a limited commitment from the developed countries to reallocate some of their aid funds to R & D objectives; a new focus within the UN system to remind all countries of the importance of science and technology in development strategies; and perhaps preliminary agreement to extend current efforts in fields such as information dissemination and remote sensing.

This may sound pitifully little for an exercise which has taken up so much time and energy over the past few years. But the real gains may be less tangible. The conference secretariat constantly reminds us that, for many countries, merely preparing a position paper for the conference has been a highly instructional exercise. In the same way the conference proceedings themselves may highlight one topic which has been dropped from the explicit agenda, namely the obstacles that obstruct the applications of science and technology to development. (For example through clarifying major areas of disagreement, and analysing the reasons). If no more, delegates will be exposed to the challenge and the difficulties of developing a common frame of international discourse embracing political and technical issues simultaneously. This in itself will do little to change the world; but it is a prerequisite for the issues that need to be negotiated in the decades ahead. □

UNCSTD: what's to be done?

The UN Conference on Science and Technology for Development starts in Vienna next week. We asked a group of scientists, politicians and administrators to pinpoint the most critical action that needs to be taken



The proper place for science in the Third World

by Joao Frank da Costa
Secretary-General of UNCSTD

I AM frequently asked "how are you going to assess if the Conference on Science and Technology for Development is a success or a failure?" It is not an easy question to answer. Obviously, the difficulty in reaching agreement on conference issues should not be the sole criterion. I believe incidentally that there are only three main issues: 'transfer of technology' — which evokes emotional overtones on both sides; the institutional mechanisms for science and technology in the UN system; and, above all, the financial arrangements. They are extremely difficult problems that have been discussed or negotiated without success for decades, and there is no objective reason why, in these times of extreme economic difficulties, dramatic breakthroughs should be considered possible.

Another criterion could be the ability and willingness to arrive at a common agreement on the text in the Draft Programme of Action. For my part, I would have liked to see a Programme of Action containing fewer brackets than the text which resulted from the fifth session of the conference preparatory committee (PrepCom), so that the conference could concentrate on only a few critical issues. The time-table for the PrepCom was however too short, and the exercise undertaken was not much more than a first reading. In addition, it seems to me that the negotiators considered the text as a kind of treaty and not, as they should have, as a general recommendation directed to member states, international organisations, and the UN system; and as a recommendation that still needs to be channelled and endorsed by proper mechanisms like the General Assembly.

In our case, neither of the criteria seem to be entirely valid. I believe that the conference will have achieved at least part of its purpose if it results in the recognition of the specificity and the importance of science and technology as tools for development, and their incorporation into national planning and international programming at all levels. I consider that this purpose has already been largely achieved at the national level. An enormous number of countries (and not only the developing ones) have studied, in the context of the preparations for the conference, the difficult problems involved in the application of science and technology for development. They have also achieved a measure of coordination internally, not only between the different government departments concerned but also between producers of science and technology (mainly the scientific and technological community) and its users (the public in general). Institutional arrangements have been initiated or established, and rudimentary structures have been replaced by more comprehensive and modulated mechanisms for development, at both the national and the supranational level.

The success of the conference could also be judged by the political will shown by member states in providing the tools required to monitor the results of the conference — whether these are programmes of action, declarations or resolutions — and the necessary financial means needed to strengthen the endogenous capacities of developing countries. It is obvious that an international high-level mechanism like an intergovernmental body is needed to monitor the implementation of the results of the conference — otherwise, these would remain on paper. Similarly, the strengthening of the endogenous capacities of developing countries would need some new international financial arrangements, and in the absence of these arrangements, it will remain a pious platitude.

Of course success will only be complete if the specificity and importance of science and technology for development are also recognised at the international level. I believe the second part of the conference (the Vienna conference itself) should be assessed by the importance accorded to those two elements.

The conference is not an end in itself. It is both a reflection on what has been done in the past (rather little I am afraid) and what needs to be done in order to provide each country with a capacity to solve its own problems and to create an international environment conducive to such an endeavour. And it should, of course, also be considered against the background of the New International Economic Order and in the context of the formulation of a new International Development Strategy.

Strengthening the scientific infrastructure

Michael J. Moravcsik

AMONG the various guidelines suggested for UNCSTD the one with perhaps the broadest worldwide support urges that UNCSTD should result in concrete steps to strengthen the scientific and technological infrastructure of the developing countries. Such an aim has a number of advantages:

- it touches on the crux of the matter and is not just a palliative;
- it is relatively low-profiled politically, since many who are reluctant to share specific knowledge or resources are nevertheless willing to share the capability of generating knowledge;
- it is relatively inexpensive and does not require large new lump sum expenditures;
- it can be implemented in a decentralised fashion, thus avoiding the creation of new international or national bureaucracies;
- it brings partial benefits if it is partially implemented, in contrast to grandiose, all-or-nothing schemes.

There are many problems in infrastructure building, and UNCSTD can address only a few of them — it does not matter much exactly which. Science and

technology education of specialists and of the public, inside and outside with technology, and technology with production; and scientific research and technological development: these are all areas in which many specific, realistic, and effective proposals await implementation. Such programmes must involve the direct participation of working scientists and technologists the world over.

The most important aim is to ensure that UNCSTD, unlike the long list of other UN conferences, produces something tangible and operationally useful, even if only on a modest scale.

Dr Moravcsik is at the Institute of Theoretical Science, University of Oregon

A ten-year ban on large and poorly prepared meetings

Ward Morehouse

Now that the final UNCSTD Preparatory Committee meeting is over, it is possible to predict with a modest measure of certainty the likely outcome of the conference — a programme of action filled with platitudinous recommendations for national governments, especially in developing countries, to do more for and with science and technology for development; a new high-level committee and some rearrangement of concerned secretariats at the UN; a charge to the UN Development Programme and the specialised agencies to pay more attention to science and technology in their activities; and possibly some very marginal increse in the resources available from development agencies, mostly by reallocating existing budgets.

In the meantime, the cost of UNCSTD is approaching $50 million — not just the direct UN secretariat and conference costs ($7.8 million) but also the lodging, food and international travel expenses of the 4,000 people expected in Vienna during the last two weeks of August ($6 million), the professional compensation of the 2,500 government delegates and UN officials preparing for and attending the conference ($3.8 million), the host country's budgetary allocation for UNCSTD ($5 million), the five PrepCom meetings ($3.2 million), the preparation of national papers and related preparatory activities of 100 governments and an assortment of UN and other international agencies ($14.6 million) and the 100 or more international conferences and seminars spawned by UNCSTD ($8 million).

The likely outcome of UNCSTD seems marginal in relation to such expenditures. Even if a four-part package involving a $200 million science and technology fund, which is now being quietly negotiated, were to happen (an outside chance), it is not clear whether the result would do more to benefit scientists in North and South who say they are concerned with development or to improve the lives of poor people by bringing them the benefits of science and technology — through, as the principal UNCSTD discussion paper expresses one of the major goals of the conference, "the establishing of a just and equitable social order."

In fact, the real danger of exercises like UNCSTD is that they divert attention from the underlying economic, social, and political causes of human suffering and deprivation by deluding us into thinking these problems can be solved by science and technology alone.

Hence I propose, as one important action to enhance the realisation of the UNCSTD goal expressed above, a ten-year moratorium on large and indifferently prepared international meetings on science and technology for development, and similar global problem-solving conferences, while we search for more cost-effective ways to link the North-South dialogue to meaningful efforts to ameliorate the human condition. The money thus saved can be used in the coming decade to get on with the task of improving poor peoples' lives by whatever means are most appropriate, including — but not limited to — science and technology.

Dr Morehouse is President of the Council on International and Public Affairs, New York

Radical changes in UN procedures and policies

A.B. Zahlan

THE deliberations that underlie UNCSTD revolve around three particular problem areas. North-South relations, internal transformations of developing countries and the UN system as an instrument of technical assistance. Although all three require attention, it is perhaps fitting that UNCSTD should be primarily concerned with how the performance of the UN system could be improved. The UN system is not merely a source of aid; it also diffuses procedures and models that are often copied by the institutions of the Third World. The subject matter concerned with each of these problem areas is extremely complex; the extent of awareness of the specifics of the processes is poor; and the vested interests in the bureaucracies of UN agencies, ministries of Third World countries, the business communities and the governments of industrial countries are such that a stalemate has been attained. This situation is not in the interest of either the world community or the UN system; and it is certainly not in the interest of the Third World.

The efforts so far deployed to effect the

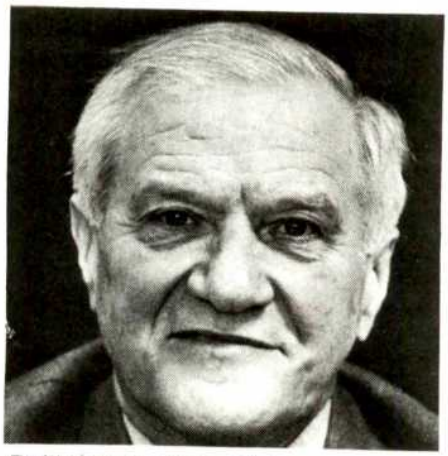


Da Costa: UNCSTD Secretary-General

changes called for by the New International Economic Order and by UNCSTD are too limited to dislodge established practices. The tens of thousands of persons employed in this multi-billion dollar 'aid industry' cannot spontaneously switch off past practices and become pioneers of the New International Economic Order and UNCSTD. The 'aid industry' operates on the basis of concepts of development that originate in both advanced and developing countries, and all these parties must share in altering accepted dogma. The management of the UN system has often attracted attention, but so far the system has not been opened up to provide factual data on its operations, performance and the policy implications of its practices. Yet the technological development of the Third World, along the lines proposed by UNCSTD, calls for radical changes in the procedures and policies underlying the operations of UN agencies. In theory the UN system is subject to scrutiny by member states at the level of the General Assembly, and during the general conferences of the respective agencies. In fact, however, very little of the necessary factual examination and analysis actually takes place at any of these gatherings. Short-term interests dominate, and few of the delegates possess the adequate information to assess the facts. Currently these international discussions are remote from the scientists, institutions and problems of the Third World. It is only through a dedicated concern about the field aspects of science and technology that the debate can be enriched with facts and realism.

The challenge facing the Third World in the UN system today is not to win a vote or to issue a new proclamation, but rather to initiate a process powerful enough to transform the practices of the system. A revitalised UN system has a good chance of accelerating the mobilisation of the internal resources of developing countries. These resources are formidable and all too often underestimated.

Prof Zahlan has been a scientific advisor to the UN (on ACAST) and Professor of Physics at the American University, Beirut. He is presently a visiting fellow at the Science Policy Research Unit, University of Sussex



Dedijer: information revolution

A basic capacity to absorb and create knowledge

Hiroshi Harada

THERE is much that can be said on the topic of 'Science and Technology for Development', but as far as developing countries are concerned it is useless unless they have in the first place a basic capacity to absorb and create scientific and technological knowledge, and to be adept in its use for productive and commercial purposes. This obvious fact seems, nevertheless, to be often overlooked, as is the fact that there is no short cut to acquiring it.

Education is the only universal means for forming this capacity from the innate intelligence available. And in this context, education must not be regarded solely as a process of passing on information and experience, but one which stimulates the curiosity of its recipient and develops a sense of applicability of the knowledge acquired. It must increase practical ability and the powers of observation and communication. It must instil mental self-discipline and ruthlessly maintain standards and integrity in study and research. These — and a willingness to be self-critical — are essential for self-reliance, both individually and nationally.

The process must be started deliberately at the primary level of education. It is too late at the secondary and higher levels, when the sheer weight of knowledge to be acquired can inhibit any burgeoning sense of mastery over it. This applies whether the product of the educational system is a professional scientist or technologist, or a layman. Indeed, an appreciation by the latter of what is involved is essential if the impact of science is to be accepted and the technological revolution necessary for development is to take place. Otherwise, scientists and technologists become, as is seen too frequently in developing countries, elitist and largely ineffectual in the development process.

Prof Harada is a Japanese plant physiologist, currently with UNESCO

Common sense, perseverance and ingenuity

John Ziman

IN the short run, the developing countries are seeking scientific and technological information; in the long run, to make use of that information and to serve their own needs, they will need research capability.

- In the short run, that means material facilities; in the long run, these are merely the instruments of scientific institutions.
- In the short run, a scientific institution is a bureaucratic organisation; in the long run it must grow into a scientific community.
- In the short run, a scientific community is made up of technically qualified personnel; in the long run, it depends on the leadership of its scientists.
- In the short run, a scientist is an expert who has passed the examinations for a PhD; in the long run, this is only an apprenticeship to the profession of research.
- In the short run, the goal of research is to make specific contributions to knowledge; in the long run, it is to exercise curiosity and to solve problems.
- In the short run, problems are often solved by the application of sophisticated, specialised expertise; in the long run this calls for common sense, perseverance, and open mind and ingenuity.

A scientific institution with some capability for research can be set up in about 5 years. Scientific workers with some research experience can be trained in about 10 years. It takes a generation or so of education and cultural change to foster the personal qualities needed to solve practical problems by scientific methods. This path is long and narrow, but it is the only known way. For the developing countries there is no time to lose: they, and we, must start now.

Prof Ziman is head of the Department of Physics, University of Bristol

Breaking the monopoly on scientific knowhow

S. Dedijer

I SEE three threats in UNCSTD to developing countries developing their own capability to find out, to discover, to invent and to innovate in both predictable and unforeseen circumstances. Two of them are strongly backed proposals for deceptive solutions to the problems which arise from the fact that almost all the global scientific and technological output, capability and knowhow is concentrated in only 30 countries, from which the remaining 80% of the world has to import.

The first threat would arise if UNCSTD set up one more UN organisation to 'deal' with the problem, without a critical evaluation of the efficacy of the existing ones. The second threat could come from the search for solutions to the problem in political attacks against 'capitalist dependence' of the developing countries without the realisation that such non-'dependent' countries as the USSR and China are almost colonially dependent on the science and technology of the democratic countries.

The most serious threat is that the developing countries, as shown by their UNCSTD national papers, have done their homework badly. Not one of them seems to have yet discovered the very fact of the new electronic information revolution, or has realised its implications for development. This includes the fact that information democracy — the right and motivation of everyone in a country to obtain, produce, distribute and withold information — is becoming essential for a country's ability to acquire and create scientific and other knowledge and knowhow. Democracy, in other words, is becoming a productive force.

UNCSTD itself offers the delegation from every developing country the opportunity to test critically the approach, the findings and recommendations in their national papers and to apply them creatively for national goals after UNCSTD.

Prof Dedijer is at the Research Policy Institute, University of Lund

Active partnership of Third World scientists

Yongyuth Yuthavong

THE challenge of science and technology today is not to show that it can give rise to social benefits, but that these benefits are produced where they are most wanted — in the Third World. There is an urgent need to direct a far larger part of world scientific and technological activities specifically towards Third World development. Solutions to many development problems have to be sought largely within the developing countries themselves, and their scientists and technologists must be given the necessary means to search for them. Solutions to many problems, for example in the fields of population, nutrition and health, are already known but lack implementation. An integrated approach including all necessary social, economic and political components is needed.

The developed countries have the capacity to find scientific solutions to many basic problems concerning the survival of mankind, and should make more effort in this direction. However, ultimately, effective action in the Third World

depends on the competence and morale of its own scientists, technologists and other colleagues, and sufficient appreciation by its general public of the value of scientific solutions to development.

Support from developed countries could take the form not only of direct financial assistance, but of collaboration between individuals and agencies concerned with issues ranging from basic research to industrial consultation, and the dissemination of packaged technology to an illiterate rural population. In short, an active partnership of Third World scientists and technologists is an important prerequisite for success of development efforts. It is, furthermore, important to make the general public in the Third World — especially the few leaders with so much influence — aware of the critical role of science and technology in development, and to make the necessary tools available.

Dr Yuthavong is at Mahidol University, Bangkok

Three imperatives: food storage, energy, work ...

Guido Brunner

A GREAT responsibility rests on those who will be taking part in UNCSTD in Vienna. They have to make a fundamental choice. They will either be drawn into a discussion of abstract principles of a new international economic order, or — and this is what I hope to see — they will get down to some practical business. The first will merely lead to yet another diplomatic deadlock of the kind we have seen already too often in meetings between the developed and developing world. The other will spark off that real scientific and technical co-operation which is so necessary for the solution of the problems of development.

This is an especially significant time for the conference. Prospects for the world economy are clouded, and governments are worried about the rising cost, and shortages, of energy. For the developing world the three imperatives are: the storage of food, energy and work. The conference must see that the proper scientific and technological resources are freed to contribute to these great tasks.



Brunner: fundamental choices

A long term effort internationally and at home will be required if the developing countries are to build up the necessary internal scientific and technological capability. The transfer of technologies from the developed to the underdeveloped is only one aspect of the problem. What is equally important is to establish the means for the developing countries to improve their educational and managerial standards.

The Community and its nine Member States will play their part in this great venture. Our association with 57 developing countries in Africa, Asia and the Caribbean within the Lomé Convention has already enabled us to make a contribution, and the new Lomé Convention will provide us with a framework for offering additional support for scientific and technical co-operation.

Dr Brunner is Commissioner of the European Communities for Energy, Research, Science, and Education

Policymaking should be at the highest level of authority

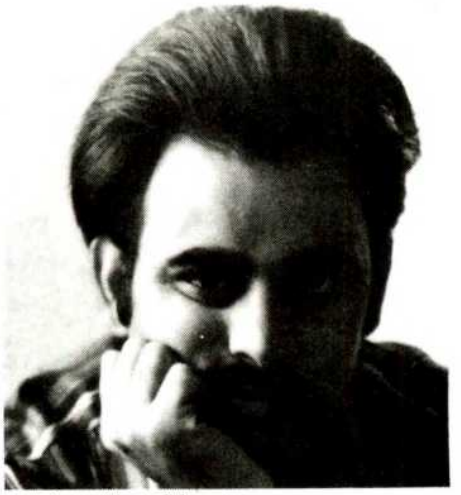
Antonio Pinilla

THE most pressing need is for a strong, deep and consistent political decision that science and technology constitute essential elements for each state to obtain:

- social integration;
- economic development;
- cultural independence;
- political coherence; and
- administrative efficiency and productivity.

Inadequate knowledge generates racial, social or religious prejudice, frustrating the national integration of countries. Economic growth requires agricultural and industrial mechanisation and rationalisation and these are impossible without a certain advanced degree of scientific and technological development. Payment of duties and royalties make national products uncompetitive in international markets. Economic dependence often generates cultural dependance, so decreasing awareness of national identity. Political and administrative sciences are key solutions to problems concerning political coherence, continuity, administrative efficiency and productivity within a free society.

For these reasons the making of science and technology policy should be the responsibility of the highest levels of authority, such as the president of the Republic (Head of State), parliament and the cabinet. The person in charge of implementing science and technology policy should have the rank of minister of state, or even better, be a full cabinet member.



Sardar: self-confidence

The usual type of problem confronting each cabinet member is something very specific and urgent. Cabinet members cannot afford to concentrate on the scientific and technological aspects of the problems they face. That is why it is critically important that present-day states create national systems of science and technology, giving them the highest level of responsibility and authority to ensure scientific and technological aspects of national plans and objectives, policies and actions are sound, do not include errors or insufficient information, and are adequate and appropriate to each national reality.

Prof Antonio Pinilla is President of the Peruvian Consejo Nacional de Investigacion Cientifica, Lima

Inculcating an appropriate sense of confidence

Ziauddin Sardar

ALL developing starts with the individual, and science development is no exception. The first and the most critical action, in my book, concerns the scientists of the developing countries themselves: every Third World scientist must consciously inculate in him/herself an appropriate sense of confidence. Science can only play its full role in development when the integrity of those who do science is preserved. And this integrity cannot be preserved when the Third World scientists themselves do not have any confidence in their ability to manage their enterprise.

There is a natural corollary to the development of confidence in Third World scientists: confidence in oneself procedes from confidence in one's society, tradition, culture and institution. If someone has confidence in his ability as a scientist and



Salam: 10 times more scientists

his culture and society, then he does not look down on the needs of the society as 'insignificant' and indigenous research as 'inferior'; nor does he apologise away his research efforts to solve local problems or place too heavy a reliance on outside help and consultants and advisors. Furthermore, a scientist with confidence in himself builds up confidence in his colleagues and does not feel threatened by younger scientists. Nor does he articulate the desire to block the paths to full realisation of those who have potentially more to contribute to science development.

As far as I am concerned, science development in the developing countries begins and ends with the confidence the Third World scientists have in themselves, their society and the science that they practise.

Dr Sardar is at the Hajj Research Centre, King Abdul Aziz University, Jeddah

Local scientific committees are too small

Abdus Salam

I HOPE the conference can focus on and initiate action on the following important area. Any declared intention of applying science and technology to development is — and will remain — meaningless until the developing countries build and deploy indigenous scientifically and technologically trained communities. At present, however, such communities simply do not exist, or their sizes are tenths or hundredths of what is needed. Their direction and deployment must be the concern of scientists and technologists themselves, and not of the economists and bureaucrats of national and international planning commissions.

It must be realised that whatever priorities the countries set themselves (food production, mineral exploitation, transport, health, manufacture or even defence), a meaningful application of science and technology needs commitment; is not cheap in money, men or time; and it brooks of no magic formulae.

Turning to the reciprocal role of developed countries, we note that the theme of world development has been woefully neglected by the scientific and technological communities of the developed countries, who have made little organised effort to help in this task, nor have they collectively shown any great vision. The same applies to their state agencies.

In addition, the international funds at the disposal of bodies like ICSU — or even UNESCO — for the development of science and technology for global concerns are pitifully small. (The entire UNESCO budget — not to restrict it to what is spent on science and technology — is smaller than that of the Ford Foundation). These international funds need to be increased by at least an order of magnitude.

Prof Salam is Director of the International Centre for Theoretical Physics, Trieste

A large debt remains for past exploitation

Joseph Hanlon

THERE is a need for a 'new scientific order', like the new economic and information orders, which attempts to break the stranglehold of the developed over the developing nations. Scientists in developed nations must realise that the science gap has not come about by accident. During the past 200 years of scientific progress, most Third World nations were colonies which were not permitted to develop an independent scientific capability. Small wonder, then, that there is a gap now.

The challenge today, especially for UNCSTD, is that the developing nations are working hard to maintain and widen that gap. The brain drain continues, with the developed nations sucking the brightest scientists into their companies, health services, and universities. Patent laws are now being used to block Third World research into key areas such as alternative energy. Journals and scientific societies, controlled by developed world scientists, define 'good' science as that which is of interest to developed nations. Tied aid ensures that experts and equipment come from such a wide variety of countries that even the most talented local scientist cannot digest and control the results.

All of this ensures that in those scientific areas which are of most importance to the developing nations — health, energy, ecology and geology — the expertise and control will remain with the developed nations.

UNCSTD can help to end this exploitation. But it will require the realisation in the developing nations that they must stop bowing to the demands of journals and aid agencies in the industrialised world. And it will require that scientists and science policy makers of the developed world understand that a large debt remains to be paid for past exploitation, and that a way of repaying is to provide increased aid — not for what they think the Third World needs, but for what local scientists and policy makers feel is required.

Dr Hanlon is a development journalist

Loosen the grip on proprietary rights in technology

Jose Goldemberg

THE most critical actions necessary to improve science and technology in the developing world are probably beyond the powers of UNCSTD, or of the United Nations.

Science, especially technology, does not flourish in the Third World — not because of incompetence amongst the people involved (although that exists too) — but because it is not really needed in the present model of development being followed in most of the Third World. Machines, technical expertise and a powerful marketing system coming from abroad inundate the developing countries, ministering to some of the needs of the people, creating others and above all generating a craving for the comforts of modern civilisation as defined by the industrial nations.

There is little that science can do to interfere with this situation, because most of the science needed exists already, and there are no fundamental new discoveries that could change the situation or the climate in which science could flourish in the Third World.

Technology, however, has to be adapted, in many cases to local conditions (of temperature, humidity, and so on) and to local fuels and materials. Native technologies have a role to play there, but in general technology is imported.

The most critical action to take in my view would be to loosen the grip on proprietary rights in technology. If these were made available and essentially free, local industries would have more room to move and more scientists and technologists would be needed to make choices and develop missing links. One thing that could accelerate this process would be to have international funding institutions which preferentially support projects that use as much native technology as possible.

Dr Goldemberg is at the Universidade de Sao Paulo, Brazil

Soviet aid: a two-way traffic

The eastern bloc's relations with the Third World stress 'real reciprocity'. **Vera Rich** reports

Science and technology transfer from the Soviet Union and other Comecon countries to the Third World is seen, not surprisingly, somewhat as an extension of such transfers within the bloc itself. That is to say, it is viewed not as "aid" — a word properly reserved in Soviet terminology for *ad hoc* measures such as the recent earthquake relief to Yugoslavia but as an essentially reciprocal process. (The official name of Comecon is the Council for Mutual Economic Assistance).

One interesting outcome of this approach is the attitude to "intermediate technology". The long-term plans of Comecon aim at integrating the economies of the member states, hence there is clearly a pressure to bring such countries as say, Hungary and Bulgaria, from a peasant-agrarian to an agro-industrial economy in the shortest possible time.

At least, this is the case in the production sector. The current Mongolian exhibition in London suggests that, although the Mongolian People's Republic boasts a state university, an academy of sciences, polytechnic, medical research institutes, a flourishing pharaceutical industry and a major programme for the exploitation of mineral resources, a significant proportion of its population still lives, by choice, in the traditional *ger* or felt tent, though, on the outskirts of the capital, Ulan-Bator, gers are frequently fitted with such modern conveniences as electgric light and television.

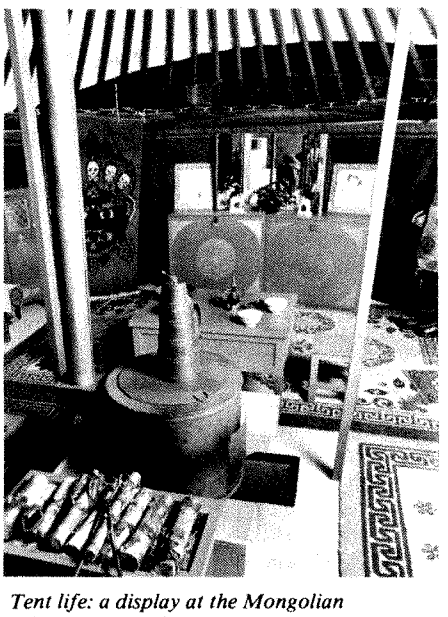
Regarding the third world, Soviet commentators stress that the bulk of what they offer is not the "relatively primitive mechanisms and implements which, in the opinion of some foreign theorists, are the most appropriate to the present requirements of the 'third world' countries", but "modern technology . . . which can contribute to the developing countries' mastering of modern scientific and technological achievements". Similarly, when co-operating with the developing countries, the Comecon countries have tended to follow current practice within the bloc as far as know-how is concerned.

As a result, the transfer of technological documents to the third world, originally free of all but incidental costs, has increasingly become replaced by the sale of know-how and licences (usually against long-term credits), and special trading organizations have been set up in the various Comecon countries to handle this. These include *Litsensintorg* for the Soviet Union, *Polservice* for Poland, and TESCO for Hungary.

Hungary is a particularly interesting case of such co-operation, since, until recently, it was itself eligible for aid under the United Nations Development programme. TESCO, the Technical and Scientific Co-operation Organisation, was founded in 1962, and at present operates co-operation agreements with 57 developing countries. One of the directors of TESCO, Mr Istvan Orosz, told *Nature* recently that Hungary cannot offer such assistance free, although sometimes it may be provided at basic costs. Another director, Mr Sándor Derzsi, stressed that in the case of countries which have recently been "liberated from colonialism" the main form of co-operation is the sending of Hungarian experts (in all, nearly 1000 annually), whose salaries are, if necessary, paid by the Hungarian government.

As the countries develop, he said, the form of co-operation can change. This was the case with Cuba, for example. Originally Hungarian experts went to Cuba, and Cuban trainees studied in Hungary. "But now we have bilateral connections and joint research projects with various Institutes". Cuba had taken 10 years to reach this stage. "But Iraq, Algeria and Nigeria have an economic basis", he added, "so they can reach the second stage more quickly".

This "tendency towards real reciprocity", said Mr Orosz, is typical of TESCO. Mexico, for example, has developed its own research in the growing of maize, a crop of considerable importance to Hungary also. A similar pattern, he explained, can be seen in Hungarian-Soviet co-operation. From 1948, when Comecon was established, until well into the 1960s, Hungary was largely the recipient. "We received some technology from the Soviet Union, and had our experts trained there. We did have some co-operation, but it was on a 20-80 basis." By the mid-1970s, however, some 50 to 60 Hungarian research institutes were engaged in joint ventures with their Soviet opposite numbers.



Tent life: a display at the Mongolian exhibition now showing in London

Basic research and exchanges involving whast Mr Orosz described as "concrete scientific themes" come not under the auspices of organizations such as TESCO, but that of the relevant Academies of Sciences. Nuclear research, for example, belongs here, and so does the Comecon space programme *Interkosmos*. The latter, it appears, (*Nature*, **280**, 94-95) is financed overall by the Soviet Union, with the other member countries paying only for their own experiments. This would explain why Vietnam, which joined Comecon last year, could this spring send two cosmonaut candidates to Baikonur for training, although the Vietnamese economy is in such an urgent state that 75% of the proceeds of this year's *Subbotnik*, the Saturday in April when Soviet citizens contribute a day's work for the good of the economy, are to be devoted to Vietnam.

Many co-operation projects bring the Soviet Union and other developed Comecon partners gains in raw materials. The Comecon survey of Mongolia, for example, will allow Mongolia to increase its exports of minerals — presumably within the bloc. Some projects such as the Afghanistan and Trans-Iranian gas pipelines were constructed on a "buy-back" basis. Soviet oil and gas prospecting in India, Afghanistan and Pakistan, or coal prospecting in Nigeria might well be compensated in some of the product. On the other hand, recent agreements to build a hydroelectric station in Costa Rica or develop the cotton and wheat production of Angola will bring a less immediate return to the Soviet Union — except in good-will.

For it is impossible to assess the impact of Soviet/Comecon assistance to the Third World without considering political implications. Soviet histories of the United Nations intended for home readership present the Soviet delegates as engaged in a "struggle" for "progressive principles of technical assistance". The more moderate language of a Soviet report to the UN notes that "Economic independence of the developing countries is unthinkable without their scientific and technological liberation from imperialism . . .".

Unfortunately, goodwill projects can be affected by subsequent political changes. For many years, the Aswan High Dam, with its associated hydrotechnical projects and irrigation schemes was regularly quoted as a prime example of such an undertaking. Last May, however, on the fifteenth anniversary of the "changing of the course of the Nile", Moscow Radio's Arabic service could only speak sadly of "some persons" who are now striving to make the Egyptians forget the Soviet contribution to this project. □

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Agroforestry: new hope for subsistence farmers

David Spurgeon reports from the first Conference on International Cooperation in Agroforestry, held in Nairobi last month

THE ancient practice of shifting cultivation has for many years been severely criticised by agriculturalists and foresters because of its destructive effects on both land and forests. Shifting cultivators fell and burn forest land, then plant food crops and raise animals and later move on when soil fertility drops. After a period of bush fallow, they return to the same site and repeat the process.

Historically, soil fertility was maintained first through the nutrients returned via the ash after burning, and later through natural processes during the long fallow periods. However, as population and economic pressures have increased, fallow periods have become progressively shorter and the land ever more exhausted of nutrients.

Within recent months, however, foresters and agriculturalists have been looking at these traditional practices with new eyes. Scattered but convincing evidence exists to show that the integration of trees, food crops and animal husbandry on the same piece of land can increase total productivity while preserving the ecosystem. Such a system could not only improve the way of life of the 250 million people in developing countries who depend on shifting cultivation for survival, but could help save the world's tropical forests from destruction. The name given to this approach is agroforestry.

The first Conference on International Cooperation in Agroforestry, held in Nairobi, Kenya, at the end of last month, was an indication of the widespread interest the concept has generated: delegates from 31 countries and eight international agencies attended. They strongly endorsed agroforestry practices, particularly in marginal or degraded areas where agriculture alone is not productive. They urged the extension of research into agroforestry systems throughout the developing world. And they recommend the adoption of education, training and extension programmes by appropriate institutions.

Much conference discussion was devoted to definitions of agroforestry. ICRAF's definition is a "sustainable land management system which increases the overall yield of the land, combines the production of crops (including tree crops) and forest plants and/or animals simultaneously or sequentially on the same unit of land, and (which) applies management practices that are compatible with the cultural practices of the local population." It was considered by some as not comprehensive or precise enough. No agreed-upon definition emerged: the conference left it to ICRAF to hammer out a final version.

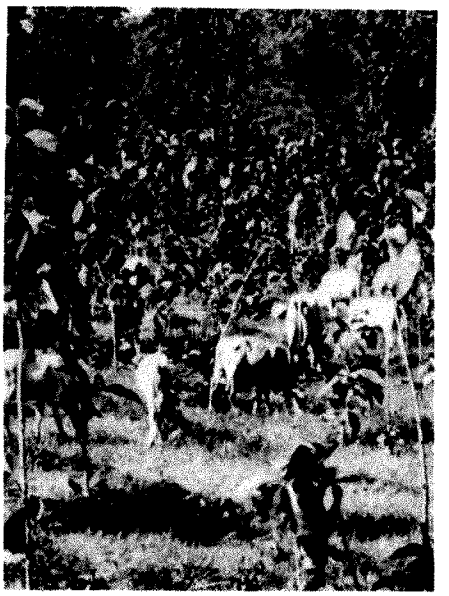
The lack of an adequate definition did not inhibit enthusiasm for the practice itself, however. Numerous examples were given of its benefits. One, from Soekiman Atmosoedaryo, president of Indonesia's state forest corporation, Perum Perhutani, was a programme that aims at developing forests not only as providers of wood and cellulose and protectors of the environment, but as sources of food, animal feed, honey, medicinal herbs, pine resin and natural silk. The programme, which involves growing rice between young forest plants, has raised paddy production from 0.7 tonnes per hectare to 1.8 tonnes within two years.

The director-general of the United Nations Food and Agriculture Organisation had told the 1978 World Forestry Congress in Jarkata that the forest areas of developing countries are being destroyed at the rate of 16 million hectares a year. At the Nairobi conference, a paper by Professors Jon Morris and Keith Openshaw of the University of Dar-es-Salaam translated this into starker terms: "Every three years an area of forest is being lost that could supply in perpetuity the wood raw material requirements of 480 million people — enough to meet all the requirements of the African continent!"

Peasants and subsistence farmers in developing countries are on the horns of a dilemma, according to Morris and Openshaw. On the one hand population pressure, rising expectations and faulty agricultural practices are cuasing them to destroy the woodlands. On the other hand, they need the products of the forest for cooking, heating and shelter, so they must preserve the trees. "A combination of agricultural practices are causing them to most logical way to do this," they said, "— having one's cake and eating it. And yet it is not universally practised."

Morris and Openshaw examined the economics of growing trees for fuel and poles together with agricultural crops in Kenya, and concluded that it "appears to be a sound economic proposition". The cost of planting a half hectare of trees would be only 740 Kenya shillings (about £49) over a 10-year rotation, with an average yearly cost of 70 Kenya shillings ($4.7) while the financial yield would be approximately 12%, "well in excess of the minimum 8% that the Tanzania government expects from projects it invests in."

They concluded: "The farmer by growing his own wood products guarantees his energy, building materials and fencing requirements as well as improving his microclimate, soil fertility, fodder and food supply. He may even produce a surplus to earn cash income."



Integrating trees and animals: part of Indonesia's forest corporation

One of the surprising revelations of the conference was the extent to which agroforestry practices are already being carried on throughout the world, and the amount of research already being conducted. Countries in which agroforestry is extensively used include Indian, Bangaldesh, Sir Lanka, Thailand, and the Philippines in Asia; Trinidad, in the West Indies; Mexico and Costa Rica in Central America; Argentina, Brazil, Chile, Colombia, Ecuador and Peru in Latin America; and many countries in Africa, including some in the Sahel.

Agroforestry research programmes were described in a number of these regions. Dr Gerardo Budowski of Centro Agronomico Tropical de Investigacion y Ensenanza (CATIE), Costa Rica, said "agroforestry has made an imposing start in the region (Central and South America), and will undoubtedly move ahead, particularly in wet areas where trees seem to be particularly indispensible ingredients. However, at this stage, it appears that scientists and administrators are more moved by wishful thinking than hard evidence. The task of designing appropriate technologies for specific ecological, social and economic conditions still remains to be done."

Dr Budowski's concern about the need for hard data was a central one at the conference. Yet some projects are already trying to obtain it. Besides those undertaken by CATIE, one was described by Abdul Malik Choudhury, deputy chief conservator of forests for Bangladesh. A pilot scheme is underway there to settle 300 families through agroforestry practices. Each family is given five acres of land on which to grow bamboo and practise

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horticulture. They are paid for land clearance and given loans for inputs. A target of 9,000 acres is to be settled in this way in ten years, with an assessment after five years and an ultimate goal of 2.5 million acres if results of the pilot scheme are positive.

Although enthusiasm ran high for agroforestry, many voiced concern that too much might be expected of it, and were cautious about the prospect of its acceptance as a new discipline. One question raised was whether promotion of agroforestry practices would condemn subsistence farmers to a life of poverty. Dr King, whose agency went into operation only a year ago, answered that "Without inputs (eg fertilisers) they are condemned to a life of misery and mere subsistence. Don't think that if we could get them inputs we would be sitting here. You can grow anything anywhere if you have the money. It is precisely because farmers will remain at this level that some of us believe we could help them in a way that might not be so costly.

"Agroforestry is not the answer to all social problems. But I really think that, given the problems in some of these countries, it ought to be tried." □

Peer seeks to improve UK animal research law

LORD Halsbury's Animal Protection Bill successfully passed its first reading in the House of Lords on 16 July. Sometime after the start of the new parliamentary session on 23 October, it will receive its second reading. If it survives that hurdle, it will then be the subject for discussion of a Lords Select Committee before it is given its third reading and eventually handed over to the House of Commons for adoption as law — a procedure which could take up to two years to complete. Earlier this week, the Research Defence Society — which exists to promote the need for research with animals — held a press conference to mark the Bill's first successful reading.

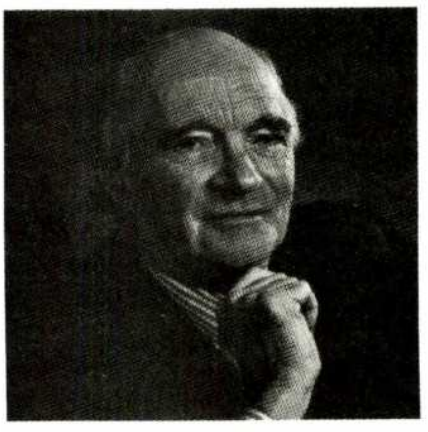
Lord Halsbury, who has been President of the Research Defence Society for twenty years and has been interested in the use of animals in research for much longer, decided to formulate and sponsor the new Bill about one year ago. A series of events had persuaded him that it was time the government "took over its responsibility" to research and opposed the allegations of cruelty to animals in many laboratories being expressed in some of the tabloid newspapers. His Bill is intended to replace the increasingly criticised Cruelty to Animals Act of 1876 as the main piece of UK legislation for ensuring the protection of laboratory animals.

Two events last year convinced Lord Halsbury that the 1876 Act — which, he says is "mostly criticised by those who don't understand it" — needed revising. One was an address to the Annual General Meeting of the RDS in which the speaker expressed his growing distaste at experimenting with animals. ("I realised that I felt that way myself", says Lord Halsbury). The other was last year's Labour Party conference which was attended by a number of slogan bearing anti-vivisectionists and resulted in the Labour government promising to set up a Royal Commission on Animal Protection to look at all aspects of animal rights (a promise which went by default when Labour lost the last election). Setting up a Royal Commission to tackle the problem would have been like "using a sledge hammer to swat a gnat", according to Lord Halsbury. The problems of animals on farms, in slaughter houses, in transit, in laboratories and as pests are all very different. In attempting to cover all five areas, the Royal Commission would be trying to be a "council of perfection".

In spite of his belief that the 1876 Act does work reasonably well, Lord Halsbury has come to the view that there is a need for more restrictive legislation. "Scientists must realise that they cannot live on the 1876 Act forever", he says.

His main criticism of the old Act is that it is too narrow; so his Bill is designed to extend the scope of legislation beyond vertebrates to include other species as and when protection is considered necessary "in the light of advancing scientific knowledge of the nervous systems of such species". Current legislation caters well for "cuddlesome pets". "But the octopus doesn't get protection. This is a highly intelligent creature — at least as intelligent as some of the minor vertebrates." Arthropods, such as crabs, also do not receive protection under the 1876 Act. "Do they have pain nerve endings under their shells?". The incorporation of new species on to the protected list would, under Lord Halsbury's Bill, be up to the relevant Secretary of State.

The new Bill would also extend the definition of a laboratory and an experiment. "All laboratory usage is included, even a green field". The word experiment has been deliberately excluded from the text of the Bill and replaced by "procedure" which should increase the number of animals entitled to protection. Lord Halsbury expects that the effect of this broadening of scope will be to increase the number of institutions applying for licences to work with animals.



Lord Halsbury: seeks support of scientists

The overall philosophy behind the Bill is not very different from that behind the 1876 Act. After all "nothing that's said now is any different from what was said then", according to Lord Halsbury who has been studying old Hansards to find out the arguments in vogue before the introduction of the first Act in the 1870s. There are differences in some of the detail, however. For example, the Bill gives power to the Secretary of State to "make regulations to control the procurement, breeding, transport, accommodation, management, veterinary supervision and care of animals for laboratory use". It also gives stronger powers to inspectors, including the right to enter premises licensed under the Bill and to order the killing of any animal thought to be suffering unduly. The Secretary of State would also be required to publish a 'guide to good laboratory practice' which would include advice on the use of alternatives to experiments with animals. The Advisory Committee to the Home Office would be extended to include advising the Secretary of State on novel or controversial procedures and on the incorporation of new species.

For the success of his Bill, Lord Halsbury believes that he must have the support of the scientific community. The Select Committee stage at the House of Lords should provide scientists and others with the opportunity to study the Bill and express their opinions. But the Bill should only be a "little more restrictive" for scientists ("there are very few people who do experiments on octopus"). The anti-vivisectionists, however, are bound to be critical. "The militants will misrepresent the Bill. They will say that the word 'experiment' was cut out to disguise what scientists are doing, whereas procedure has been used to get animals protected".

The UK has amongst the toughest legislation of any country on the protection of laboratory animals and Lord Halsbury believes that it should take a lead in the Council of Europe's deliberations to formulate European guidelines. "The government says let's wait and see what the Europeans do. But the UK should take the lead. I make up my own mind first and other people's later". **Judy Redfearn**

news in brief

Tropical legumes can aid Third World economies: A report issued by a panel of the US National Research Council describes almost 200 species of underexploited tropical legumes that could prove valuable to "the economies of developing countries." The legumes, which include root crops, pulses, fruits, forage crops, fast-growing trees, luxury timber and fibre and gum producing legumes, can reduce dependence on costly chemical fertiliser, widen the range of agricultural products and increase the production of food proteins. Of the thousands of known leguminous species only 20 are used extensively. The 200 plants selected by the panel could produce edible oils, nutritious forage, paperpulp and firewood as well as resins, tannin, spices and food. The panel notes: "Of all plants used by man, only the grasses are more important than the legumes. However while enormous resources have been expended in recent decades on grasses like rice, wheat, corn, sorghum and barley, among the legumes only soybeans and groundnuts (peanuts) have received much attention." The report is critical of the 'green revolution' for not having decreased protein malnutrition in the Third World and calls for an equal effort to be spent on the production of pulses which "could quickly eliminate protein malnutrition for the immediate future."

J.P.M. Brenan, Director of the Royal Botannic Gardens at Kew near London and a member of the NRC panel cautions that there are difficulties to be solved before legume production becomes a reality. These include studies to determine whether plant breeding techniques can work to select good strains, whether the plants can be adapted to arid and semi-arid tropical environments and whether the plants could become pests when introduced to new areas. "It's a very large programme and we are too small and too poorly funded to carry it out quickly" he said.

UK research and development funding falls below 1964 levels: Research and development expenditure trebled in the UK between 1964 and 1975 but fell in real terms, according to an article in *Economic Trends,* published by the Government Statistical Service. Using 1975 price as a base, the article says £2,200 million was spent on R&D by government and industry in 1964 but only £2,151 million in 1975. The number of people employed in R&D has also fallen. In 1967, industry had 219,000 researchers compared with only 180,600 in 1975.

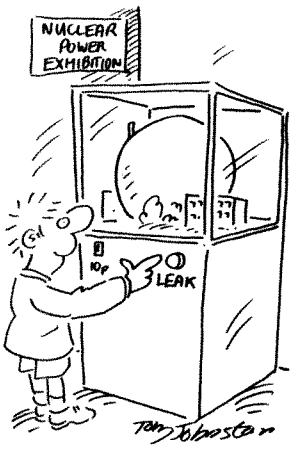
R&D expenditure peaked at the end of the 1960s and steadily fell from 1970 to 1975. Industry's expenditure fell particularly sharply in the early 1970s, whereas central government R&D expenditure rose between 1970 and 1975 but then fell from 1976-1977. In volume terms, however, R&D carried out by the government changed little, because between 1970-1975 the government financed more R&D actually carried out by other sectors. Defence research suffered the biggest cuts in central government spending falling from 0.86% of the gross domestic product in 1964 to 0.16% in 1977.

Wider brief for cholera lab: The Cholera Research laboratory in Bangladesh has been re-named the Internatioanl Centre for Diarrhoeal Diseases Research, putting an end to last year's rows over the laboratory's future. Research and training on the diarrhoeal diseases and their related nutrition and epidemiology will be carried out at the centre, and finance and organisation will be multi-national. David Bradley of the London School of Hygiene and Tropical Medicine said he was pleased the work done at the Cholera Research Laboratory would continue. "It is a lab which has done superb work in the past and much of our knowledge of cholera has come from there. Dacca is a good place to have the new centre. There are over 100,000 people to work on there in an area where cholera is highly endemic." The laboratory had been reconsitituted on a firmer international basis in order to expand its remit and funding.

China begins construction of its 50 GeV synchrotron: Surveying has begun near the Ming Tombs, Changing County, Beijing (Peking), for construction of China's first 50 GeV proton synchrotron. A preliminary design for the machine has been completed after several years of effort by 500 scientists and engineers in China. The final decision to build the facility was announced by vice-Premier Fang Yi in March 1978.

Since then about 100 factories and scientific institutions have been producing parts for the synchrotron. The Ministry of Metallurgy and the first and fourth machine-building industries, the High Energy Physics Institute of the Chinese Academy of Sciences and the Beijing Electrical Heavy Machinery Plant have already produced some of the required equipment.

China continues to push towards increasing its scientific and technical relationship with the West. At the Canton Trade Fair in April, China offered its nuclear instrumentation for sale abroad for the first time. A total of 14 equipment categories including instruments for handling radioactive isotopes and for measuring radioactivity, and remote handling devices, piping and valves for nuclear industry are being sold by the China Nuclear Equipment Corporation.



UKAEA launches informational campaign: The UK Atomic Energy Authority is opening a nuclear power exhibition in London beginning on 20 September for two weeks. Designed to take the "myth and mystery out of nuclear power" the exhibition will show that nuclear power is a tried and proven technology now providing 14% of the UK's electricity at a price that "industry can afford." A UKAEA official said the exhibition will present the facts about nuclear power with no need for a "recourse to opinion." The official told *Nature* that there will be no information about Three Mile Island in the exhibit as "UK reactors are not of that type." The Secretary of State for Energy will open the exhibition and scientists and engineers will be on hand to answer questions. A large number of schools is expected to organise parties to the exhibition.

NRC invites public submissions on Three Mile Island: The US Nuclear Regulatory Commission is inviting the public to help it decide a complicated legal question of liability in the Three Mile Island accident of last April. According to the provisions of the Price-Anderson Act claimants for damages caused by an "extraordinary nuclear occurrence" do not have to prove negligence on the part of the company if there was (a) a substantial release of radiation off-site and (b) there were substantial off-site damages. If the commission were to make these findings a claimant would only need to prove injury or damage, the value of the damage and that the damages were caused by the accident. If the commission does not make these findings, the claimant has to prove not only damages but also that the company was negligent. NRC staff have given preliminary advice that the radiation released appears to be several hundred times less than the criterion set in the Act.

Eruption over Atlantis: Soveit oceanographer Andrei Aksenov announced recently that Western news agency reports last March of Soviet claims to have discovered Atlantis were a "misunderstanding". The underwater photographs which gave rise to the reports (of walls and buildings) now prove to show Mount Ampere, a submerged volcano some 380 km south west of Portugal.

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correspondence

Dangers of low doses of radiation

SIR, — David Dickson's account (19 July, page 180) of the controversies surrounding the BEIR III report is evidently a fair and unusually competent job of science reporting. However, it should be made clear that the disagreement on the validity of the "linear hypothesis" when applied to carcinogenesis by low LET radiation does not, strictly speaking, concern the "filling" of a "gap". This terminology might be interpreted to indicate an interpolation while there is in fact the need for (much more uncertain) extrapolation. It is the magnitude of the risk at about 0.1 Gy (10 rad) that is in dispute. As stated by Dickson, there is agreement on even greater uncertainties at lower doses. I, for one, could not object to various surmises (including linear extrapolations) on what might happen below that level in view of the virtual absence of acceptable data on the carcinogenetic effects, if any, of such small doses.

By contrast, information on high LET radiations, and especially the data for Hiroshima, where neutrons plainly posed the principal hazard, indicates approximate linearity down to doses less than 0.1 Gy.

It should also be borne in mind that any fit of the data by linear or quadratic functions is probably at best a first approximation. Animal experiments show complicated — and sometimes inverse — correlations between radiation dose and carcinogenesis which vary not only between species and strains, but also between types of cancer. It would seem unrealistic to assume that the same does not apply to man.

HARALD H. ROSSI

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Nautilus records Earth's second moon

SIR, — I quite understand the need to close the debate on the fascinating Kahn-Pompea-Runcorn dialogue concerning the possibility that *Nautilus* has quietly recorded the lunar recession rate through the ages (31 May, page 452). However, it should be noted that (as in many debates) the participants, including *Nautilus*, may be arguing from different premises. That is to say, while Pompea, Kahn, and Runcorn are clearly discussing our present moon, let us say Luna, *Nautilus* may in fact have recorded the recession of a sister moon, let us say Selena, from Ordovician until rather recent geological time.

If Selena had been in orbit at 12 Earth radii in Ordovician time, as Pompea and Kahn propose for Luna, she could have dominated the *Nautilus* recording apparatus even with a mass much less than her sister Luna, who could well have been orbiting far out near her present position of 60 Earth radii. Perhaps a later Precambrian continental emergence had greatly increased tidal fricition, so that light-weight Selena, receding from the Earth at the required ten-times greater rate than that of present-day Luna, finally approached her sister too closely, and was either devoured by collision or ejected to the firmament. In any event, a small Selena could have orbited the Earth more closely, and have retreated much more rapidly than her larger sister, without dumping the excessive heat noted by Runcorn into the Earth-Luna system.

If Selena vanished before mid-Tertiary, then Runcorn's objection that there is no record of the maxiumum 33-day month which Luna should have produced at 45 Earth radii is of no concern; Luna would have been far beyond that orbit when she took over regulation of the Nautilian cycle, leaving every vestige of Selena forgotten except for a few shells and perhaps a very-young lunar mascon.

It is worth noting that H. Alfven and G. Arrhenius (*Evolution of the Solar System*, NASA, 1976) have proposed, by analogy with the Martian satellites, that Earth once had a second moon at a predicted orbital distance of 13.5 Earth radii, very close to the inferred Nautilian Ordocician value of 12. Perhaps Selena finally perished simply because Earth had oceans of water; the persistence of two Martian satellites may thus mean that Mars has never had an ocean at all.

HARMON CRAIG

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Nuclear power: an evil necessity for some countries

SIR, — In a letter under the title "Nuclear power does not lead to nuclear weapons" (5 July, page 10), Mr Greenhalgh, speaking for the nuclear lobby group "A Power for Good", argues that there is no direct link between peaceful nuclear power and nuclear weapons. It is tragic that in this day and age such absurd views should be advanced, which fly in the face of convictions held by the majority of nations. The Non-Proliferation Treaty and the safeguard systems of the International Atomic Energy Agency are the best evidence of the concern which people all over the world have for years felt about the link between nuclear power and nuclear weapons. More recently, and in recognition of the weaknesses and shortcomings of the NPT and the safeguards systems, a number of new initiatives were taken. The London Club was set up to provide additional guarantees. The International Nuclear Fuel Cycle Evaluation, with 56 nations participating, is still studying ways and means of reducing the danger of proliferation arising from the use of nuclear power. In the United States a special act was brought into being for the same purpose. Why would this enormous effort be needed if there were no danger of nuclear power leading to nuclear weapons proliferation?

Mr Greenhalgh bases his argument on past history, that countries developed nuclear weapons before starting nuclear power programmes. But the fact that something has not happened in the past cannot be used as an argument that it will not happen in the future, particularly if the opportunities for it were greatly increased. One of the chief characteristics of beings endowed with thinking power is that they can look ahead, anticipate possible trouble and take steps to prevent it.

A nation could, of course, obtain nuclear weapons by means other than power reactors, but the existence of one danger does not justify the introduction of another danger. Moreover, the setting up of a nuclear power programme, while not being a pre-requisite, is the most likely route to the acquisition of nuclear weapons, because the technology and the materials become legitimately available to a nation possessing nuclear facilities, including a reprocessing or an enrichment plant.

Mr Greenhalgh points to the existence of 100,000 MWe of nuclear power in the world as evidence of no direct link with nuclear weapons, but he forgot to add that this contributes less than 1% to the world's annual energy consumption. The situation would be radically changed if nuclear power increased by two orders of magnitude, as it would have to do (to meet the energy needs of the developing countries) in order to become the chief source of energy as envisaged by Mr Greenhalgh and his ilk.

Fortunately this is not likely to happen. The danger of proliferation, together with other well known negative aspects of nuclear power, as recently illustrated by the Three Mile Island accident, have now convinced public opinion that, at most, nuclear energy should be considered an evil necessity for some countries. Except for those with a vested interest, very few now believe that nuclear energy is a power for good.

J. ROTBLAT

London, UK.

Democracy cannot be defended by security police

SIR, — " 'Just imagine yourself sitting there below the judges' table . . . squashed like a lemon'. 'Just tell us, are you a communist?'. 'What do you think of the GDR?'. 'If as a christian you are an anti-fascist — should you not be anti-communist as well?' 'As a christian, you say love your neighbour. But how about the fascists?'."

These quotations come from the official report mentioned in *Nature* (31 May, page 363), which to your correspondent has been "cleverly invented by leftist totalitarians". They are in fact excerpts from the questions asked of Heinrich Haberlein, a pacifist who was found by the constitutional courts unfit to be a teacher, because "while not belonging to those groups hostile to the constitution, his employment could be rejected, if he, while not actively combating the free and democratic constitution, could be reasonably supposed to be simply indifferent towards the latter." (Page 37 of the DGVT report). I suppose that this is an example of what your correspondent calls the 'right to select', and it is of little surprise that the German Society for Behaviour Therapy's report carries the results of this policy.

Your correspondent seems to miss the point that you don't defend democracy by creating a vast security police to hound out 'totalitarian radicals' because you intimidate and pollute its concept.

C.N.M. POUNDER

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news and views

Stops and starts in microtubules

from Dennis Bray

THE conventional wisdom that microtubules run the entire length of nerve axons and dendrites probably comes from a paper by Weiss and Mayr (*Acta Neuropath.* supp V, 198; 1971). On page 200 these authors conclude that "each tubule is a single unitary entity, extending in uninterrupted and undivided continuity from its base in the cell body to its peripheral tip". Clear enough one would think, but alas, the data are far less so. The evidence amounts to no more than the approximate constancy of tubule number in cross sections of six rat motor axons taken before and after bifurcations — a result that could obviously have arisen in several ways. Tubular continuity was presumably chosen as the explanation because it was consistent with the prevailing view of axonal dynamics. Tubules were believed to be synthesised at the soma and pushed out into the process; they were the continuous rails on which the fast transport ran. The whole story made such a neat package that it would have been churlish to worry about such corollaries as that the tubules in many parts of the mature nervous system would then be metres long. Best not to think of the recurrent laryngeal nerve of the giraffe.

Questions of continuity and point of origin also exist, although in a less extreme form, in non-neuronal cells. In fibroblasts, microtubules wander within the cytoplasm and can be seen by immunofluorescence as fine threads in tangled skeins. The method is sensitive enough to detect single tubules (Osborn *et al. J. Cell Biol.* **77**, R27; 1978) but cannot resolve those in bundles. In consequence it is impossible to say in most preparations where the tubules begin and end. In 1976, however, three laboratories described a way to cut this Gordian knot. The tubules are depolymerised with colchicine or other antimitotic agent, the drug is washed out, and the cells fixed as the tubules begin to grow back again and then stained with antitubulin. When this is done the reforming tubules are first found in discrete star-like bodies in the cytoplasm, usually close to the nucleus (all of this applies to interphase cells; mitosis is different). The tubules lengthen from these centres until eventually the original network is regained. There is usually one, sometimes two, of these centres in a cell and it seemed most reasonable to suggest that they were the centrioles or cytocentres which have sometimes been seen by electron microscopy within arrays of microtubules in interphase cells. The implication was then that in such cells all tubules lead to the cytocentre which serves as a nucleation point for their growth. This was analogous to the role of the nuclear assembly point envisaged for the nerve cell.

The picture at this time was simple and satisfying. There were one or two structures in a fibroblast, epithelial cell or neurone which are responsible for the initiation and subsequent growth of all the microtubules in that cell. A simple picture, satisfying — and shortlived. A few months ago in *Cell* (**16**, 239; 1979) Spiegelman and colleagues showed that a minor variation in the staining procedure can produce not 1 or 2, but 5 to 10 centres in the average fibroblast, all close to the nucleus and each with 10–30 radiating microtubules. The main change in procedure was a previous extraction with non-ionic detergent and the new result was attributed to the loss of background staining — the new centres emerging from the background like stars at dusk, so to speak. Even worse, the same approach when used on human fibroblasts or epithelial cells showed one or two major centres together with many single points so small that they seemed little more than individual tubules growing in the cytoplasm. There are a number of imponderable factors, as always. Is it possible that the preparation of cytoskeletons might disrupt the organising centres and scatter the newly growing pieces through the gutted cell? Or, to go back a step, how do we know that the recovery from antimitotic agents will follow the same course as growth in a healthy cell? Within the limitations of the method, however, the picture obtained by immunofluorescence is of multiple nucleating centres, possibly of several distinct types.

Back at the nerve cell, a cultured neuroblastoma when treated by the above procedure shows a single organising centre at the base of its one or two neurites (Spiegelman *et al. op. cit*). Dividing neuroblastomas look more like a fibroblast in that they have an average of 12 smaller sites distributed around the nucleus. Obviously as the cell 'differentiates', the multiple centres coalesce to a single one and from a timed series of specimens Spiegelman *et al.* show that this occurs, on average, before the neurite grows out. The intriguing possibility is thereby raised that this coalescence in some way instigates neurite outgrowth. At any event, the tubules in this study are still firmly anchored to organising centres, small or large, in the soma.

The hint that even this tenet might be on shaky ground existed in an ultrastructural analysis of crayfish nerve (*J. Neurocytol.* **3**, 73; 1974). Nadelhaft found that sections of the same axon at different points along its length could show a significant variation in microbutule number. His polite, but firm conclusion was that the hypothesis of microtubular continuity could not be universally valid. A conclusion that received a thumping validation in the July issue of the *Journal of Cell Biology* (**82**, 278; 1979). Here Chalfie and Thomson present a serial analysis of certain axons in the world's most photographed worm. These axons are notable for their distinct tubules which are larger than tubules elsewhere in the animal and very highly ordered. Serial sections show major fluctuations in tubule number along the length of these axons and frequent terminations. The tubules are in short lengths, between 6 and 27 μm, distributed in staggered arrays along the axon. Even more interesting is the observation that the two ends of each tubule are not the same. The distal end, furthest from the cell body, terminates in a cloud of light staining that is always right up against the plasma membrane. The proximal end can be anywhere in the cytoplasm but is characterised by central density in the tubule. Assuming that these features are not freaks of these specialised axons — and breaks can be found in other tubules in the animal — the Weiss and Mayr picture of continuity is roundly wrong. And the question of how microtubules grow, and from where, in neurones as in other cells, is back to the starting point. Perhaps the rabid reductionists are right and we will have to wait until the analysis of tubulin polymerisation *in vitro*, under controlled conditions and with defined components, provides the answer. There have been many such studies and they have revealed a rich and multifarious set of phenomena. It is true that they have yet to point to a single mechanism, but that is another story. □

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Conformational flexibility in protein molecules

from Robert Huber

IN describing protein molecules the term flexibility is used with quite different meanings. A precise definition for a particular protein molecule would require the determination of the number, energy and geometry of the various stable conformational states the molecule might have, the kinetic parameters of interconversion and the thermal motion of all the atoms in each conformer: a considerable task, requiring a great variety of experimental techniques, if feasible at all. Several rather different experimental approaches have provided evidence that protein molecules are indeed flexible: deuterium–hydrogen exchange under non-denaturing conditions[1], fluorescence quenching of internal tryptophan residues by oxygen diffusing into the protein molecule[2], ESR, NMR, and fluorescence depolarisation. These last spectroscopic techniques allow one to determine the mobility as a function of temperature of certain residues whose spectroscopic signal can be identified. The power of these methods is clearly demonstrated by recent experiments, using NMR techniques, which show that the aromatic rings of tyrosyl and phenylalanyl residues in pancreatic trypsin inhibitor (PTI) flip[3-5]. Model calculations show that aromatic ring flipping is a consequence of substantial breathing motions of the protein, which include movement of the polypeptide backbone[6, 7]. The mobility of tryptophanyl residues has been probed, using very short and intense light pulses to measure fluorescence depolarisation[8]. Motions in the picosecond time range are found, but the degree of flexibility is different for different protein species, some being rather rigid, some soft. Recent molecular dynamics calculations on PTI[9] show structural fluctuations in the picosecond range of a size in rough agreement with the observed crystallographic temperature factor[10] and promise to provide the theoretical background to motions in proteins. However, the computational problems are enormous and, so far, allow the analysis of a small protein molecule such as PTI for a few picoseconds only. This makes extrapolation to equilibrium properties and comparison with the time-averaged crystal structures problematic, as does the neglect of solvent effects in calculations performed to date. More refined dynamics calculations are currently being pursued.

Protein crystallography as a means to gain information about flexibility is unique in providing us with an overall view of the molecule in contrast to the spectroscopic techniques which focus on a particular label. However, there are a number of theoretical and experimental problems. As the physical diffraction process is instantaneous compared with lattice vibrations, the 'temperature' factor determined crystallographically does not differentiate between thermal motion and static disorder. Static disorder may have components from conformational micro-heterogeneity of the protein molecules (substates or microscopic states) and from lattice defects. The interesting parameters, thermal motion and substates are difficult to determine accurately and still more difficult to separate from one another. The experimental problem has been partly overcome since the development of techniques for refinement of protein crystal structures, some five years ago.

Protein structures were successfully refined[10-12] using principles borrowed from small molecule crystallography, extensively modified and adapted to the much larger problem[13]. From these studies it was obvious that the 'temperature' factors of individual atoms were important variables for the correct description of the protein structure. However, the values were regarded with some suspicion, as they changed from atom to atom in a rather erratic way due to the limited resolution of the diffraction data. But, when the 'temperature' factors were averaged for rigid groups and smoothed along the main and side chains, physically reasonable features were obvious: 'temperature' factors increased in external polypeptide loops and external side chains were more mobile than internal ones.

Several examples of protein crystal structures are now known where disorder (either dynamic or static) is a dominating aspect: antibody molecules [14, 15], trypsinogen [16], citrate synthase[17], tobacco mosaic virus protein[18, 19] and tomato bushy stunt virus[20]. In these molecules substantial parts show no significant electron density indicating disorder in the crystalline state. In some of the molecular species rigid states are known with different functions, providing evidence for the functional significance of large scale disorder[21]. Except in the case of TMV protein, where NMR experiments have demonstrated increased thermal mobility of the disordered segments[22], it is unclear which type of disorder (conformational heterogeneity or thermal motion) prevails in these examples. Lattice defects involving the molecule as a whole are excluded, of course, as most parts of the molecules are well ordered.

Two reports in this issue of *Nature* are centred on the question of conformational disorder and its static and dynamic components determined by crystallographic methods in two different systems: lysozyme (Artymiuk *et al.* page 563) and myoglobin (Frauenfelder *et al.* page 558). Two different lysozyme species crystallising in different crystal forms have been carefully refined and atomic 'temperature' factors determined. When these were averaged for the main chain atoms of each residue a close correspondence between the two crystal structures was obvious, suggesting that the variation in 'temperature' factor along the chain is a molecular property and not seriously influenced by crystal packing. A detailed mathematical analysis indicates a rigid body translation and libration of the molecule as a whole, overlaid with additional flexibility of some external loops. Among these are the loops determining the substrate binding cleft in lysozyme. A positive correlation between ordered secondary structure and 'rigidity', and between shielding of amino acid side chains and their 'mobility' is obvious. The slightly enhanced 'mobility' of the substrate binding area is intriguing, in particular, if it were to be found that these segments 'rigidify' on substrate binding; one wonders why nature utilises such small effects, having in mind the drastic segmental flexibility in the examples discussed before. Artymiuk *et al.* do not and cannot dissect the 'temperature' factors observed into their static and dynamic contributions. This problem is the main object of the paper by Frauenfelder *et al.* who analysed and carefully refined

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myoglobin crystals at various temperatures from 300 to 220 K, with the ultimate goal of differentiating static and dynamic disorder by their low temperature limit. As protein crystals contain solvent which may not be allowed to freeze, the temperature range that is covered is small as is the effect on the 'temperature' parameters determined.

The principal result of Frauenfelder *et al.*, that 'temperature' factors are increased in the peripheral parts of myoglobin and correlate with the secondary structural elements, agrees with the observations on lysozyme and other proteins discussed. The authors also follow and compare the variation of the 'temperature' factors with temperature for internal and peripheral atoms: they make the novel and interesting observation that internal atoms 'freeze' more than peripheral ones. This leads the authors to the view that conformational heterogeneity (substates) is a dominent type of disorder, at least for the outer parts of myoglobin. As the temperature range covered is small and the effects measured marginally above the level of significance, it would be highly desirable to back up these results by going to substantially lower temperatures, perhaps using the shock-freezing technique developed by Parak *et al.*[23, 24]. These authors had shown by Mössbauer absorption spectroscopy on myoglobin crystals that about half of the crystallographic 'temperature' factor is due to static disorder. Mössbauer absorption measurements are insensitive to static disorder as long as the equilibration between different conformers is slow compared with the life time of the excited Mössbauer nucleus. The question of whether a protein molecule is to be regarded as a vibrating aperiodic crystalline solid or a 'semiliquid' 'glass', characterised by a larger number of substates of similar energy, is a topic of lively discussion at present. Of course, there is clear evidence for alternative side chain conformations in protein crystals (see for instance Epp *et al.*[24] who observed conformational heterogeneity of an internal cysteine residue; or His E7 in erythrocruorin (Steigemann & Weber[25])). However, the uneasy feeling of a protein crystallographer about the 'semiliquid', 'glassy' protein picture comes from the fact that proteins are tightly packed[26], multiply linked and cross-linked molecules which may have problems finding efficient alternative packing except, of course, for residues and loops interacting with solvent. It would, perhaps, be appropriate to defer these arguments until it is possible to define more precisely the extent to which substates exist in a particular type of disorder. The differentiation between a single vibrating conformer and an assemblage of a huge number of closely related substates separated by low energy barriers and in rapid equilibration becomes a matter of semantics, except close to 0 K, a rather unphysiological temperature. □

Meteoroid fragmentation in the auroral zones

from David W. Hughes

SATELLITE measurements of the micrometeoroid dust particles in the Earth's vicinity have produced many interesting results in the last two decades. One of the more fascinating observations has come from experiment S 215 on the HEOS-2 spacecraft. Not only did this experiment measure the normal sporadic flux of these particles but it also found groups and swarms of micrometeoroids which had apparently been produced as lunar ejecta, and also as the debris from the electrostatic disruption of larger meteoroids.

H. Fechtig, E. Grün and G. Morfill of the Max-Planck-Institute for Nuclear Physics, Heidelberg, have produced a detailed analysis of these results in a recent edition of *Planetary and Space Science* (**27**, 511; 1979). We can dismiss the sporadic particles fairly quickly. They arrive randomly, (as expected) the number observed per unit time obeys Poisson statistics (as expected) and the flux is what one would expect from the zodiacal dust cloud, allowing for the enhancement produced by the gravitational field of the Earth.

A sizeable fraction of the observed particle events did not occur randomly, however, but came in correlated bursts, leaving the researchers with the problem of finding the sources of the particles responsible for these bursts. Two sources immediately sprang to mind — because there were two types of bursts. Swarm bursts lasted for less than 40 min and the time interval between consecutive particles was less than 10 min. This was taken to indicate that the source was comparatively close by. Group bursts had durations between 40 min and 13 h and the time interval between consecutive particles was less than 6.5 h. The source of the particles responsible for the group bursts was obviously further away.

A meteoroid impacting on the Moon will produce a crater. Ejecta from this crater will consist mainly of lunar crust material, some of it having a velocity in excess of the lunar escape velocity, while the velocity dispersion will also be of the order of the escape velocity. The HEOS-2 satellite is in an Earth orbit with a perigee of 10,000 km and an apogee of 244,000 km. The lunar ejecta has to travel about 384,000 km to reach the satellite, by which time it will have spread out in space as a result of the velocity dispersion. Reasonable assumptions show that it will take about 7 h to sweep past the spacecraft. The experiment was working between 7 February, 1972 and 2 August, 1974 and during this time 19 group bursts were seen, individual groups taking between 40 min and 13 h to pass the spacecraft. Fechtig, Grün and Morfill tried to correlate the observation times of the groups with the occurrence of seismic events on the Moon recorded by apparatus left behind by the Apollo astronauts. There were ten times more seismic events than group events. This fact is not too disheartening, however, for fast ejecta would leave the impact point at an angle of less than 10° to the surface, so only the lunar rim regions can provide ejecta detectable at the spacecraft.

The source suggested for the swarm bursts is the Earth's auroral zones. About 30% of the meteoroids in space with masses above 100 g have densities less than 1 g cm^{-3}, and these meteoroids are thought to be fragile, crumbly, loose conglomerate bodies made up of collections of individual particles in the micron and even submicron range. In interplanetary space meteoroids are positively charged with potentials in the range from +3 to +20 V. The main cause of this is the photoelectric effect. Solar quanta of sufficient energy (more than about 10 eV) may dislodge electrons from the surface layers of the meteoroid and sufficient numbers of these electrons escape the Coulombic back-attraction to leave the meteoroid positively charged. This charge is somewhat reduced by the capture of solar wind electrons and there are other much less dominant effects such as proton capture, secondary electron emission, sputtering and thermionic emission that can be neglected.

However when this meteoroid enters the ambient plasma of the Earth's magnetosphere at an altitude of about 60,000 km it quickly becomes negatively charged. The dominant charging process here is collection of electrons and protons from the Maxwellian plasma. Remember that the thermal electron and proton velocities are much greater than the meteoroid velocity. The majority of electrons and protons that hit the meteoroid stick to it. Also a meteoroid of radius greater than 10^{-5} cm can completely stop electrons of energies less than 10 keV and protons of energies less than 70 keV. S.P. Wyatt (*Planet. Space Sci.* **17**, 155; 1969) estimates that an iron, nickel or silicate meteoroid in sunlight at the Earth's orbit would be losing about 2.5×10^{10} electrons per second per cm^2 due to the photoelectric effect, so this has to be taken into account too.

Fechtig, Grün and Morfill consider two very dissimilar regions in the magnetosphere — first, the plasmasheet, a region outside the dipolar field regime. Here the electron number densities are

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between 0.1 and 10 per cm^3 and the electron and proton energies are about 1 and 10 keV respectively. The second region is the inner magnetosphere, in the field line regime connected to the auroral zones. Here, especially during geomagnetically active times, the fluxes can be as high as 2×10^{10} particles cm^{-2} s^{-1} and the electrons have energies of about 40 keV. Although meteoroids seem to traverse the first region without harm, dust grains larger than 0.002 cm radius can be charged to very high potentials in the second region.

The authors estimate exactly how high this potential is from the HEOS-2 results. The internal disrupting stress, due to electrostatic repulsive forces, which acts on a meteoroid is proportional to the square of the ratio of surface potential to radius. For example, a surface charge of -500 V will break up a 100 μm diameter dust ball, a 1 μm diameter meteoroid made of compact stone or a 0.05 μm iron particle. The fragments of this electrostatic explosion have a dispersion velocity inversely proportional to the square of the radius. Now the swarms observed by HEOS-2 are made up of fragments of radii about 10^{-5} cm (mass about 10^{-14} g) and the maximum time taken for a swarm to pass the satellite was 90 min. An analysis of the fragment dispersion indicated that the mean surface potential required to break up the meteoroids was about -600 V and 90% of the meteoroids producing swarms broke up at potentials in the range between -300 and -960 V. It was obvious that in most cases the breakup only took place after the meteoroid had entered the inner dipolar field region of the magnetosphere, the region in which intense fluxes of energetic auroral electrons have been observed. A particle of radius r will charge up to -600 V in 0.13 r^{-1} s on entering the auroral zone.

The swarm data indicate that the primary particles are interplanetary in origin. From the area of the detector, the exposure time and the total number of swarms observed, the authors have estimated what percentage of the large interplanetary meteoroids incident on the Earth's atmosphere actually break up as they pass through the auroral zone. The swarm parent bodies have estimated masses in the range 10 g to 10^6 g. When the flux of swarm-producing meteoroids is compared with the flux of similar mass objects that produce lunar seismic events and fireballs visible to the Prairie Network of all-sky cameras, it is found that only between 1 and 10% of the incident bodies actually fragment into micron and submicron particles at auroral zone heights.

The parent bodies are from the same group that produce type III fireballs having low densities (<1 g cm^{-3}), low geocentric velocities (< 20 km s^{-1}) and orbits with semi-major axes around 1 AU. So in these few incidences the Earth's atmosphere is not hit by a single large meteoroid but is peppered by millions of submicron particles which will not produce meteors in the atmosphere but will simply be retarded and then drift slowly to the ground. Fragmentation takes place at a height of about 10 Earth radii. The average meteoroid observed was moving at around 8 km s^{-1} when it broke up and the fragments produced dispersed at about 1 km s^{-1}, thus peppering an area of about 10,000 km in diameter, of the same order of magnitude as the cross sectional area of Earth.

Fechtig, Grün and Morfill are continuing their research and expect to see similar effects in the vicinity of Jupiter which has a strong magnetosphere and a superfluity of moons. They have already designed a suitable dust experiment for inclusion in the Jupiter Orbiter Mission scientific package. □

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Superfluid vortex waves

from P.V.E. McClintock

RADIO frequency vortex waves have been detected on quantised vortex lines in superfluid ^{4}He, according to a report by R.A. Ashton and W.I. Glaberson of Rutgers University in a recent issue of *Physical Review Letters* (**42**, 1062; 1979).

Vortex lines form in liquid ^{4}He if attempts are made to rotate a container of the liquid when it is below its superfluid transition temperature of 2.2 K. In this respect, as in many others, the superfluid behaves in a manner strikingly different from classical liquids. Whereas an ordinary liquid can rotate with its container much in the manner of a solid body (apart from acquiring a parabolic upper surface) superfluid helium is unable to rotate. In fact, for low enough angular velocities of the container, the superfluid apparently remains at rest relative to the fixed stars and does not rotate at all, no matter how long the experimenter rotates the container. If the container is speeded up, however, a single vortex line eventually appears; but the liquid still does not rotate as a whole. The vortex line is a microscopic quantal analogue of the eddy which is often seen as water runs out of a bath plug-hole; it is a line, parallel to the axis of rotation of the container, around which the liquid circulates with a tangential velocity which is inversely proportional to the distance from the axis, or core, of the vortex.

Speeding up the rotation of the container still further causes additional vortex lines to appear, all identical, because each one is singly quantised. At 1 rev s^{-1}, there are about 10^4 cm^{-2}. Since each line exists in the flow field of all the others, the whole array of lines rotates at the same rate as the container, thus to some extent simulating solid body rotation of the liquid as a whole and producing what appears to be a conventional parabolic surface (although, on the microscopic scale, one expects dimples where the cores of the lines touch the surface). Support for this general picture comes from a number of sources, but the most direct evidence is from the beautiful experiments performed at Berkeley by Packard and his co-workers, who have managed to take photographs of the vortices. One of their latest results (Yarmchuk, Gordon & Packard *Phys. Rev. Lett.* **43**, 214; 1979), reproduced in Fig. 1, provides a striking illustration of the structure of the rotating liquid.

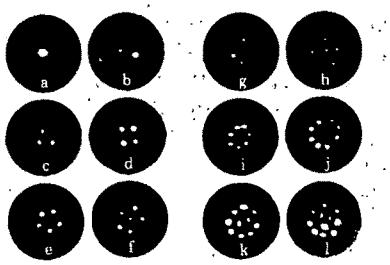


Fig. 1 Photographs of stable vortex arrays, looking along the axis of rotation. The angular velocities with which the 2 mm bucket was rotated varied from 0.3 rad s^{-1} to 0.86 rad s^{-1}.

The lines are potentially capable of supporting vortex waves: travelling helical deformations in which each element of line moves in a circle, though in the opposite sense to that of the vortex flow field. The phenomenon has been known for some time in relation to classical fluids, the first detailed study being due to Kelvin (*Phil. Mag.* **10**, 155; 1880). Early attempts to observe vortex waves in helium were based on torsional pendula, and were restricted to frequencies below 1 Hz; they yielded somewhat ambiguous results. More recently, however, it has been suggested that vortex waves of much higher frequency might be both excited and detected with the aid of negative ions.

Much of our knowledge of the quantised vortices has, in fact, been discovered through ion experiments. The negative 'ion' in liquid helium — in reality an electron confined within a 20 Å radius bubble — is easily trapped on a vortex line. Although it cannot escape in the direction perpendicular to the line, it readily slides along it under the influence of an electric field and, by measuring the velocity of the ion as a function of field, it has been possible to deduce information about the properties of the vortex itself. (The photographs in Fig. 1 were taken by shooting ions right off the top of the vortex lines to strike a detector placed above the liquid surface, thus revealing the position of each line.) Halley and Cheung suggested

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(*Phys. Rev.* **168**, 209; 1968) that one way of exciting vortex waves might be to subject the line to a transverse oscillatory electric field while ions were travelling along it. This resonant excitation of vortex waves would have a corresponding effect on the motion of the ion. To achieve it, several conditions must be fulfilled. In particular, the ions should be moving along the line at the same speed as the propagation velocity of the vortex wave corresponding to the frequency of the applied electric field. Halley and Osermeier were subsequently able to calculate in detail (*J. Low Temperature Phys.* **26**, 877; 1977) how the velocity of the ion might be expected to vary with the d.c. electric field propelling them along the line. They predicted that there should be a kink in the velocity-field characteristic at a certain critical velocity, which depends on the frequency of the transverse oscillatory electric field.

Ashton and Glaberson have now performed this experiment, using a rotating ^{3}He cryostat in which the ^{4}He sample can be cooled to 0.3 K while being rotated at speeds of up to 10 rad s^{-1}. The transverse electric field was circularly polarised, so that resonant vortex wave production was expected to occur for only one polarisation for a given sense of cryostat rotation. Their experimental results are shown in Fig. 2. It is quite clear that the radiofrequency (RF) electric field has a considerable influence on the ionic velocity V, and that entirely different effects are obtained when the applied field is changed from a clockwise (CW) to a counterclockwise (CCW) sense relative to the rotation of the cryostat. An anomalous kink and plateau occurs in the velocity at about 3.6 m s^{-1}, in tolerable agreement with a theoretical prediction of 3 m s^{-1} for the RF frequency being used (10 MHz). This result can be regarded as a vindication of the principal conclusions of the Halley and Ostermeier theory. But the theory does not give a satisfactory account of the detailed shape of the velocity-field curve; nor is it able to explain the reduced values of V found in the CW rotation.

A good deal more work, both theoretical and experimental, will be required to sort out the details. Even on the basis of the present results, however, it seems clear that high frequency vortex waves really do occur in superfluid ^{4}He. □

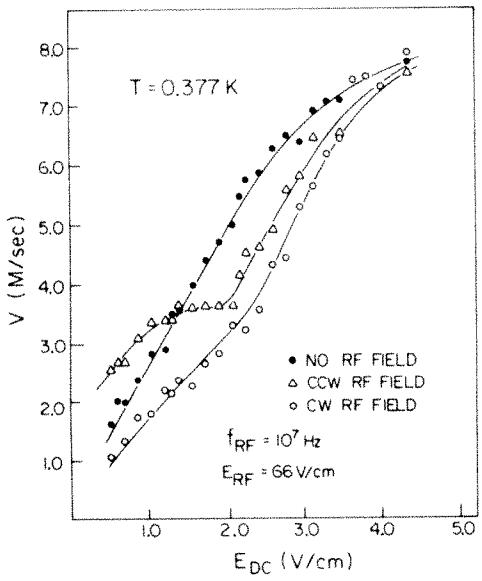


Fig. 2 Measurements of the ionic velocity V along a vortex line as a function of the longitudinal electric field E_{DC}: for zero applied radiofrequency (RF) field; and with the latter field polarised clockwise (CW) or counterclockwise (CCW) relative to the rotation of the experimental cell. The anomalous plateau and kink at about 3.6 m s^{-1} in the CCW curve is interpreted as evidence of a coupling of the ions to vortex waves.

Erratum

IN the article 'Hypercycles and the origins of life' in last week's *News and Views* (*Nature* **280**, 445; 1979), two lines were inadvertently transposed from the foot of the second column on page 445 to the middle of the second paragraph in the third column. Starting from the last line of column 2 the correct reading is "Then before long only one quasi-species would remain — GOD only, or THE only, if short words replicate faster." and in the middle of paragraph 2, column 3, the correct reading is "I_1 and I_2 are two RNA quasi-species. Both the + and − strands must be good replicators."

The cladistic debate continued

The critical view of the cladistic method of classification taken by L.B. Halstead in his report of a meeting on Vertebrate Palaeontology published in News and Views *some months ago (***276**, *759; 1978) and the rejoinder by a group of supporters of 'cladism' (***277**, *175; 1979) provoked a large correspondence, a selection of which is published below.*

There can be few disagreements on methodology in the recent history of biology which have been pursued with such passion by the protagonists, while leaving biologists not involved (in this case in systematics) so unmoved by, or even ignorant of, all the fuss. There is fruitful territory for psychologists here, but I would like to confine myself to the methodological rather than the theological aspects of the affair.

The fundamental tenet of cladism, is that in systematic biology the reconstruction of phylogeny is logically prior to the construction of a classification and that the latter is a redundant expression of the former. Unlike all other taxonomists, the cladists characterise phylogeny solely as the result of speciation or cladogeneses (the splitting of one species into two or more in evolution). They ignore the results of evolutionary change within evolving species (phyletic evolution or anagenesis). Furthermore in reconstructing phylogeny they allow division only by dichotomy: that is, speciation, according to them, is always the splitting of one species into two daughter species, sister species of one another. Primitive cladists held this as a dogma, which, with our current knowledge of evolutionary genetics, is patently absurd. Sophisticated advocates use it only as an operational principle. Nevertheless the rationale of the method depends on evolution by speciation which results in reproductive isolation between sexually reproducing organisms. Cladistic classification is therefore inapplicable to non-sexual organisms and will presumably never be used for microorganisms such as Protozoa, bacteria and viruses.

The recognition of sister species (or groups of higher rank) is by shared derived characters unique to the sister groups and thus presumed present in their (always hypothetical) unique common ancestral species. Shared primitive characters, those also present in collateral groups outside the pair, are ignored. Unfortunately if a large number of character states is considered, as it should be by any competent taxonomist, contradictions will arise due to convergently evolved characters mimicking true synapomorphies (shared derived ones). The method of resolving this difficulty destroys the whole rationale of the method of recognition of sister groups. The inconsistencies are resolved by using the principle of parsimony. By parsimony cladists mean that the sister-grouping which yields the smallest ('most parsimonious') number of contradictory characters compared with the number of apparent shared derived characters, *of those the cladist has studied,* is the correct one. This preposterous assumption could only be true if, first, an organism (be it lungfish, salmon or cow) could be analysed into a finite number of discrete characters, and, second, if a competent cladistic taxonomist could be reasonably sure that the tiny number of shared characters he can study in that organism has a similar ratio of true derived to convergent characters as does the class of all its characters.

Admittedly, traditional and also phenetic ('numerical') taxonomists use parsimony in an informal way, but they also use the criterion of predictability, perfectly described in the last century by John Stuart Mill, as an extrinsic criterion to test their classifications. Despite the fact that many cladists pay lip-service to the hypothetico-deductive method usually associated with the name of Sir Karl Popper, their only test of a cladogram appears to be a comparison, based on parsimony, with other contradictory cladograms. It is perhaps for this reason that a number of incompatible schemes for the mutual relationships of the major groups of fish-like vertebrates have been proposed by cladists, without any successful criterion within the system to choose between them.

At the risk of earning the opprobrium of your cladistic correspondents, I should like to end by pointing out that the problem I have touched on was well known to that very pre-Darwinian taxonomist Aristotle. In *de Partibus Animalium* (Book 1, paragraphs 2-4) he notes that a dichotomous classification based on a single character state will inevitably lead to an artificial classification (as it did recently when one eminent palaeontologist used it in the fundamental division of the Class Mammalia), but that the use of several character states leads to contradictions which cannot be resolved.

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Cladism defended

THE contribution of L.B. Halstead to your journal was so misinformed about the assumptions and benefits of phylogenetic analysis that we are compelled to comment. A thorough presentation of phylogenetics ('cladistics') is inappropriate, but we do wish to make some remarks.

Phylogenetic methods are used to determine the most parsimonious arrangement of the patterns of character distribution among taxa (groups of organisms). These patterns are assumed to be the results of evolutionary processes and to reflect natural, monophyletic groups. The result of the analysis is a cladogram, a branching diagram with each branch justified by a character thought to be a unique evolutionary innovation. It should be noted that these diagrams are in fact hypotheses about relative recency of common ancestry among the taxa considered. 'Relationship' is thus defined by a genealogical criterion rather than such vague concepts as proportion of shared genes, as evidenced by supposed morphological similarity, or estimation of overall morphological similarity.

Dr Halstead's remarks about the phylogenetic system being designed such that fossils cannot be included is simply uninformed: many phylogeneticists have dealt with this issue, including one of us (E.O.W.) who published a phylogenetic analysis of fossil and Recent gar fishes.

Dr Halstead seems to be most interested in those evolutionary changes that 'allow' organisms to enter new 'adaptive zones', concepts widely used by grade-oriented systematists. He states, "the fact that many parallel changes can be related to adaptations to similar conditions, is more significant than listing trivial trademarks from which cladograms can be constructed." But, with some reflection, that statement yields a simple fact: parallel changes in evolution are not discernable, indeed are incomprehensible, without a genealogical hypothesis of relationship. Put simply, how can we know that similarity shared between two organisms is a parallelism without a phylogenetic tree which demonstrates that the character was not, in fact, found in the common ancestral species of the two taxa? This concept is so basic that we find it difficult to understand the misconceptions concerning it. Further, the "trivial trademarks" which mark monophyletic groups are often the very characters which are associated with new "adaptive zones" (for example, feathers of birds and mammary glands of mammals).

Dr Halstead states that if one could trace the ancestry of man back in time to a rhipidistian fish, the whole sequence of changes would, by definition, be a single monophyletic species. He adds that "most zoologists and palaeontologists" would surely use arbitrary divisions to partition that lineage. This misses another basic biological fact. The lineage is broken up, from the Devonian to the Recent. If this were not so, we would observe only one tetrapod species, *Homo sapiens*, and not the diversity of land vertebrates that we observe today. (Wiley (*Syst. Zool.*, 11-21, 1978) has shown that conclusions such as Halstead's stem from an artificial concept of classification.)

Since "the issue was summed up for many by R. Parrington's exasperated exclamation that according to the cladists a lungfish is more closely related to a cow than to a salmon," it is appropriate to end with a comment on that statement. It is not the cladist who 'makes' a lungfish more closely related to a cow than to a salmon any more than it is a cladist who 'makes' *Hyracotherium* more closely related to *Equus* than to Eocene condylarths. Evolutionary history as exemplified by genealogical descent with modification (a Darwinian concept) 'makes' it so. Lungfishes and cows are more closely related to each other than lungfishes are to salmons because lungfishes and cows share a common ancestor that is not shared with salmons. That this is somehow 'wrong' indicates a confusion on the part of critics about the meaning of genealogical relationship. If cladograms, with their clearly defined data and clearly stated hypotheses, "are difficult enough for experts in the field to comprehend fully," then we suggest that the shortcomings lie with the 'experts' and not with phylogenetic methods or their results.

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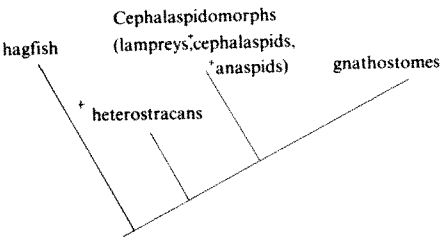
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As a non-participant in the scientific meeting of which Dr Halstead's report has stirred such sharp reactions in your columns, but as a previous exponent (*Classification and Biology*, Heinemann, 1970) of the principles excoriated by Halstead under the name of 'cladism', I would like to address four questions to him. First, would he agree that there is not, and probably cannot be, an objective measure of 'degree of similarity' between organisms in respect of phenotypic characters of the type studied by museum systematists, and in particular that the 'unit character' of the numerical taxonomists has proved to be undefinable? Second, would he accept that the most fundamental type of similarity or difference between organisms is that of their genotypes, as represented primarily by their nuclear DNA? Third, would he accept that we now have objective methods of measuring DNA similarity, either directly (by DNA hybridisation and similar techniques) or indirectly (by comparative protein studies) and that the range and reliability of data from these techniques is likely to increase? Fourth, if by such methods it could be shown that a lungfish was 'nearer' to a cow than to a salmon, would Dr Halstead then be prepared to accept the 'cladist' case in this instance. And if the answer to this question were "yes", would this alter his general attitude to phylogenetic classification in the sense proposed by Hennig (and myself)?

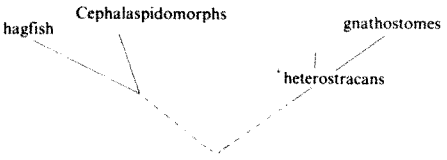
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HALSTEAD states that the phylogenetic ideas illustrated in the "...cladograms presented by P. Janvier (University of Paris) on agnathans and B. Gardiner (Queen Elizabeth College, London) on choanichthyans ... were demolished within hours of their being proposed". I wish to reply to this statement, which is designed to dismiss cladistics in general and Dr Gardiner and myself in particular.

I proposed the following phylogeny based on cladistic methodology.



An objection was raised by Dr Halstead who did not agree with the proposed affinities of the Heterostraci, a group of poorly known fossil agnathans. He pointed out that heterostracans probably had paired nostrils and nasal sacs (diplorhiny) and were therefore related to gnathostomes and not to other agnathans which are monorhinous. This information is supposed to demolish my phylogeny and to support his own 'evolutionary tree' given below (with permission):



We agree that the diplorhinal condition is a primitive craniate character-state. Therefore, Halstead must believe that primitiveness is a criterion by which we recognise relationship. As a cladist I cannot accept this view.

If heterostracans did have paired nostrils (and not all palaeontologists accept this interpretation) then I have to recognise that monorhiny arose twice; in the hagfish and in cephalaspidomorphs. To counter this objection I could lean on detailed embryological and anatomical information to argue that the hagfish monorhiny had arisen independently of that seen in the lamprey. But this approach is both fruitless and unnecessary in the light of other information which I presented. Furthermore, basing one's case on whether heterostracans were monorhinal or diplorhinal is a particularly unfortunate tactic. Heterostracans are represented by thin bony shields and the interpretation of the soft anatomy is governed by the recent model we choose. This is amply demonstrated by the differing views of heterostracan morphology proposed by Stensiö[1] and Halstead[2] for instance.

Unless Halstead can provide derived character-states to show that heterostracans are more closely related to gnathostomes than to other agnathans then I submit that the 'demolition' amounts to no more than a dislike of my idea because it differs from his.

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2. Halstead, L.B. *Biol. J. Linn. Soc. Lond.* 5: 339-349 (1973).

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review article

Superheavy-element research

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The existence of an island of relatively stable elements beyond the present Periodic Table has been predicted by theoretical extrapolations of nuclear properties. During the past 12 years vigorous efforts have been made to discover these superheavy elements in nature and to produce them by nuclear reactions.

ALTHOUGH the existence of chemical elements much heavier than the known elements had already been considered more than two decades ago[1-3], these early studies did not receive much attention because for the heaviest known elements half lives in radioactive decay and production rates in nuclear reactions decrease steeply with increasing atomic number. Thus, there seemed no hope of proceeding to much heavier elements. In 1966, Myers and Swiatecki[4] pointed out, however, that the stabilising effects of proton and neutron shell closures should be strong enough for certain elements beyond the explored Periodic Table to ensure a local stability against spontaneous fission comparable to or even greater than that of uranium. Furthermore, Meldner and Röper[5] predicted that the next proton shell closure should occur at atomic number 114, a region not too far from the already synthesised heaviest elements.

The first detailed theoretical investigations of nuclear properties in this region[6-8] revealed the topology of an island of relatively stable nuclei (Fig. 1) as a consequence of shell closures at atomic number 114 and neutron number 184. The elements forming this island are called[3] superheavy elements. First estimates indicated half lives comparable to or even larger than the age of the Earth for nuclides in the centre of the island. Thus, superheavy elements could even exist in nature. The predicted long half lives encouraged many groups to search for such elements in terrestrial and extraterrestrial samples. The first negative results were published in 1969 by Thompson *et al.*[9,10] who looked for element 110; the first positive results were reported for element 114 in the same year by Flerov and Perelygin[11] but later work showed that the background induced by cosmic rays had been underestimated in the latter measurements. First attempts to synthesise superheavy elements in the laboratory by fusion of ^{248}Cm with ^{40}Ar projectiles had been reported a year earlier, again by Thompson *et al.*[10,12]. In contrast to the search in nature, attempts to produce such elements by nuclear reactions can presently be carried out at only a few places, namely with the heavy-ion accelerators at Berkeley (USA), Dubna (USSR) and Darmstadt (FRG).

Here the most recent results will be discussed. Detailed treatments of earlier and recent[16] work can be found elsewhere.

Nuclear and chemical properties

The decay mode ultimately terminating the Periodic Table will be prompt fission of the atomic nucleus. In terms of the well established liquid-drop model of the nucleus, the stability of heavy nuclei is a delicate balance between two forces, the short-range attractive nuclear force between nucleons and the long-range repulsive electrostatic force between protons. In the heaviest known nuclei the attractive force dominates slightly, leading to a fission barrier which keeps the nucleus together. However, an excitation energy of only about 6 MeV, small compared with the total potential energy of 1,800 MeV of such nuclei, is sufficient to overcome the fission barrier, as evidenced for example by the slow-neutron fission of ^{235}U and ^{239}Pu. Furthermore the nucleus may tunnel through the barrier from its ground state. This process, called spontaneous fission, becomes more and more likely towards the end of the present Periodic Table. Extrapolation of the liquid-drop model beyond the known elements shows that the fission barrier should vanish in the region around element 114 leading to prompt fission. Only the action of nuclear shell closures will overcome this trend by keeping the nuclei spherical and stiff against deformation, producing a fission barrier which is now entirely due to shell effects[17].

Most theoretical studies[17,18] agree that the next shell closures beyond the existing elements should occur at proton number $Z = 114$ and neutron number $N = 184$. Hence a maximum

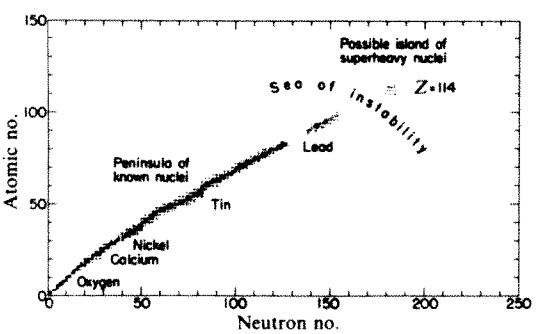


Fig. 1 Location of the island of superheavy elements relative to the peninsula of known nuclei. The heaviest points represent naturally occurring nuclei, the medium-weight points nuclei with half lives greater than 1 yr, and the lightest points nuclei with half lives less than 1 yr. The labelled elements correspond to nuclei with closed shells (from ref. 13).

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stability against spontaneous fission is expected for the isotope of element 114 with mass number $A = 298$. In addition to spontaneous fission, other decay modes must be considered—α decay, β^- decay and electron capture. Whereas the spontaneous fission half lives decrease from their peak value at $^{298}_{114}X^{184}$ rather uniformly in all directions, the α decay half lives show a rather continuous decrease with increasing atomic number so that this decay mode already dominates around element 114. Hence the longest overall half lives are shifted to lower atomic numbers. The other two modes, β^- decay and electron capture, keep the neutron-to-proton ratio of the most stable superheavy nuclei within a certain region. Figure 2 gives two examples of overall half lives calculated for superheavy nuclei with even proton and neutron numbers. The results[19] presented in Fig. 2*a* show an island with a maximum half life of 10^9 yr for $^{294}_{110}X^{184}$. A broad shore to the west is important since in the synthesis of superheavy elements by heavy-ion reactions, one approaches the island at this side. According to a more recent calculation[20] (Fig. 2*b*) the shape of the island remains nearly the same but the half lives decrease by orders of magnitude with a maximum value of 10^3 yr for $^{296}_{112}X^{184}$. Odd proton or neutron numbers should increase the half lives but the effect is expected to be small, about one order of magnitude[19]. A rather different and more unfavourable shape of the island was obtained in another calculation[21] yielding a steeper decrease at the west side due to the considerably lower fission barriers. For example, the 1 s contour line is already crossed at neutron number 180 for the elements 106–114. The longest half life, 10^5 yr, is calculated for $^{294}_{110}X^{184}$. In any discussion of half lives one should keep in mind the large uncertainties of about a factor of $10^{\pm 10}$ for spontaneous fission and $10^{\pm 3}$ for α and β decay half lives[13].

After evidence that elements around atomic number 126 exist in nature[22], the question of a proton shell at this number, in analogy to the well known neutron shell at $N = 126$, was reconsidered. Most calculations show[18,23,24] that the shell effect at proton number 126 should be small but stability in this region may be due to a strong neutron shell at neutron number 228.

When superheavy nuclei are formed in nuclear reactions, they carry a certain excitation energy and rotate with a certain angular momentum. Both these effects decrease the effective fission barrier. As theoretical calculations[25,26] indicate, the barrier will disappear at about 50 MeV excitation energy and 30 units of angular momentum.

In addition to the half lives for different decay modes, the energy released in these processes is of interest since it forms the basis for specific and sensitive detection techniques. The average total kinetic energy of fragments formed in spontaneous fission of superheavy elements (200–230 MeV) and the number of neutrons being evaporated from the fragments (about 10) should exceed[27,28] the corresponding values for any long-lived lighter nucleus. Hence fission events with high total kinetic energy and high neutron multiplicity should constitute a characteristic fingerprint for superheavy elements. Recent experimental data on fission of heavy nuclei indicate that the kinetic energy may even be higher, about 270 MeV, but the neutron multiplicity may be lower, about five neutrons per fission[29]. Calculated α-decay energies[19] are 6–10 MeV and lie within the range of other natural and artificial α emitters.

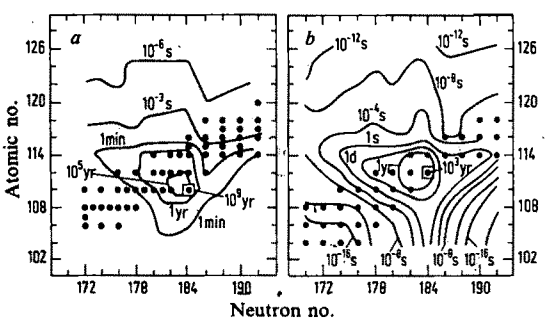


Fig. 2 Calculated half lives for superheavy nuclei shown as contours of constant overall half life plotted versus proton and neutron number. The points indicate nuclei calculated to be stable against β decay and electron capture: *a* is from ref. 19; *b* from ref. 20.

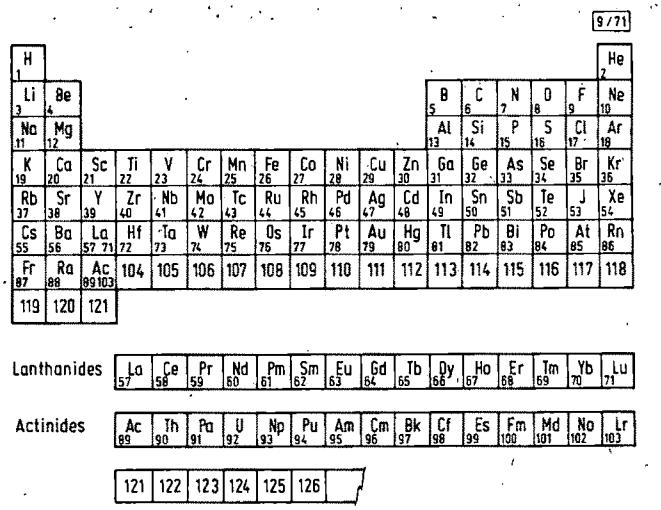


Fig. 3 Continuation of the Periodic Table of the elements into the region of superheavy elements according to quantum-mechanical calculations of their ground-state electronic structures.

The chemistry of superheavy elements was discussed[30,31] on the basis of their position in the Periodic Table and the systematic trends of chemical properties within the Table. Calculations of their ground state electronic structure[30,31] with the usual quantum-mechanical methods support a continuation of the Periodic Table as shown in Fig. 3. Accordingly, element 110 is expected to be a homologue of platinum, element 112 a homologue of mercury, and element 114 a homologue of lead. Element 126 should belong to a group of super-actinide elements in which the inner 6f and 5g electron shells are filled, and may thus behave like plutonium. The predicted high volatility of several superheavy elements[30–33] is interesting and may be enhanced by relativistic effects so that elements 112 and 114 might be expected to be volatile liquids or even gases as a consequence of relativistic stabilisation of the closed $7s^2$ electron shell in element 112 and a relativistic splitting between the $p_{1/2}$ and $p_{3/2}$ energies giving the filled $p^2_{1/2}$ orbital in element 114 the character of a closed electron shell[33]. Such predictions of chemical behaviour are relevant for the selection of natural samples in a search for superheavy elements and form the basis of chemical separation schemes applied in their isolation from such samples and from irradiated targets[31,32,34,35].

Atomic binding energies for inner-shell electrons, and hence transition energies of characteristic K- and L-series X rays have also been calculated with high accuracy[36] together with X ray transition rates[37]. Observation of a series of such characteristic X rays with the predicted energies and relative intensities in a sample would be considered as strong evidence for the presence of superheavy elements. General criteria for the discovery of new elements have recently been proposed[38].

The search in nature

If superheavy elements exist in nature they were probably produced in supernovae by a sequence of neutron captures and β^--decay processes, the latter increasing the atomic number stepwise. It is still debatable whether this *r*-process proceeds into the region of superheavy elements since it has to pass through a region of nuclei having a low fission barrier. After a neutron is captured in such a nucleus, the excitation energy gained from the binding of the neutron may exceed the fission barrier so that prompt fission will occur. Precise treatments of the *r*-process in the very heavy element region are hampered by

the comparable magnitudes of neutron binding energies and fission barriers and by the need to extrapolate over a large, unexplored region. Most of the recent studies consider the production of superheavy elements in the *r*-process to be unlikely[39–41].

The large uncertainties in the predicted half lives as well as the open question of production in nucleogenesis have not discouraged experimentalists from examining hundreds of samples for superheavy elements, including lead from old cathedrals, manganese nodules from the ocean floor, water from hot springs, meteorites, and samples from the lunar surface. Extremely sensitive techniques were developed for such studies[42], most of them being based on the direct or indirect detection of fission events. In counting untreated natural samples detection limits of 10^{-12}–10^{-14} g of superheavy elements per g of sample can be achieved for superheavy nuclei of 10^9 yr half life. The most common methods are counting of fission fragment tracks produced by radiation damage in glass, mica or plastics, and counting of neutrons emitted in the fission process. The sensitivity of such methods can be increased by chemical enrichment and mass separation. In an extreme case[43] samples collected during the industrial processing of 10^3–10^4 tons of lead were further concentrated by chemical and mass separation, and a detection limit of 10^{-19}–10^{-23} g per g was estimated for element 114.

Most of these searches produced negative results, but there are some observations which deserve attention and we report here on three: the hot spring water from the Cheleken peninsula, the Allende meteorite, and the Madagascan monazite.

In the hot springs from the Cheleken peninsula in the Caspian Sea a spontaneously fissioning activity was observed after passing about 2,000 m^3 of water through 850 kg of an anion exchange resin[44]. This particular spring water is known to be rich in volatile elements and is thought to collect material escaping from large depth in the Earth's mantle. Part of the resin was eluted with water and different acids followed by precipitation steps in the eluates. The samples were inspected with neutron-multiplicity counters which record the number of neutrons emitted in time coincidence. These neutrons are traced back to spontaneous fission events in the sample. The rates, about 0.5 fissions per day for a 9-kg sample of the resin and 5 fissions per day for the precipitates isolated from 170 kg of the resin, are much higher than what has been reported previously for any other sample inspected in low-background conditions. This allowed measurements of the neutron multiplicities with good statistics. Figure 4 compares the multiplicity distribution obtained[44] by summing the data for the resin and precipitate samples with the known distributions for several heavy nuclides and with some hypothetical distributions. Evidently the measurements rule out ^{238}U but agree with heavy actinides such as ^{252}Cf as a possible source of neutrons which may be present in the sample due to contamination in the laboratory or by fallout from nuclear weapons tests. From a small fraction of the combined precipitates, actinide elements were chemically isolated and inspected for α activity. Flerov *et al.*[44] argue that from these measurements, actinide nuclides except pure ^{252}Cf can be excluded as the source of the neutrons. Further experiments seem to be necessary in order to identify the spontaneous fission activity in the Cheleken hot spring waters.

Evidence for now-extinct superheavy elements in a certain class of primitive meteorites was presented by Anders *et al.*[45,46]. These carbonaceous chondrites contain xenon fractions with an excess of neutron-rich xenon isotopes. Such isotopes could stem from spontaneous or neutron-induced fission but the $^{136}Xe/^{132}Xe$ ratio observed is much larger than can be accounted for by any known fission reaction. The concentration of this fission-like xenon in different meteorites was observed to be correlated with the concentration of volatile elements such as thallium, bismuth and indium[45,46]. Fission xenon plus volatile elements were found to be strongly enriched in a mineral fraction comprising less than 1% of the meteorite and consisting of carbon, chromite, spinel and an ill-defined mineral, probably a sulphide[47,48]. From the parallelism in the condensation history of the fission-like xenon and of volatile elements as inferred from such data, it was concluded that the neutron-rich xenon isotopes stem from spontaneous fission of a volatile superheavy element, most likely from element 115, eka-bismuth, but elements 114 and 113 are other possible candidates[48]. Alternative explanations have been proposed mainly because the anomalous xenon fractions are also enriched in neutron-deficient isotopes[49,50] and similar enrichments both of neutron-excess and neutron-deficient isotopes are also found for other elements[51]. It has also been pointed out[52] that the predicted yields of xenon isotopes in spontaneous fission of superheavy nuclei would not fit the observed isotopic ratios. Whereas Anders *et al.* consider the progenitor of the fission-like xenon to be extinct, the Dubna group has reported weak spontaneous fission activities, about one event per month and kilogram, in several meteorites including Allende[53–55]. An attempt is being made to concentrate the activity from Allende by evaporation–condensation techniques to determine its atomic number[56].

Unexpectedly, evidence for element 126 and possibly 124 and 127 in nature was reported in 1976 by Gentry *et al.*[22] who examined small monazite inclusions surrounded by giant haloes in Madagascan biotite. Radioactive haloes[57] are spherical zones of discolouration of the host mineral caused by radiation damage due to α particles emitted from the central inclusion. Cuts through such haloes show a circular ring structure corresponding to the ranges in the host mineral of the α particles from different members of the radioactive decay series descending from uranium and thorium deposited in the inclusion. In the giant haloes, however, the ranges correspond to α-particle energies around 14 MeV which are much higher than the energies of any known natural α-particle emitter but agree with predicted α-particle energies in the region of element 126. By bombarding the inclusions with a sharply collimated proton beam, characteristic X rays of the elements contained in the inclusions were excited and small peaks were seen at the expected positions of the $L\alpha_1$ X rays of elements 126, 124 and 127. The concentrations for these elements as estimated from the peak intensities were surprisingly high, about 10–100 p.p.m. However, the strongest peak attributed to element 126 could be accounted for by a nuclear γ ray from the $^{140}Ce(p, n)^{140}Pr$ reaction already occurring at the proton energy chosen[58], and other peaks could be assigned to K X rays of ordinary elements such as antimony and tellurium[59]. The X ray spectra of the inclusions were remeasured[60,61] with a different technique using a tunable monochromatic X-ray source for excitation, thus circumventing the interference by proton-induced nuclear reactions and by excitation of the mentioned ordinary elements

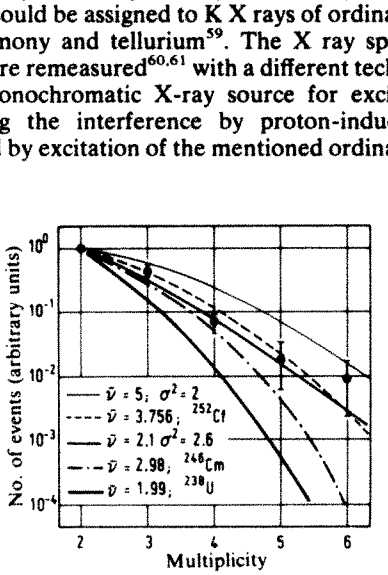


Fig. 4 Spontaneously fissioning activity in hot spring water from the Cheleken peninsula. Multiplicity distribution of fission neutrons emitted from samples obtained by ion-exchange and precipitation methods (●) compared with the measured multiplicity distribution for various actinide nuclides and with calculated distributions. The inset gives the corresponding values of the average number $\bar{\nu}$ of neutrons per fission and of its variance σ^2 (from ref. 44).

because of the differences in absorption edges. No evidence for superheavy element $L\alpha_1$ X rays was found[61] at a concentration level about a factor of 100 below that previously reported[22]. Although Gentry[62] agrees that the evidence was not confirmed, other members of the original group[22] argue that problems in reproducing the earlier measurements are due to spatial inhomogeneities of the superheavy element distributions in the samples and to their volatility[63,64]. Attempts to enrich superheavy elements from bulk monazites of the same origin by mass separation[65] and chemical separation[66] have failed. Surprisingly, evidence for high-energy α rays necessary to understand the giant haloes has recently been reported for the Madagascan monazite[67] in contrast with other negative findings[68,69]. There seems to be no convincing evidence that giant haloes have anything to do with superheavy elements but none of the alternative explanations[62] for the origin of these haloes has generally been accepted.

Searches at accelerators

Heavy-ion reactions seem to be the only practical way of producing superheavy elements in the laboratory. As shown in Fig. 1, one would jump in one step from the peninsula of known nuclei to the superheavy island. In heavy-ion reactions[70], three principal mechanisms play a part. The first is an exchange of a few nucleons between the two colliding nuclei in a rather distant collision and is called quasi-elastic transfer. The other two processes occurring in closer collisions are outlined[71] in Fig. 5 with their characteristic time scales in units of 10^{-21} s. The upper branch illustrates complete fusion[72] of the reacting nuclei to form a nearly spherical, excited compound nucleus which gains its energy from the fact that generally more kinetic energy is required to overcome the mutual electrostatic repulsion between the interacting nuclei than is consumed in the fusion process. The excitation energy is large enough to evaporate from the compound nucleus several neutrons, protons, or α particles followed by emission of γ rays to reach the ground state of the final evaporation residues which have the same or somewhat lower atomic number than the compound nucleus. For heavy compound nuclei, fission into two fragments of comparable size competes with evaporation and dominates for the heaviest known nuclei. Fusion to a compound nucleus occurs preferentially in reactions with relatively light projectiles such as carbon, oxygen or argon.

The third process[73–75] is shown by the lower branch of Fig. 5. The two interacting nuclei stick together for a very short time, about 10^{-21} s, forming a composite system which separates again into two nuclei roughly identical with the interacting nuclei in the entrance channel. In spite of the short contact time, the kinetic energy of the projectile can be completely transformed into internal excitation and into rotation, and hence the process is called strongly damped or deep inelastic collision. During such a reaction a substantial number of protons and neutrons can be transferred between the interacting nuclei. The reaction

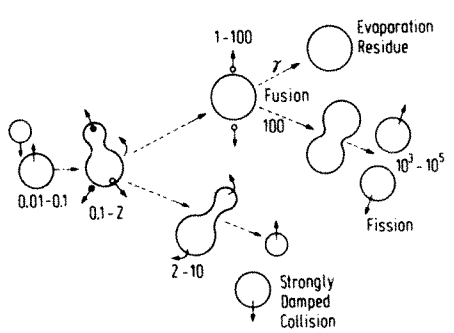


Fig. 5 Interactions between heavy nuclei leading to fusion into a compound nucleus (upper branch) and to damped collisions via a composite system (lower branch) with characteristic times in units of 10^{-21} s (after Brix[71]).

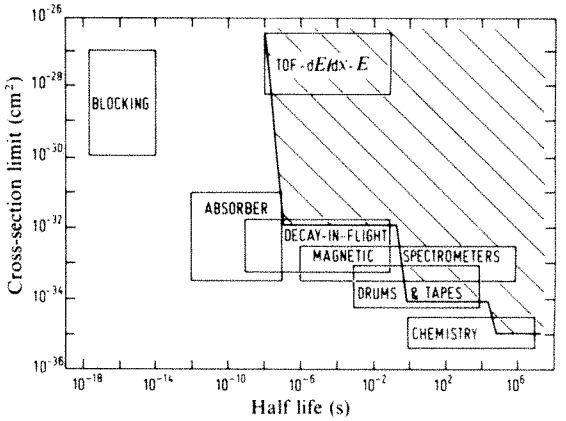


Fig. 6 Half-life region covered by various techniques applied to or applicable to the search for superheavy elements at accelerators, and upper limits for production cross-sections achievable with these techniques (from ref. 78).

products de-excite by emission of neutrons, protons, and γ rays and, in heavy systems, by fission. Although this novel type of nuclear reaction was discovered two decades ago[76,77] it has received widespread interest only recently, after its important role in the interaction of heavy nuclei was recognised.

A wide variety of techniques has been developed and applied in searches for superheavy elements at accelerators[78]. Figure 6 shows that techniques applicable to very short-lived nuclides are in general less sensitive than those suitable for longer half lives. Chemical methods combined with fission-fragment detection reach down to production cross-sections of 10^{-36} cm^2 corresponding to production rates of about one superheavy atom in 10^{12} nuclear collisions, but require half lives of at least a few seconds. Gas-jet systems for rapid transport of the activity from the production site to the detector system extend the half life range to shorter half lives by about one order of magnitude. Mechanical transport systems like tapes, drums or disks which are applicable to species of 10^{-3} s half life are even faster. The region down to 10^{-6} s is the domain of instruments based on the deflection of reaction products in magnetic and electric fields. Measurements of the time-of-flight (TOF) for a given distance and of the specific energy loss plus total energy ($dE/dx - E$) are widely used in studying heavy-ion reactions to obtain mass and atomic number distributions of the reaction products. When applied to superheavy nuclides they permit the detection of species with half lives down to 10^{-8} s. Detection of fission fragments emitted in flight from a superheavy nucleus passing a gas-filled tube extends the half life range which can be covered by one order of magnitude. By stopping superheavy atoms in a metal foil and observing their fission fragments in a direction perpendicular to the original flight path, nuclei with half lives down to 10^{-12} s can be detected. The extreme region, 10^{-14}–10^{-18} s, becomes accessible by the blocking technique in which the reaction products are produced in a single crystal and the probability of escape of their fission fragments is measured for different crystallographic directions, indicating the distance traversed by the fissioning species in the crystal; thus the half life can be deduced since the velocity of the recoiling nucleus is known.

Because most of the heaviest synthetic elements were produced by heavy-ion fusion reactions it was quite natural to use fusion in attempts to synthesise superheavy elements. The problem with this approach lies in the extreme neutron excess of the most stable nuclei in the centre of the island which makes it impossible to reach those nuclei by any realistic fusion reaction. For example the first attempt[10,12] to produce element 114

$$^{248}_{96}Cm^{152} + ^{40}_{18}Ar^{22} \rightarrow ^{288}_{114}X^{174}$$

leads to a hypothetical compound nucleus with 174 neutrons only, which should further evaporate four neutrons due to its

excitation energy yielding the isotope $^{284}_{114}X^{170}$ far away from the $N = 184$ neutron shell. After detailed half life estimates for superheavy nuclei became available it seemed to be possible to overcome this problem by overshoot reactions, that is by producing nuclei beyond the region of highest stability but with rather high neutron number which should then decay by electron capture (EC) towards the centre of the island. To illustrate this a reaction is considered which has a crucial role in attempts to synthesise superheavy elements:

$$^{248}_{96}Cm^{152} + ^{48}_{20}Ca^{28} \rightarrow ^{296}_{116}X^{180}$$

After evaporation of four neutrons the following decay chain would be expected, with half lives generally increasing along the chain[19]:

$$^{292}116^{176} \xrightarrow{\alpha} ^{288}114^{174} \xrightarrow{EC} ^{288}113^{175} \xrightarrow{EC} ^{288}112^{176}$$

the latter nuclide should decay by spontaneous fission with a half life of ~1 h.

Table 1 surveys fusion reactions which have attempted to produce superheavy elements. The upper limit of the production cross-section is listed together with the half life range covered and an indication of whether chemical separation methods were applied. Table 1 re-emphasises the dilemma of fusion reactions: in the vicinity of element 114 the achievable neutron numbers are far below 184 whereas this closed neutron shell can only be reached with atomic numbers as high as 122. A more detailed analysis of these reactions in terms of excitation energy of the compound nuclei and survival against fission in the neutron evaporation chain can be found in a recent review[90].

Among the systems listed in Table 1 the reaction between ^{248}Cm and ^{48}Ca has been studied the most intensively since this reaction provides the closest approach to the island if both the proton and neutron numbers are considered. Experiments with this reaction are difficult, however, since neither the target, the artificial nuclide ^{248}Cm with 3.6×10^5 yr half life, nor the projectile ^{48}Ca, which has to be enriched from its low natural abundance of 0.19%, is generally available. The results reported so far for the ^{48}Ca-on-^{248}Cm reaction are summarised in Fig. 7 where the upper limits for production cross-sections are plotted against the half life range covered with a particular technique. A variety of techniques has been applied. The Dubna group[81] (D in Fig. 7) applied two different chemical separation methods and inspected the samples by spontaneous fission (SF) and α-particle counting (α). At Berkeley (BL) two different chemical procedures (chem) were used[91] and an experiment designed to detect extremely volatile elements during and after bombardment was performed (gas)[92]. Attempts were also made to detect short-lived species by counting without chemical treatment the catcher foils in which the reaction products recoiling out of the target were collected (foils)[91]. In further experiments, the recoil atoms were stopped in a gas and transported with a gas jet to a rotating-wheel counting system (VW)[93]. Finally spontaneous-fission decay in flight was searched for (dif)[93].

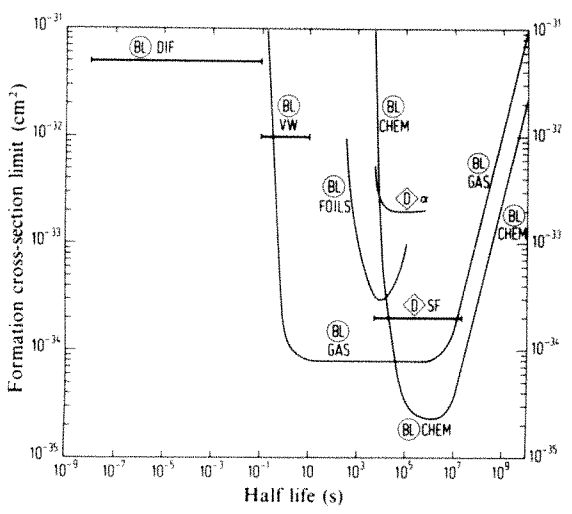


Fig. 7 Upper limits of the production cross-section for superheavy elements in the reaction of ^{248}Cu with ^{48}Ca versus the half-life range covered with different techniques. The data are from work of the Berkeley–Livermore group[91–93] (BL) and the Dubna group[81] (D); for details see text.

Table 1 Attempts to synthesise superheavy elements by complete fusion reactions

System*	Compound nucleus	Upper limit for production cross-section‡ (cm²)	Half life range covered	Ref.
$^{232}_{90}Th + ^{48}_{20}Ca$	$^{280}110^{170}$	3×10^{-35}	$\geq 3 \times 10^{-3}$ s	79
$^{231}_{91}Pa + ^{48}_{20}Ca$	$^{279}111^{168}$	4×10^{-35}	$\geq 3 \times 10^{-3}$ s†	79
$^{233}_{92}U + ^{48}_{20}Ca$	$^{281}112^{169}$	7×10^{-35}	≥ 2 h†	79
$^{248}_{96}Cm + ^{40}_{18}Ar$	$^{288}114^{174}$	2×10^{-32}	10^{-9} s–1 d	10, 80
$^{242}_{94}Pr + ^{48}_{20}Ca$	$^{290}114^{176}$	1×10^{-35}	2 h–1 yr†	81
$^{243}_{95}Am + ^{48}_{20}Ca$	$^{291}115^{176}$	2×10^{-35}	2 h–1 yr†	81
$^{246}_{96}Cm + ^{48}_{20}Ca$	$^{294}116^{178}$	2×10^{-35}	2 h–1 yr†	81
$^{248}_{96}Cm + ^{48}_{20}Ca$	$^{296}116^{180}$	See Fig. 7		
$^{208}_{82}Pb + ^{84}_{36}Kr$	$^{292}118^{174}$	1×10^{-30}	$\geq 6 \times 10^{-7}$ s	82
$^{208}_{82}Pb + ^{86}_{36}Kr$	$^{294}118^{176}$	6×10^{-35}	3×10^{-3} s–100 d	83
		1.5×10^{-30}	2×10^{-9} s–5 h	84
$^{238}_{92}U + ^{59}_{27}Co$	$^{297}119^{178}$	4×10^{-33}	1 s–30 h†	85
$^{238}_{92}U + ^{(60)}_{28}Ni$	$^{298}120^{178}$	2×10^{-33}	1 s–30 h†	85
$^{238}_{92}U + ^{(65)}_{29}Cu$	$^{303}121^{182}$	8×10^{-33}	1 s–30 h†	85
		2×10^{-33}	2 h–1 yr†	86
$^{238}_{92}U + ^{65}_{29}Cu$	$^{303}121^{182}$	4×10^{-34}	2×10^{-6} s–10 h	87
$^{232}_{90}Th + ^{76}_{32}Ge$	$^{308}122^{186}$	1×10^{-34}	5×10^{-3} s–1 yr	88
$^{136}_{54}Xe + ^{170}_{68}Er$	$^{306}122^{184}$	1.5×10^{-33}	2×10^{-6} s–10 h	87
$^{238}_{92}U + ^{76}_{32}Ge$	$^{314}124^{190}$	1×10^{-34}	5×10^{-3} s–1 yr	88
$^{243}_{95}Am + ^{68}_{30}Zn$	$^{311}125^{186}$	5×10^{-32}	10^{-9} s–5 d	89
$^{232}_{90}Th + ^{84}_{36}Kr$	$^{316}126^{190}$	5×10^{-30}	$\geq 6 \times 10^{-7}$ s	82
$^{238}_{92}U + ^{84}_{36}Kr$	$^{322}128^{194}$	8×10^{-29}	$\geq 6 \times 10^{-7}$ s	82

* If the projectile beam was not isotopically clean the mass number used here is in parentheses.

† Denotes studies in which chemical separation methods were applied at least in part of the measurements.

‡ Note that in calculating cross-section limits different authors use different assumptions about the width of the excitation function for the nuclear reaction involved; this introduces an uncertainty of about one order of magnitude[86].

Why these experiments have failed has been discussed in detail by Seaborg *et al.*[90]. The bombardments were carried out at an average energy exceeding the coulomb barrier by a factor of 1.09 resulting in some 40 MeV of excitation energy of the compound nucleus. Based on the measured production cross-section of the fusion product $^{254}_{102}$No in the ^{48}Ca + ^{208}Pb reaction, a fusion cross-section of at least 10^{-27} cm² was estimated for the ^{48}Ca–^{248}Cm system. Thus the reasons for negative results were sought in the evaporation chain. Using two sets of calculated fission barriers the overall survival probability was estimated. Whereas the optimistic set[19] predicts that 96% of the compound nuclei should arrive at $^{292}_{116}X$, the more pessimistic set[21] predicts less than 4×10^{-9}%, corresponding to a production cross-section of the order of 10^{-38} cm². One may attempt to increase the survival probability by lowering the bombarding energy in order to produce less excited compound nuclei. In theoretical studies[94,95] of fusion reactions it is pointed out, however, that the bombarding energy in the performed experiments was still too low[95] by 10–30 MeV since an extra push of energy is required to fuse two nuclei. A general consequence of this analysis[90] is that it

may indicate a steeper descent of the island on its west shore than found in calculations as shown by Fig. 2. If this is the case, to produce superheavy elements by complete fusion is apparently hopeless, a result which agrees with the numerous negative results listed in Table 1. Oganessian *et al.*[81] argue that either a low fusion cross-section or short half lives may explain the negative results with the $^{48}Ca + ^{248}Cm$ reaction.

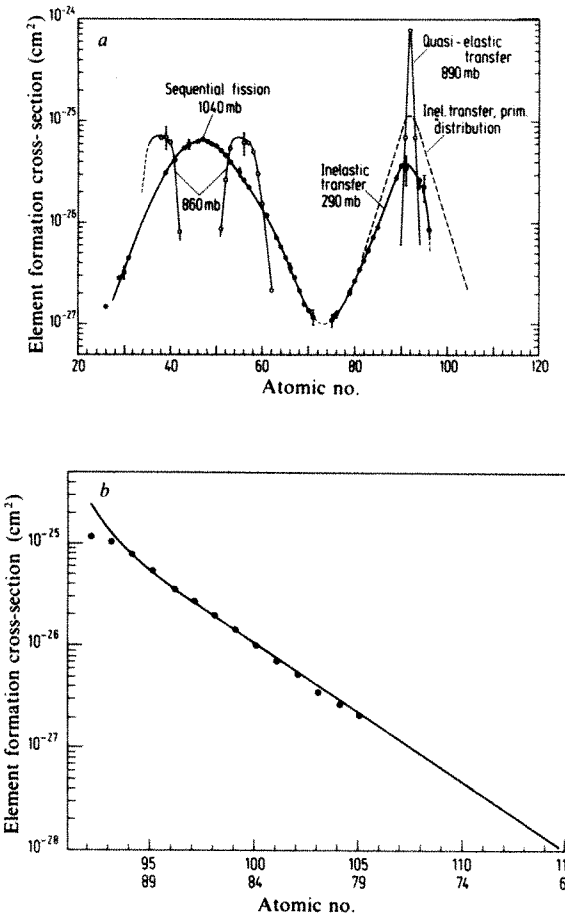


Fig. 8 *a*, Element formation cross-sections in the reaction of ^{238}U with ^{238}U as obtained by a radiochemical analysis of the reaction products produced in thick uranium targets bombarded with 7.5 MeV AMU^{-1} uranium ions (from ref. 102). *b*, Element formation cross-sections for complementary fragments in the $^{238}U + ^{238}U$ reaction deduced from experimental data as shown in *a* (●), compared with values calculated (solid line) using the diffusion model of damped collisions (after Riedel and Nörenberg[103]).

Are there any alternatives? A first hint in other directions seemed to be the observation of a 150-d spontaneously fissioning activity[96,97] produced with a cross-section of 10^{-33} cm^2 in bombardments of ^{238}U with ^{136}Xe and attributed to a superheavy element because of its chemical behaviour which was tested in a few bombardments. Flerov *et al.*[96,97] assumed that a fusion–fission process occurred but this is an unlikely mechanism for reactions between very heavy nuclei, as more recent studies have shown. Attempts to reproduce these findings, failed[85,98–100], however, in spite of an increase of the sensitivity by two orders of magnitude[99].

An alternative pathway to the superheavy elements was opened[101,102,35] by the first studies of the reaction between two colliding uranium nuclei carried out at the Darmstadt UNILAC accelerator when, for the first time, uranium beams with energies sufficient to overcome the interaction barrier even for uranium became available. These studies[101,102] demonstrated that the superheavy island might be reachable by exchange of nucleons in damped collisions. The evidence is still indirect and is based on the observation of complementary products. One of the colliding uranium nuclei increases its proton and neutron number at the expense of the other uranium nucleus

$$^{238}_{92}U^{146} + ^{238}_{92}U^{146} \rightarrow ^{298}_{114}X^{184} + ^{178}_{70}Yb^{108}$$

This implies, of course, that such damped collisions are binary reactions and that charged-particle evaporation plays only a minor part. There is strong evidence for these assumptions[73–75].

Figure 8*a* shows the element distribution in the reaction between two ^{238}U nuclei as obtained in radiochemical studies[102] by measuring a network of formation cross-sections for individual nuclides. The element yields peak at uranium in a narrow distribution due to quasi-elastic transfer. The underlying, broader distribution results from nucleon transfer in damped collisions. Reaction products from both types of reactions undergo fission as is evident from the broad distribution of fission products between element 30 and 70 and the steep decrease of the cross-sections beyond uranium due to increasing fissionability of transuranium nuclei. This decrease was followed over eight orders of magnitude up to fermium ($Z = 100$) whose element formation cross-section is 8×10^{-33} cm^2. Before fission occurs the element distributions both in the quasi-elastic and in the damped collisions should be symmetric around uranium for reasons already mentioned. The primary distribution originating from damped collisions can easily be reconstructed and is shown by the dashed curve in Fig. 8*a*. Extrapolation of this distribution to element 70 results in a cross-section of 10^{-28} cm^2 for the element 114–70 combination. A more sophisticated extrapolation is shown in Fig. 8*b* where the element cross-sections obtained experimentally are compared with values calculated [103] using a model which treats the nucleon transfer in damped collisions as a diffusion process[104]. The agreement between experimental and calculated cross-sections is very satisfying. For the element 114–70 combination again a production cross-section of 10^{-28} cm^2 is calculated.

But what fraction of element 114 may survive fission? It is an attractive feature of damped collisions that the reaction products are formed with very broad distributions in excitation energy and angular momentum, tailing to low values which are essential for survival. Counter experiments[101] and an analysis of production cross-sections of the highly fissionable transcurium nuclides[102] have shown that the excitation energies of fragments originating from the uranium-on-uranium reaction are unusually low if compared with other reactions between heavy nuclei. Calculations[103] with the diffusion model predict at 8.3 MeV AMU^{-1} bombarding energy production cross-sections of about 10^{-34} cm^2 for element 114 nuclei with <30 MeV excitation energy, an energy range for which the survival probability is estimated[25] to be >50% for the first neutron-evaporation step provided the neutron number is close to 184. It has been shown for damped collisions with light[75,105] and heavy[106] projectiles that the most probable neutron numbers for a given element split corresponds to a maximum mass difference between interacting and product nuclei with a minor correction for nuclear pairing energies. Applying this concept to the uranium-on-uranium reaction leads to $N = 180$ and $N = 182$ as the most probable neutron numbers for element 114 fragments. Taking all these estimates together one may expect production cross-sections of about 10^{-35} cm^2 for element 114. This corresponds, with the accessible beam intensities, to production rates of a few atoms per day or per week a rate just exceeding the detection limit of the most sensitive methods available at present.

Direct searches for superheavy elements in the uranium-on-uranium reaction have so far been negative. Looking for spontaneous fission events from reaction products implanted in a semiconductor detector gave an upper cross-section limit of 2×10^{-32} cm^2 for half lives between milliseconds and months[101]. With the gas-jet technique an upper limit of $1.5–3 \times 10^{-33}$ cm^2 has been reported for half lives between 1 and 10^5 s[85], and 10 and 10^3 s[107]. A more sensitive chemical experiment with a separation method based on volatilisation gave an upper limit of

10^{-35} cm^2 for half lives between several hours and one year, but further experiments using modified techniques are underway[108].

The use of heavier elements is obviously very attractive. Calculations with the diffusion model predict[103] an increase in the production cross-section for element 114 by two orders of magnitude when ^{248}Cm instead of uranium is bombarded with uranium projectiles. On the other hand the use of ^{244}Pu as projectile as proposed by Seaborg *et al.*[90] offers no advantages if compared with uranium[103]. Another factor of seven may be gained with a ^{249}Cf target but the gain may be illusory because of the limited availability of such exotic targets. Furthermore the estimated most probable neutron number moves away from the 184 neutron shell for targets heavier than ^{244}Pu.

Another mechanism for formation of superheavy elements has been suggested[109]. When fissile nuclei are used as target or projectile they may undergo fission under the influence of the other partner, and one of the fission fragments may fuse with the projectile to form a superheavy nucleus. Experiments[110] following this suggestion in which lead was bombarded with ^{136}Xe yielded negative results with an upper cross-section limit of 1.2×10^{-30} cm^2 for half lives between 10^{-12} and 10^4 s.

Outlook

There is no convincing evidence that superheavy elements have been discovered. On the contrary, the negative results produced by strong efforts in many laboratories during the past dozen years are discouraging. Yet, there is at least one natural source, the hot spring water from the Cheleken peninsula, which deserves attention, and similar springs may exist elsewhere. Also, reactions between very heavy nuclei have not yet been fully explored as a possible pathway to the superheavy elements in the laboratory. Whatever the final outcome, the intense exploration occasioned by the search for superheavy elements is bound to lead to a better understanding of the forces that terminate the Periodic Table at its upper end.

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articles

Boninites, komatiites and ophiolitic basalts

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The rare Phanerozoic rock type boninite petrographically resembles Archaean basaltic komatiites and the range of boninite compositions overlaps that of basaltic komatiites. Quench amphibole and hydrous glass in the groundmass of boninites confirm earlier ideas that they may be the products of partial melting of peridotite in hydrous conditions, whereas basaltic komatiites have been assumed to result from fractionation of dry melts. Where field relationships are known, boninites are found either in ophiolites or a fore-arc tectonic environment.

BONINITES, first described in 1890 (ref. 1) in Cenozoic lavas from the Western Pacific, have chemical compositions similar in some respects to magnesian andesites. They bear a remarkable resemblance to basaltic komatiites which form a substantial component of Archaean vulcanism (Fig. 1). Rocks which we consider to be boninites, containing rapidly grown pyroxene and microphenocrysts of olivine and/or low-calcium pyroxene set in a glassy matrix are relatively widespread amongst ophiolitic basalts. This article offers new petrological information on these important rocks with implications for the origin of at least some ophiolites.

The use of the term boninite requires explanation. Kikuchi[1] described two rocks from Chichijima in the Bonin or Ogasawara group, Western Pacific. One contained phenocrysts of orthopyroxene (bronzite) and skeletal augite set in a dark glassy matrix "comparatively free of the palagonitic alteration product". The chemical analysis (analysis 1, Table 1) was of a "glassy portion" of the rock which we interpret as meaning close to the liquid composition. The other rock had phenocrysts of bronzite, augite and plagioclase feldspar in a groundmass of augite, plagioclase and glass (analysis 2). Petersen[2] introduced the name boninite and presented a third analysis of a soda-rich, probably altered rock (analysis 3) which he described as a feldspar-free, glass-rich variety of bronzite–andesite with olivine and augite.

Later, Tröger[3] gave analysis 1 as the type boninite and mentioned possible groundmass olivine as part of the mode. We concur with Tröger's choice and redefine boninite as follows: a highly magnesian but relatively siliceous, glassy rock containing one or more varieties of pyroxene, some or all of which have a morphology characteristic of rapid growth, accessory magnesiochromite and, commonly, minor amounts of olivine. Laths of amphibole or plagioclase microlites are rare.

Although boninites could be called high-magnesium andesites[4] their mineralogy, texture and high chromium and nickel contents differ so markedly from those of typical andesites that we consider the separate term justified.

Samples conforming to our definition of boninite were studied from the type locality, Chichijima in the Bonin islands[1,2,5,6] (Oligocene), the Dabi volcanics, Cape Vogel peninsula, eastern Papua[7–9] (?Oligocene), the uppermost Upper Pillow lavas of the Troodos massif[10,11] and Arakapas Fault belt[12], Cyprus (Cretaceous), the Agrilia Formation of the Othris Mountains, Central Greece[13] (Triassic) and from dredge haul 1403 at 12° N on the inner wall of the Mariana Trench[14,15] (Upper Eocene, unpublished data). Boninite has recently been recovered from a similar tectonic environment at 18° N in Hole 458 on Leg 60 of the IPOD drilling programme[16]. Palaeozoic rocks with similar

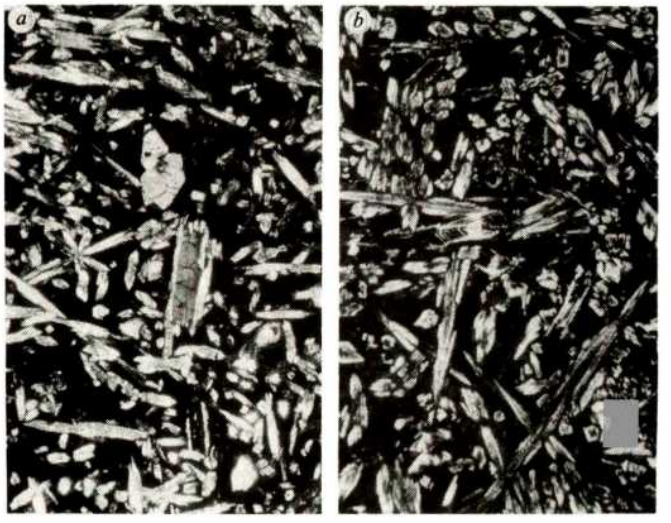


Fig. 1 Comparison of textures of boninite and basaltic komatiite. *a*, Boninite, specimen 29473, Upper Pillow Lavas, Cyprus. Microphenocrysts of olivine, orthopyroxene jacketed by augite. Groundmass pyroxenes are mainly augites, but a few are zoned from pigeonite to augite. 1.5 mm wide, crossed polars. *b*, Basaltic komatiite NG137 from a 2-m thick flow (Fig. 2, ref. 22), Belingwe greenstone belt, Rhodesia. Skeletal olivines and groundmass augite surrounding cores of pigeonite, now altered. 2.5 mm wide, plane polarised light.

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Table 1 Chemical compositions of boninites and basaltic komatiites

	1[1]	Original boninites Bonin Islands 2[1]	3[2]	Inner wall Mariana Trench, 12°N[15] 1403–45	Dabi volcanics C. Vogel, Papua[9] LB105	Upper Pillow Lavas, Cyprus 29473	Arakapas Fault Belt, Cyprus[12] 1303	Belingwe Greenstone Belt, Rhodesia[21] NG137	Munro Township, Northern Ontario Canada[23] P9–185
SiO_2	54.4	53.2	53.9	55.9	54.1	51.4	52.2	50.9	47.2
TiO_2	ND	ND	ND	0.21	0.30	0.36	0.20	0.52	0.43
Al_2O_3	12.9	16.2	18.0	10.8	9.5	12.1	12.1	10.3	11.9
Fe_2O_3	7.1	10.3	ND	2.9	2.5	1.8	2.4	1.3	1.8
FeO	ND	ND	4.9	6.0	6.5	5.6	6.5	10.0	9.5
MnO	ND	ND	ND	0.18	0.15	0.14	0.17	0.16	0.20
MgO	12.7	6.7	4.6	11.8	13.0	10.5	12.1	12.8	12.6
CaO	5.1	10.1	7.6	6.0	5.5	9.8	10.0	8.6	9.9
Na_2O	2.1	1.8	3.9	1.8	0.75	1.4	0.70	2.0	1.9
K_2O	0.35	0.35	1.1	0.7	0.41	0.27	0.25	0.23	0.01
P_2O_5	ND	ND	ND	0.03	0.07	0.03	0.04	0.03	ND
H_2O	5.5	1.6	4.6	4.2	7.4	5.7	3.5	3.5	3.9
CO_2	ND	ND	ND	0.0	0.14	0.30	ND	ND	0.02
Cr_2O_3	ND	ND	ND	0.15	0.12	0.09	0.12	0.20	ND
NiO	ND	ND	ND	0.03	0.03	0.03	0.05	0.03	ND
Total	100.1	100.2	98.6	100.7	100.4	100.3	100.3	100.5	99.3

ND, not determined.

textures occur in the Betts Cove ophiolite[17] and within the Central Heathcote 'greenstone' belt, Victoria[18], but these contain augite and chromite as the only relict phases. Probable equivalents from Rambler, Newfoundland[19], and the Dundas Trough, Tasmania[20], have been completely recrystallised.

Basaltic komatiites with good textural and mineralogical preservation are rare; our analytical study involved specimens from the Belingwe greenstone belt Zimbabwe-Rhodesia[21,22], and was supplemented by data from equally fresh material in the Abitibi belt of northern Ontario[23,24]. Texturally, basaltic komatiite flows contain clinopyroxene, some or all with a morphology indicative of rapid growth; olivine, in the more magnesian lavas; and commonly groundmass plagioclase which varies from microlitic to intergranular. Pigeonite cores to augite are relatively rare and orthopyroxene is restricted to the cumulus zones of very thick flows.

Mineralogy

The compositional ranges of the primary minerals are given in Table 2; the complete analyses will be presented elsewhere. Olivine compositions in the boninites were found to vary from Fo_{93-83}, depending largely on crystal size. Phenocrysts (generally 1.0–10 mm long) found in the 'ultramafic lavas' of Cyprus[25,26] and Othris are Fo_{93-91}. Microphenocrysts (0.2–0.6 mm) form 5–10% of the mode in pseudomorph or unaltered form in almost all the other samples studied; the vast majority have compositions between Fo_{90} and Fo_{87}. Tiny skeletal olivines occur in the glassy matrix of several of the Cyprus boninites and have compositions between Fo_{88} and Fo_{83}. Some of the phenocrysts but few of the microphenocrysts are corroded and none shows a reaction relationship to calcium-poor pyroxene.

In basaltic komatiites, olivine (almost invariably pseudomorphed) typically occurs as microphenocrysts or skeletal grains 0.2–0.4 mm long. One rock from Belingwe, Zimbabwe-Rhodesia, has completely unaltered olivine microphenocrysts of composition Fo_{88} and abundant groundmass olivine, sometimes euhedral, with compositions as iron-rich as Fo_{82}. In a thick basaltic komatiite flow (19% MgO) from Munro Township cumulus olivine of comparable composition (Fo_{89-87}) was found[27]. Olivines in both the Belingwe komatiite and boninites generally have high nickel and calcium contents (>0.20%) but boninitic olivines have lower chromium content (<0.08%), suggesting that the order of crystallisation we observe petrographically in komatiites, olivine→chromite, is reversed in boninites.

Calcium-poor pyroxene occurs in all boninites except those from Othris, either as clinoenstatite (Cape Vogel, Mariana Trench) and orthopyroxene, or exclusively the latter. Phenocrysts (up to 3 mm) are present in most of the Cape Vogel rocks and in a few of the Mariana Trench and Bonin samples. Their Al_2O_3 contents are low, 0.1–1.1%, although they have very high Mg/(Mg+Fe) ratios (Fig. 2, open symbols). Microphenocrysts of orthopyroxene are common in the Cyprus rocks and have 'quench' overgrowths of augite. Groundmass orthopyroxene consists of laths intergrown with pigeonite and augite, or discrete cruciform shapes. Magnesian pigeonite has either nucleated on orthopyroxene grains of slightly higher Mg/(Mg+Fe) ratio or from the liquid. More often augite mantles orthopyroxene which may in turn be intergrown with clinoenstatite. Phenocrysts of calcic pyroxene are rare both in boninites and komatiites; their compositions lie close to $En_{52}Wo_{41}Fs_7$.

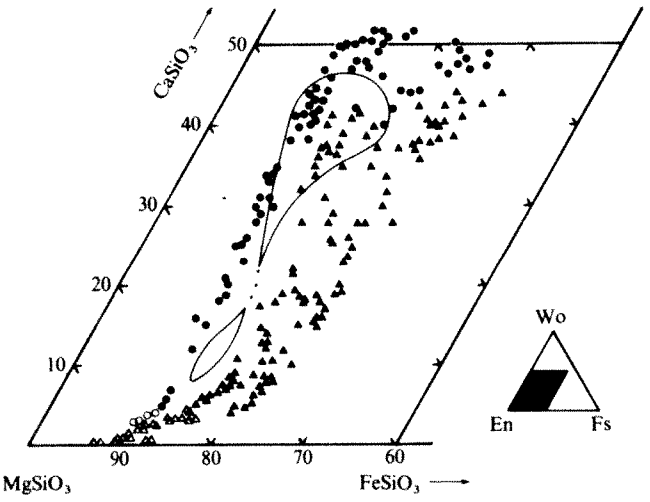


Fig. 2 Compositional variation of calcium-poor (open symbols) and groundmass calcic pyroxenes (closed symbols) within portion of the system $MgSiO_3$–$FeSiO_3$–$CaSiO_3$. All Fe is expressed as Fe^{2+}. Origin of specimens; ○●, Cyprus and Othris; △▲, Bonin Island, C. Vogel, Mariana Trench. Only a representative portion of augite analyses are shown. Field (enclosed) for basaltic komatiites from ref. 27 and this work. Analyses by energy-dispersive electron probe operated at 20 kV and 30 nA. Trace elements mentioned in the text were determined on a Geoscan instrument operated at 20 kV.

Groundmass calcic pyroxenes, however, are ubiquitous and typically have skeletal forms[28] and metastable compositions, evidence of supercooling and rapid growth. In boninites, the bulk of the quench pyroxene is aluminous augite but in a few examples from each locality except Othris, pyroxene is zoned continuously from pigeonite to augite; spot analyses selected to show the range of compositions are given in Fig. 2.

Table 2 and Fig. 2 show that samples from the Western Pacific have a different compositional trend with crystal growth from those associated with ophiolites. The Bonin Islands calcic pyroxene trend is the most magnesium-rich of the Pacific types whereas the Cape Vogel one is the least. More magnesian by far is the Cyprus trend which meets the least calcic of the Othris pyroxenes at about Wo_{38}. The crystallisation trends are specific to each locality and probably represent differing thermal histories just before eruption as much as fundamental differences in parent liquid compositions.

Orthopyroxene is rare in komatiites and so far has been positively identified only as a cumulus phase in the thick submarine flows of basaltic komatiite composition from the Abitibi belt of Ontario[24,27]. The variation in composition of groundmass pigeonite–augite is very similar to that of boninites. The data given in Fig. 2 are for pyroxenes in a pyroxene–spinifex top to a thin flow from the Belingwe belt and in ref. 24. Most basaltic komatiites, however, and all peridotitic komatiites, contain only augite and sub-calcic augite.

Deep red-brown magnesiochromites occur in almost every section of boninite or basaltic komatiite. Typically they are <0.03 mm across, euhedral and surrounded by glass. We detected zoning in one phenocryst only, from $Mg/(Mg+Fe^{2+}) = 0.683$ at the centre to 0.669 on the margin. All spinels are very chromium rich (Fig. 3) and have $Fe^{3+}/(Fe^{3+}+Al+Cr)$ ratios <0.1. Those in boninites from the Western Pacific have higher $Cr/(Cr+Al)$ ratios than those in the komatiite samples, whereas those from ophiolites span the whole range. Chromites in basaltic komatiites were found to have $Mg/(Mg+Fe^{2+}) < 0.6$. Figure 3 emphasises the peculiar composition of these spinels contrasted with those found in typical Mid-Atlantic Ridge basalts[29–31]; comparable compositions occur in the Great Dyke[32] and the Troodos plutonic complex itself[33].

Basaltic komatiites of comparable MgO contents to boninites commonly have a groundmass of microlitic plagioclase (0.2 by <0.01 mm) which we have determined, in one sample, to be An_{64}. Samples from the cores of boninitic pillow lavas from Othris and Cyprus display a similar plagioclase morphology but only rarely is the feldspar unaltered. In the Upper Pillow Lavas from Cyprus values of An_{65-75} were obtained and more calcic compositions (up to An_{95}) have been reported from ultramafic lavas[25]. Several boninite specimens from each locality, except the Bonin Islands, contained tiny laths, stellate aggregates or dendrites of green hornblende concentrated in the centres of pools of glass. The textures indicate that they are of primary origin, demonstrating the hydrous nature of the magma. In addition, rosettes of biotite up to 0.3 mm across occur in one specimen from the Mariana Trench[15] and several of the ultramafic (olivine–phyric) lavas from Othris. Perfectly isotropic glass in the boninites has a water content of 6–8% (microprobe, by difference) but it is unlikely that all of it is original.

Primary hydrous phases are very rare in komatiites. Arndt[23] has described cumulus brown hornblende from close to the centre of one of the basaltic komatiite layered flows mentioned earlier but we could find no evidence of original groundmass hornblende in the least altered Rhodesian samples.

Boninites and komatiites are very similar in both mineralogy and texture. With the exception of the spinifex olivine rocks from Gorgona Island[34], which we do not consider are boninites because neither are they quartz normative nor do they have large modal amounts of hydrous glass, boninites are unique in this respect amongst Phanerozoic rocks. The only notable mineralogical differences between basaltic komatiites and boninites lie in the relative paucity of orthopyroxene and hornblende and high chrome contents of olivine in the former.

Chemical composition

The chemical compositions of boninites and basaltic komatiites are similar (Tables 1 and 2) and both have $Mg/(Mg+Fe)$ ratios appropriate for primitive liquids in equilibrium with mantle olivine[35]. The lavas of the Arakapas fault belt have been called basaltic komatiites, largely on geochemical grounds[12], but this was rejected[4] because of the significantly lower incompatible element contents, notably titanium, of those rocks and what we term boninites from Cape Vogel and the Mariana Trench. The TiO_2 contents of the Othris samples (Table 2), however, assuming that titanium is a relatively immobile element during alteration, are comparable with those of basaltic komatiites, as are their CaO/Al_2O_3 ratios calculated from the less altered ultramafic (olivine–phyric) lavas. The CaO/Al_2O_3 variation between different boninites is faithfully recorded in the pyroxene compositions: in Othris there are no low-calcium pyroxenes or sub-calcic augites, in the Cape Vogel and Mariana Trench rocks clinoenstatite occurs while the Bonin and Cyprus

Table 2 Mineralogical and chemical characteristics of basaltic komatiites and boninites

	Northern Ontario	Belingwe	Cape Vogel	Mariana Trench	Bonin Islands	Cyprus	Othris
Olivine							
Phenocrysts	Fo_{89-87}	—	—	Fo_{92}	—	Fo_{93-91}	Fo_{92-91}
Microphenocrysts	—	Fo_{88}	Fo_{92}	Fo_{89-88}	Fo_{89-87}	Fo_{90-86}	altd
Groundmass	altd	→Fo_{82}	—	—	—	→Fo_{83}	—
Calcium-poor pyroxene							
Phenocrysts	En_{88-82}	—	En_{93-91}	En_{91-88}	En_{89-87}	En_{89-88}	—
Microphenocrysts	—	—	En_{92-86}	En_{88-85}	En_{88-85}	En_{88-87}	—
Groundmass	—	—	→En_{81}	→En_{83}	→En_{84}	→En_{84}	—
Pigeonite–Augite							
Groundmass	$En_{78}Wo_8Fs_{14}$–$En_{40}Wo_{45}Fs_{15}$	$En_{73}Wo_{12}Fs^*_{15}$–$En_{40}Wo_{43}Fs_{17}$	$En_{76}Wo_4Fs_{20}$–$En_{26}Wo_{44}Fs_{30}$	$En_{80}Wo_5Fs_{15}$–$En_{31}Wo_{44}Fs_{25}$	$En_{81}Wo_5Fs_{14}$–$En_{42}Wo_{41}Fs_{17}$	$En_{83}Wo_5Fs_{12}$–$En_{28}Wo_{50}Fs_{22}$	$En_{54}Wo_{38}Fs_8$–$En_{32}Wo_{52}Fs_{16}$
Representative Compositions							
$Mg/(Mg+Fe^{2+})$	0.70–0.74	0.67–0.75	0.73–0.83	0.72–0.79	0.71–0.77	0.65–0.77	altd
CaO/Al_2O_3 (av.)	0.83	~0.80	0.55	0.53	0.53	0.83	>0.9
Ti (%, av.)	~0.5	~0.60	0.33	0.19	0.24	0.28	0.7
Cr (p.p.m., av.)	1400?	~1000	850	820	710	780	970
Ni (p.p.m., av.)	640?	~ 400	300	280	250	340	330

altd, mineral in pseudomorph form only; —, mineral or pseudomorph not observed; *, larger crystal. Mineral compositions are from this study except for the Canadian data (refs 23, 24 and 27); whole rock compositions include data from refs 6, 9, 11, 12, 15, 21 and 23; Fe^{2+} calculated on the basis of $Fe^{3+}/(Fe^{2+}+Fe^{3+}) = 0.1$.

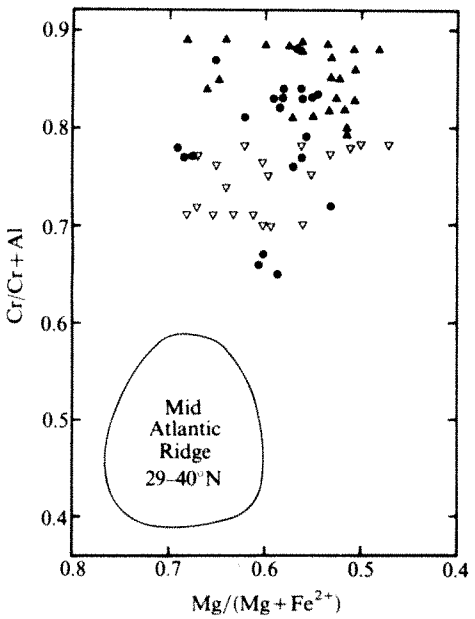


Fig. 3 Compositions of chromites from boninites (● and ▲ as Fig. 2) and basaltic komatiites (▽), projected onto the Fe^{3+}-free face of the spinel prism. Analyses by energy-dispersive electron probe, Fe^{2+} estimated by assuming stoichiometry. Field of Mid-Atlantic Ridge spinels from refs 29–31.

material has orthopyroxene as the low-calcium phase. In this context, basaltic komatiites are intermediate between the Cyprus and Othris examples. We conclude that a spectrum of titanium contents and CaO/Al_2O_3 ratios exists in boninites and each overlaps the range found in basaltic komatiites. This may result from differing degrees of melting, mantle inhomogeneity or chemical evolution of the mantle with time.

We are not entirely convinced that boninites have much lower Ti/Zr than chondritic values[4]. The Upper Pillow Lavas of the Troodos Massif, those in the Arakapas Fault bewt and Agrilia Formation of the Othris Mountains have average Ti/Zr ratios of 100, 105 (ref. 12) and 94 respectively, close to chondritic values and basaltic komatiites (ranging from 60 to 140 in 50 samples).

Origin

Curiously, the origin of boninite would seem to be among the least controversial of any rock type. Green's carefully controlled experiments on water-saturated partial melting of pyrolite[36] produced a liquid at 1,200 °C, 10 kbar (~35% melting) which he calculated to have 54% SiO_2, 14% MgO, an Mg/(Mg+Fe) ratio of 0.73 and $CaO/Al_2O_3 = 0.88$, the chief characteristics of boninite. Melting of a mantle segment that has been depleted in incompatible elements by previous melting episodes would require very high temperatures[37] to produce such magnesian liquids. It is difficult to imagine such temperatures in Phanerozoic mantle melts, thus we postulate that this could be achieved only by a 'wet' process, whereas in the Archaean with less depleted mantle and possibly higher geothermal gradients, melts of similar or even more magnesian composition were generated by 'dry' melting. Basaltic komatiites were probably comagmatic with peridotitic komatiites, having arisen by low-pressure fractionation first of olivine, then augite from parental magmas with about 25% MgO[23,21,38]. Sun and Nesbitt suggested that the term komatiite should have the petrogenetic constraint that it is derived by dry melting of a mantle (with relatively undepleted incompatible element contents)[4]. Two observations are unexplained: the rare appearance of hornblende during fractional crystallisation of basaltic komatiites[23,27] and the apparent scarcity of rocks with compositions lying between 16 and 20% MgO; basaltic and peridotitic komatiite[21].

There are great similarities between boninites and basaltic komatiites but the association of the latter with liquids of ultramafic composition is characteristic of the Archaean alone. It is tempting to speculate that the petrogenesis of basaltic komatiites and boninites differs from that of peridotitic komatiites by involving water, but the evidence presented above is inconclusive. We regard boninite therefore as petrographically, mineralogically and chemically the closest Phanerozoic equivalent to basaltic komatiite; the fundamental differences arising from changes in the Earth's mantle and thermal and tectonic regimes between the Archaean and the Phanerozoic.

It is significant that boninites are almost exclusively associated with ophiolites (but not necessarily vice versa). Troodos, Othris and the Canadian Palaeozoic examples[17,19] form part of the extrusive sequence in well documented ophiolites. The igneous rocks from the Mariana dredge at 12° N are all of the 'ophiolite association'[14].

Boninites from the Western Pacific are in identical fore-arc tectonic settings on the 'mid-slope high' (ref. 39). It is possible that boninites, and by implication ophiolites containing boninitic lavas, were formed *in situ* by partial melting of peridotite overlying the downgoing slab and that the associated two-pyroxene andesites and dacites[5] are manifestations of a steadily evolving boninitic liquid from an island-arc setting[6]. There are two main problems with this view: the fact that the active volcanic chain in the Western Pacific is 150 km further away from the trench than the mid-slope high and that the overlying slab would be too cool. One possibility may be that boninites are erupted in the initial stages of breaking an older oceanic plate. It is very unlikely that boninites represent material scraped off the oceanic plate—they share none of the petrographic or geochemical characteristics of basalts generated at mid-ocean ridges over the past 180 Myr.

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Disappearance of pink-pigmented *Globigerinoides ruber* at 120,000 yr BP in the Indian and Pacific Oceans

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The planktonic foraminiferal species Globigerinoides ruber *with pink-pigmented tests occupied a worldwide warm-water belt during much of the Pleistocene. This variety was exterminated from the Indian and Pacific Oceans at about 120,000 yr BP, based on the oxygen isotope stratigraphy in 11 Indo-Pacific deep-sea cores, but it continued to live on to the present in the North and South Atlantic Ocean and the Mediterranean Sea. The disparate Atlantic and Indo-Pacific records reflect faunal provincialism that has been developing in the two regions since the Pliocene.*

THE pink-pigmented variety of *Globigerinoides ruber* (d'Orbigny) forms a large proportion of the planktonic foraminiferal fauna of the modern Atlantic Ocean[1–11] and Mediterranean Sea[12], but is completely absent from the present-day Indian and Pacific Oceans[13–18]. This foraminiferal variety did, however, occur in a circum-global warm-water belt during the late Pliocene and Pleistocene. Here we document the timing and geographic extent of the disappearance of pink-pigmented *G. ruber* as a synchronous extermination throughout the tropical and subtropical Indo-Pacific at 120,000 yr BP (the beginning of the last full interglacial and the lower part of oxygen isotope stage 5). We have relied on $\delta^{18}O$ curves for direct worldwide correlation of this biostratigraphic event because the fluctuations of these curves reflect global ice volume[19] and are nearly isochronous within the time constraints of ocean mixing[20]. The apparent diachroneity of the Atlantic and Indo-Pacific stratigraphic records of coloured *G. ruber* since 120,000 yr BP may be related to faunal provincialism which has been developing in tropical latitudes since the Pliocene[21].

Indo-Pacific sediments

Although pink-pigmented *G. ruber* has not been reported from either the plankton or Recent sediments of the Indian and Pacific Oceans, it is occasionally listed from the Pleistocene and rarely from older sediments. Cushman *et al.*[22] and later Todd[23] reported small red and faintly pink specimens from Bikini and Eniwetok Atolls. Brönnimann and Resig[24] found pink *G. ruber* in the Pleistocene of Deep Sea Drilling Project Site 62 in the western equatorial Pacific and Jenkins and Orr[25] found it in the Pliocene and Pleistocene of eastern equatorial Pacific DSDP sites. Thompson[26] reported an acme of pink-pigmented *G. ruber* between solution cycles III_p and II_p (250,000–124,000 yr BP) in the western tropical Pacific, and placed the top of its range near the 6/5e oxygen isotope boundary.

We have taken samples at 10 cm or closer intervals for $\delta^{18}O$ measurements and total faunal analyses from many piston cores (Table 1; Fig. 1) to delineate the extermination datum level. The samples for isotopic analyses were roasted under vacuum at

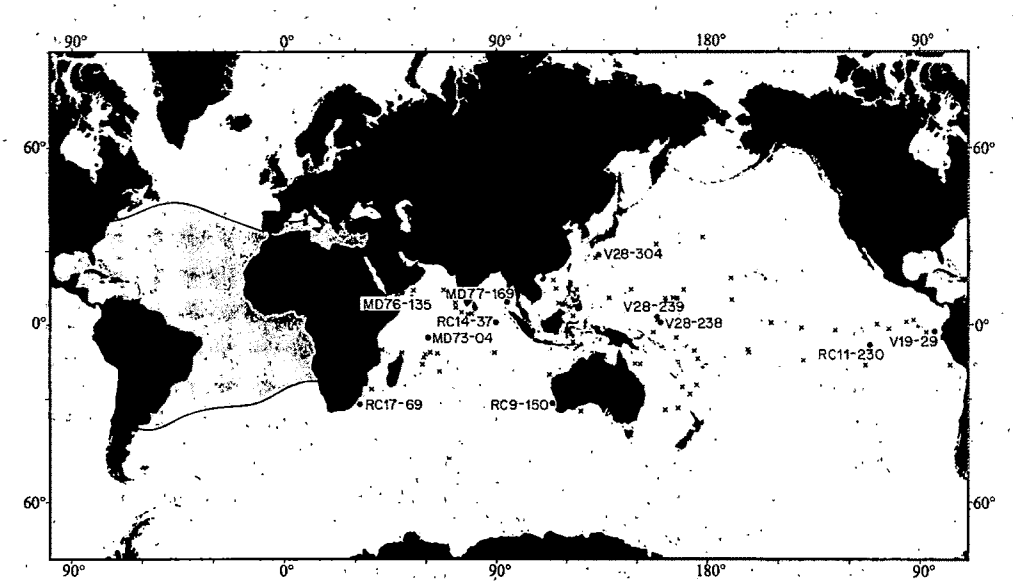


Fig. 1 Locations of piston cores with pink-pigmented *G. ruber* 120,000 yr BP datum and oxygen isotope records (dots), and other downcore occurrences of pink-pigmented *G. ruber* (crosses) in the Indo-Pacific. Pink-pigmented *G. ruber* survived in the Atlantic Ocean and Mediterranean Sea where it occurs in Quaternary sediments and present-day warm-water region (shaded).

Table 1 Cores used in this study

Core	Lat	Long	Water depth (m)	Core length (cm)	Isotopic reference	Occurrence level of 6/5e* boundary in core (cm)	Top occurrence of *G. ruber*† in core (cm)	Age of datum‡ (yr BP)
RC17-69	31°30 S	32°36′ E	3,308	1,130	31	205	170–180	109,170±3,120
MD76-135	14°27′ N	50°31′ E	1,895	1,125	Duplessy, this work	885	860–870	125,100±720
MD73-04	04°58′ S	61°40′ E	3,440	1,700	Duplessy, this work	212	212	128,000
RC14-37	01°28′ N	90°10′ E	2,226	605	32	190	160–170	111,160±3,370
MD77-169	10°12′ N	95°03′ E	2,360	1,460	Duplessy, this work	910	880–890	124,900±1200
RC9-150	31°17′ S	114°36′ E	2,703	512	31	270	240–250	116,150±2,370
V28-304	28°32′ N	134°08′ E	2,942	824	Shackleton, this work	455	426–430	120,460±620
V28-238	01°01′ N	160°20′ E	3,120	1,609	19	217	201–202	119,760±1,200
V28-239	03°15′ N	159°11′ E	3,490	2,102	30	127	110–120	115,900±5,030
RC11-230	08°48′ S	110°48′ W	3,259	480	28	245	220–230	117,030±2,090
V19-29	03°35′ S	83°56′ W	3,157	1,680	27	865	850–855	126,150±370

* Given by Shackleton and Opdyke[19] as 128,000 yr BP.
† Pink-pigmented form.
‡ Interpolated from 6/5e boundary and sample interval.

400 °C for 45 min and reacted under vacuum with 100% H_3PO_4 at 50 °C; the $^{18}O/^{16}O$ ratio is reported as $\delta^{18}O$ relative to the Chicago Pee Dee Belemnite (PDB-1) standard:

$$\delta^{18}O = \left[\frac{(^{18}O/^{16}O)_{sample}}{(^{18}O/^{16}O)_{standard}} - 1\right] \times 1{,}000$$

Faunal studies are based on a minimum count of 300 individuals greater than 149 μm in diameter to ensure statistical reproducibility. To take into account the effect of carbonate dissolution on the absolute abundances of total *G. ruber* populations through time[26], downcore variations have been expressed as the ratio of pink to total *G. ruber*.

The oxygen isotopic records[27–32] and stratigraphic ranges of pink *G. ruber* for six Indian Ocean and five Pacific Ocean cores are shown in Figs 2 and 3. The curves were plotted to align isotopic stage 5e. Marked stage-to-stage sediment accumulation variations point to the effects of local depositional characteristics. The lithostratigraphic top of the range of pink *G. ruber* consistently lies within stage 5. Furthermore, cores with slow deposition rates, such as RC17-69, RC14-37, V28-238 and V28-239, show higher vertical occurrences than cores with high rates of accumulation such as MD76-135, MD77-169 and V19-29.

Isotopic measurements on both pink and white forms from stages 7 to 5e of core MD77-169 are shown in Fig. 4. Pink *G. ruber* is 0.17‰ lighter (s.d. 0.14) than the white form. Too few specimens were recovered from samples at the 5e maximum (880 and 890 cm) to analyse, but the light isotopic composition of the pink shells at 900 cm shows that these individuals lived after the 6/5e transition. The abundance of pink *G. ruber* shells decreases from 950 to 900 cm; at 900 cm, bioturbation will introduce isotopically heavy shells deposited in stage 6, but the same bioturbation cannot introduce isotopically lighter shells from higher up in stage 5e because the variety has already disappeared. Therefore, bioturbation will produce isotopically heavier measurements if the pink form is analysed rather than the white form, which can still be contaminated from stage 5. The true (chronostratigraphic) level for the top of the range of pink *G. ruber* is probably above the 5e/6 transition but before the 5d interglacial stadium. The occurrence of pink specimens above this level is attributed to sediment mixing by bioturbation[33–35]. Thus, in cores with rapid sedimentation rates, the level of highest lithostratigraphic occurrence is closer to the true disappearance level than it is in cores with slower accumulation rates, where an equivalent amount of bioturbation can disturb sediments representing a longer period of time.

The ratio of coloured-to-total *G. ruber* shows a consistent pattern down the cores. In the late portion of oxygen isotopic stage 6, the ratio averages 10%. It drops to 0% in the early part of stage 6, quickly returns to 10% or more in stage 7, and climbs to 20–25% in stage 8. In the two subtropical cores RC17–69 and RC9–150, the overall proportions of *G. ruber* are lower than in the more tropical cores reflecting the higher latitudes of the sites, but the downcore pattern of pink *G. ruber* ratios is similar to other cores studied. In all cores, abundances decrease rapidly below isotopic stage 10. In the early Brunhes and late Matuyama sections of V28–238 and V28–239 pink *G. ruber* was noted only from intervals of low carbonate dissolution. Specimens from these samples show a very faint colouration, fading downcore to the extent that they become indistinguishable from the colourless (white) form. Thus, we conclude that first occurrences in the middle of the Pleistocene do not necessarily mark an abrupt evolutionary appearance at this time, but are probably due to a progressive improvement in preservation of the pigment.

There is a possibility that pigmented and non-pigmented *G. ruber* may be differentiated on a morphological basis. If, as Hecht[36] suggests from a study of living Atlantic forms, the pigmented varieties are larger in size with wider apertural openings and a higher spire than the white (non-pigmented) type, this morphology may enable us eventually to place a lower age limit on the range of the coloured type despite its fading.

Atlantic sediments

In the North and South Atlantic, pigmented *G. ruber* occurs in the modern-day plankton and in sediments from middle Pleistocene to the Recent. It is most abundant in the boundary currents including the Gulf Stream, Antilles, Canaries, and Brazil Currents, and near Caribbean islands; it seems to be less abundant in regions away from land masses, such as the central Sargasso Sea, the central equatorial Atlantic, and the central South Atlantic[1–11,37]. The distribution pattern generally coincides with temperature maxima of surficial waters, and seems to avoid upwelling areas[38,39]. A strong seasonality has been observed in middle latitude regions[40,41] where the white form predominates during the colder times of the year and the pink form in warmer periods. The pigment intensity increases from pink to deep ruby red with increasing temperatures. Bé and Tolderlund[10] observed a seasonal 'migratory' trend of pigmented

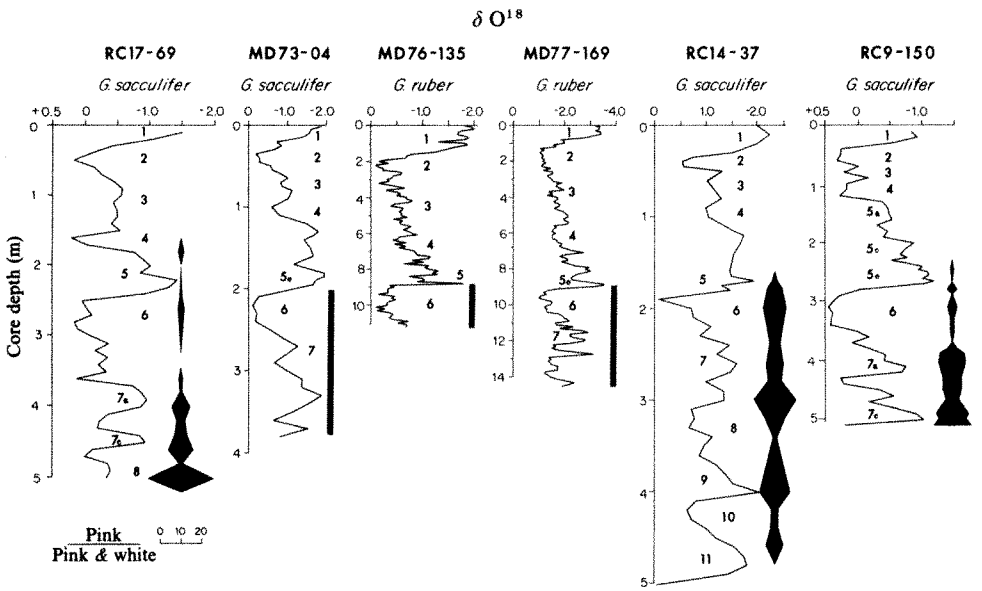


Fig. 2 Indian Ocean cores showing extermination datum of pink-pigmented *G. ruber* and oxygen isotope records (relative to PDB). Dark shading is the % ratio of pink to total *G. ruber*; light shaded line is occurrence of pink *G. ruber* in the core without quantitative measures.

G. ruber populations in the North Atlantic between the Caribbean and regions north of Bermuda. A front of pink *G. ruber* 'moved' northwards from spring to late autumn due to increasing numbers of pink *G. ruber* that developed pigmentation in response to locally elevated temperatures. A reverse southward 'migration' was observed from late autumn to early spring.

Surface sediment distributions of pigmented *G. ruber* closely follow plankton distributions in the North[1–11] and South Atlantic (Fig. 1). The northern limit runs approximately from Nova Scotia in a slightly northerly bulging arc to Spain, whereas the southern limit runs obliquely from Golfo San Matias (Argentina) to Walvis Bay (Namibia). Pink *G. ruber* is absent south of Walvis Bay both in the plankton and surface sediments, indicating that a barrier presently exists which prevents the South Atlantic population from entering the Indian Ocean. Similarly, the South American continent's extension to 55 °S latitude effectively blocks pink *G. ruber* from the Pacific Ocean. The highest proportions of the coloured variety underlie tropical areas of high surface temperature. Both pigmented and non-pigmented *G. ruber* occur in highest percentages on topographically shallow areas where carbonate dissolution is relatively low.

The tendency for pigmented tests of *G. ruber* to be associated with warm oceanic regions seems to be substantiated by downcore studies. Most studies that have distinguished pink from white *G. ruber* have noted two trends reflecting latitudinal occurrence. Low-latitude cores from the Gulf of Mexico, Caribbean and central Atlantic contain both types of *G. ruber* throughout glacial and interglacial stages[42–45]. Cores from subtropical and temperate regions, the Mediterranean[46], and the late Pliocene–early Pleistocene Le Castella section in Italy[47,48], contain pigmented forms only during warm, interglacial stages.

Discussion

The pigmentation in *G. ruber* has been preliminarily identified as the organic compound pheophytin, a carotene[7], incorporated into the test wall. The intensity of colouration is greatest in the earliest-formed chambers and typically becomes progressively lighter in later chambers. The final chamber may vary from

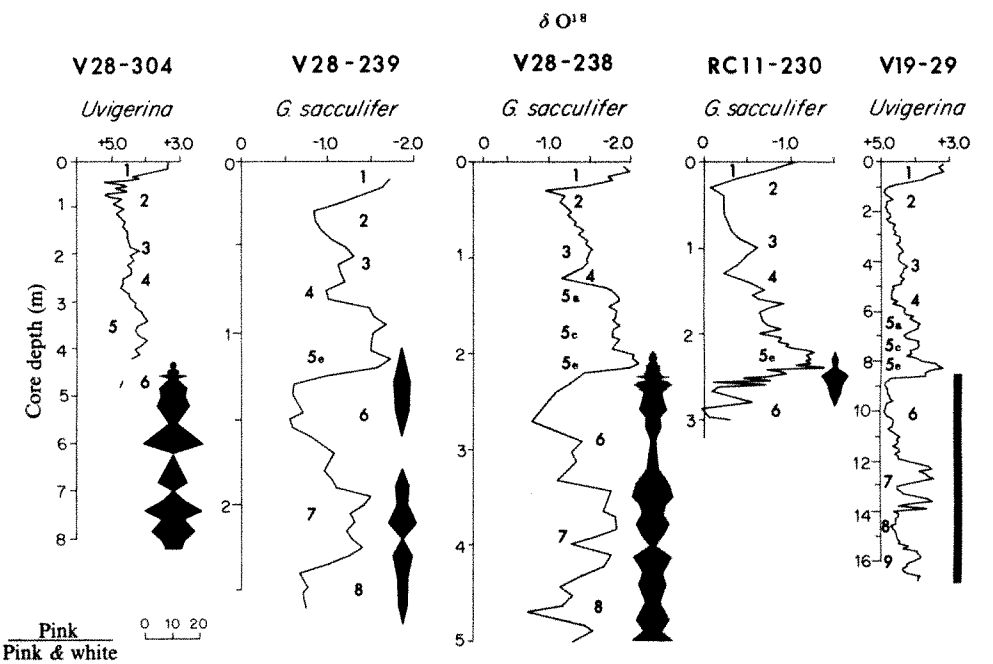


Fig. 3 Pacific Ocean cores with pink-pigmented *G. ruber* datum and oxygen isotopes. See also Fig. 2. Note that *Uvigerina* is absent during stage 5e (420–460 cm) in V28-304.

intensely pink to colourless. The origin and function of the pigmentation are unknown, but may be related to the large quantity of symbiotic zooxanthellae harboured within the protoplasm[49,50]. This light-dependent symbiosis requires *G. ruber* to have a near-surface warm-water habitat, which has been verified by plankton tows[7,10] and, ultimately, gives the species its relatively light oxygen isotopic composition[51,52].

In the Indo-Pacific sediments examined, the pigment intensity is generally much less than in the Atlantic. The Pacific forms are either light pink throughout or are light red in the early chambers, later becoming white. The deep red colouration and large test size common to the tropical Atlantic and West Indies are rare in the Pacific. We suggest that the intensity of colouration is related to vital (and perhaps genetic) factors controlling the production of the pheophytin such as nutrient supply, light intensity, surface temperature, and possibly symbiont type and proximity to land masses. Orr[25,49] has also interpreted the intensity of pigmentation as a qualitative indicator of sedimentation rate.

The oxygen isotopic change of 1.5–1.8‰ in most records across the 6/5e transition demonstrates a drastic, worldwide ice-volume shift in a period of only several thousand years. Isotopic values (see Figs 2 and 3) indicate that stage 6 was one of the longest glacial periods of the Quaternary. The white *G. ruber* form can be found throughout the Pleistocene and Recent Indo-Pacific warm-water region and seems to have suffered no detrimental effects. The persistence of pink forms to the base of stage 5e (Fig. 4) indicates that it lasted through stage 6 to the time of initiation of stage 5 conditions. Again we return to the nature of the pigment and its source. If dinoflagellate symbionts produce the pigment, the extermination may be due to the elimination of the symbionts and thus their host, whereas the species of symbiont in white *G. ruber* would have been unaffected. If production of the pigment is operated by *G. ruber* itself, a case can be made that pink *G. ruber* may be a separate subspecies from the white form. In either case, the disappearance of pigmented *G. ruber* is an important palaeoecological indicator of some natural condition(s) that dropped below, or was raised above, a critical tolerance.

The absence of pink *G. ruber* in the Quaternary and modern Indo-Pacific region may be further evidence of a faunal provincialism that developed between the Atlantic and the Indo-Pacific Oceans after the separation of the Atlantic from the Pacific by the emergence of the Panama Isthmus in the late Pliocene[21]. Rare occurrences of pink *G. ruber* are reported from the late Pliocene from both sides of the Isthmus[25,42,47]. Other evidence of Quaternary faunal provincialism is in a reversed sense of that of pink *G. ruber*. Thus, the planktonic foraminiferal species *G. adamsi*, *G. hexagona*, *G. conglomerata*[45,53], *G. pseudofoliata* (ref. 26) and *Globorotalia menardii neoflexuosa*[54–56] occur in Quaternary sediments and present-day waters

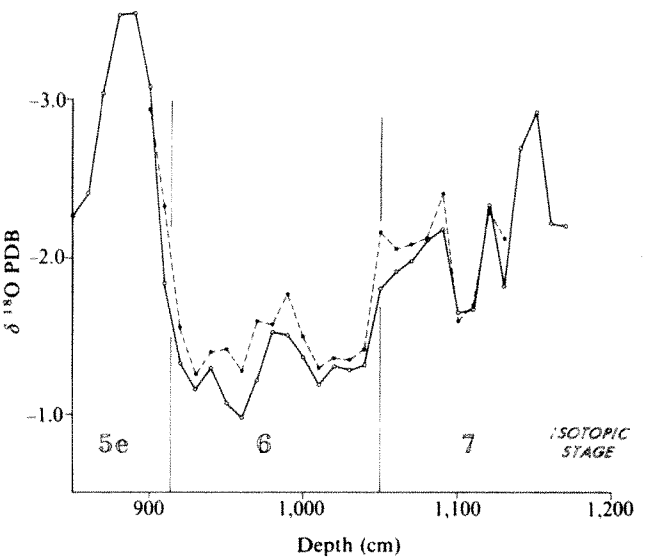


Fig. 4 Isotopic values of pink (●) and white (○) *G. ruber* in core MD77-169. The values show that the pink form lasted into stage 5e.

of the Indo-Pacific, but are absent in the Atlantic during this period. In addition, the coiling direction histories of *Pulleniatina* have diverged into two distinct patterns since the closing of the Panama Isthmus[57,58]. We suspect that the Panama Isthmus emergence, the migrational barrier presented by the Agulhas Current flowing around the Cape of Good Hope with the relatively high latitudinal location of the southern boundary of the African continent at ~35 °S, and the extension of South America to 55 °S, may all have led to the development of genetic differences between Atlantic and Indo-Pacific populations of *G. ruber* which proved fatal to the latter during the earliest portion of oxygen isotope stage 5e. Their disappearance occurred soon after a rapid climatic transition which probably placed a certain stress on the population. Whatever the exact cause of the extermination of pink-pigmented *G. ruber* at 120,000 yr BP, stratigraphers now possess a most useful and widespread datum for the identification of the oxygen isotope 5e/6 transition throughout the Indo-Pacific area.

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Temperature-dependent X-ray diffraction as a probe of protein structural dynamics

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X-ray diffraction at four temperatures from 220 to 300 K coupled with crystallographic refinement yields the mean-square displacements and conformational potentials of all 1,261 non-hydrogen atoms of metmyoglobin. The results are interpreted to indicate a condensed core around the haem, semi-liquid regions towards the outside and a possible pathway for ligands. It is concluded that X-ray diffraction can provide the spatial distribution of the dynamic features of a protein.

TRADITIONAL X-ray models present proteins as static molecules of unique structure. Experimental findings and theoretical arguments, however, indicate that they are fluctuating systems[1,2]. A protein in an equilibrium conformation can exist in a very large number of conformational substates; proteins in different substates have the same coarse structure but differ in local configurations. Rotations about single bonds or shifts of hydrogen bonds may occur. The experiments of Austin *et al.* imply that all conformational substates have the same biological function, but possibly at different rates[3]. As transition from one substate to another generally occurs by overcoming a potential energy barrier, the dynamic behaviour of proteins depends on temperature. At sufficiently high temperatures, a protein molecule moves rapidly from one substate to another, breaking and reforming non-covalent bonds and changing the structure continuously. At low temperatures, much of this conformational relaxation is lost and each molecule remains in a particular substate. The experimental evidence for a fluctuating protein comes from many different techniques. Flash photolysis data at low temperatures imply the existence of many conformational substates[3,4]. Intramolecular motions have been inferred from fluorescence quenching[5] and relaxation[6], NMR[7–9], and isotope exchange[10,11]. Theory implies that a molecular system of N components (amino acids) has approximately e^N states with energies close to that of the ground state[12] and experiences sizeable fluctuation in internal energy, entropy and volume[13]. Because binding is 'strong' ($\gg k_BT$) along the polypeptide backbone and 'weak' ($\approx k_BT$) in other directions, a biopolymer may form a globule, with a condensed core and a diffuse outer phase[14]. A computer solution of the equations of motion of the atoms in a globular protein suggests a fluid-like interior, atoms displaced from their equilibrium position by up to 2 Å, and fluctuations on a picosecond time scale[15].

The experimental evidence for the effect of substates on the binding of carbon monoxide to myoglobin[3] has prompted us to investigate whether conformational substates can be observed by X-ray diffraction. Consider a typical globular protein such as myoglobin. X-ray diffraction permits the determination of the mean-square displacement, $\langle x^2\rangle$, of all atoms except hydrogen. The temperature dependence of $\langle x^2\rangle$ should reveal regions of conformational flexibility. In this article, we outline the features underlying the determination of $\langle x^2\rangle$, describe the experimental approach and present data which support the picture of a fluctuating protein.

Atomic displacements from X-ray scattering

X-ray scattering can determine not only the position, but also the mean-square displacement, $\langle x^2\rangle$, of a scattering particle[16]. For an atom in a protein crystal, $\langle x^2\rangle$ can at a first approximation be written as

$$\langle x^2\rangle = \langle x^2\rangle_c + \langle x^2\rangle_d + \langle x^2\rangle_{ld} + \langle x^2\rangle_v \qquad (1)$$

The subscripts stand for conformation substates, diffusion, lattice disorder and vibration, respectively. The term $\langle x^2\rangle_d$ is due to translational and rotational diffusion and depends on T/η, where T is the temperature and η the viscosity of the crystal. The term $\langle x^2\rangle_{ld}$ is caused by macroscopic disorder in the protein crystal. Even if all protein molecules were identical, the crystal would possess some disorder. Disorder should be almost temperature independent and, if purely translational, contribute equally to all atoms in the protein. The vibrational term, $\langle x^2\rangle_v$, contains components from individual atoms[17], groups of atoms, and the entire molecule (rigid-body motion). The main contribution comes from the vibration of groups of atoms. In the Debye approximation, $\langle x^2\rangle_v$ is constant well below the Debye temperature T_D and proportional to T above T_D (Fig. 1*b*)[16].

The conformational term, $\langle x^2\rangle_c$, is of central interest here. If proteins are fluctuating systems with many conformational substates, each atom or group of atoms will be able to occupy different substates, as shown in Fig. 1*a*, which gives the conformational potential, V_c, as a function of the distance from the mean position. The substates correspond to local minima in V_c. Above a relaxation temperature, T_r, each atom will rapidly move from one substate to another; the substates are in equilibrium and populated according to a Boltzmann distribution. Below T_r the atoms can no longer overcome the barriers between substates and each atom remains in a particular substate. X rays interact with a rapidly fluctuating and a 'frozen' protein in the same way: X-ray diffraction measures the spatial distribution over conformational substates in the fluctuating and the frozen state. The temperature dependence expected for $\langle x^2\rangle_v$, for $\langle x^2\rangle_c$, and for their sum is shown in Fig. 1*b*. The conformational potentials and the barriers between substates depend on the local structure and may change with temperature. The relaxation temperature may differ from atom to atom; the value indicated in Fig. 1*b* implies an average.

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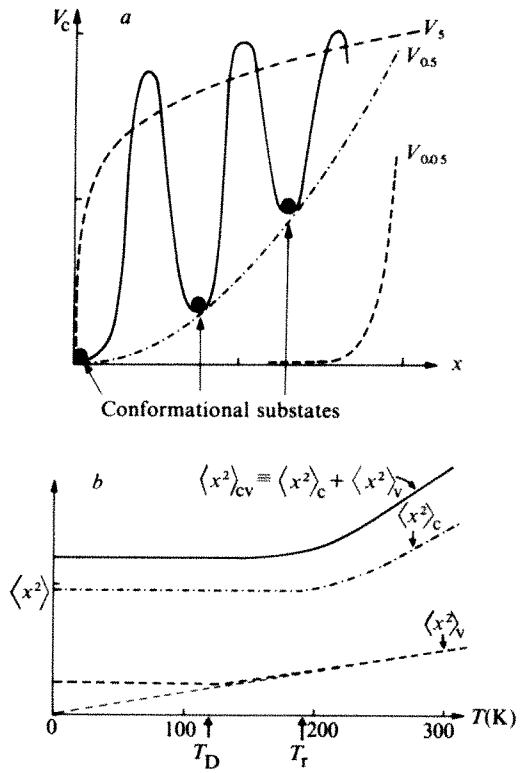


Fig. 1 *a*, The local minima in the conformational potential V_c, given by the solid line, represent conformational substates. The bottoms of the wells are parametrised by power laws, $V_v \alpha x^{1/\nu}$, and three cases are shown as dashed lines. *b*, Temperature dependence of the conformational ($\langle x^2\rangle_c$) and vibrational ($\langle x^2\rangle_v$) mean-square displacement.

The distinction between vibrational and conformational contributions to $\langle x^2\rangle$ is to a certain extent a matter of definition. One way of separating the two terms is by their low-temperature limit. Vibrational contributions should converge towards a value typical for zero-point vibrations, whereas conformational substates can lead to a much larger value of $\langle x^2\rangle$.

Mössbauer effect

Independent information on $\langle x^2\rangle$ for some nuclei (for example, ^{57}Fe) comes from the recoil-less fraction, *f*, of the Mössbauer effect[18,19]. Each nucleus absorbs randomly, stationary disorder does not decrease *f*, and only conformational substates, diffusion[20] and vibrations must be considered. The time scale in the Mössbauer effect is set by the nuclear lifetime, τ_N (140 ns for ^{57}Fe); $\tau_c(T)$ will denote the temperature-dependent mean time for conformational relaxation. At a characteristic temperature T_c, τ_c is equal to the nuclear lifetime

$$\tau_c(T_c) = \tau_N \tag{2}$$

Well below T_c, τ_c is very long relative to τ_N and the Mössbauer effect is sensitive only to $\langle x^2\rangle_v$.

$$\langle x^2\rangle_{M\ddot{o}}(T \ll T_c) = \langle x^2\rangle_v \tag{3}$$

Well above T_c, τ_c is short relative to τ_N and the Mössbauer effect is sensitive to all terms in equation (1) except $\langle x^2\rangle_{id}$.

$$\langle x^2\rangle_{M\ddot{o}}(T \gg T_c) = \langle x^2\rangle_c + \langle x^2\rangle_d + \langle x^2\rangle_v \tag{4}$$

Data collection and structure refinement

Crystals of metmyoglobin have the symmetry of space group $P2_1$, with cell dimensions $a = 64.31$ Å, $b = 30.85$ Å, $c = 34.85$ Å, $\beta = 105.85°$, and one molecule of molecular weight 17,900 per asymmetric unit. One crystal of dimensions $1.1 \times 0.8 \times 0.5$ mm^3 was used for the measurements at 300 K, 275 K and 250 K. The crystal was in contact with its normal mother liquor containing 75% saturated ammonium sulphate and 0.1 M phosphate, *p*H 6.1 Measurements at 200 K were made on a different crystal of identical size and shape in which the normal mother liquor was replaced by 75% 2-methyl-2,4-pentanediol, 30% water and 0.02 M cacodylate buffer with proton activity $p\mathrm{H}^* = 6.1$ (ref. 21). This mixed solvent is a cryoprotectant for the crystals and permits measurements below the freezing point of the ammonium sulphate mother liquor[22].

X-ray diffraction data were recorded with a Syntex $P2_1$ diffractometer equipped with an LT-1 low-temperature device, both modified for protein crystallography. Data were collected by a limited ω-step-scan procedure with continuous checking for crystal movement. No movement was detected. An empirical background curve was fitted to the data[23], which were also corrected for absorption[24]. The crystals were chosen so that the absorption effect was small. The data set at each temperature consisted of over 22,000 independent measurements to 1.5 Å resolution. Only reflections with intensities greater than twice their estimated standard deviations were retained for the refinements, approximately 16,000 per data set. To ensure that the effects of radiation damage were not misinterpreted as increased *B* factors on going to 300 K, data at 300 K were collected first, followed by the sets at 250 K and 275 K. Only 4% radiation-induced decay in diffraction intensities was observed at 300 K, and no significant radiation damage was detected at the lower temperatures.

Refinement of the metmyoglobin structure by the method of Konnert[25] as programmed by Hendrickson (personal communication) involves least-squares minimisation of the weighted sums of $|F_o - F_c|$ plus $|d_o - d_c|^2$. *F* denotes the structure factor, *d* the inter-atomic distance; the subscripts indicate observed and calculated. Proper choice of the relative weights produces a restrained least-squares fit in which the atoms are tethered to positions that maintain ideal bond lengths and angles. Refinement with restraints provides a sufficient ratio of observations to variable parameters to allow the refinement of positional coordinates and an isotropic displacement parameter, $B = 8\pi^2\langle x^2\rangle$, for every non-hydrogen atom. The starting point at each temperature was the refined 2.0 Å resolution structure of metmyoglobin determined by Takano[26], with a discrepancy index

$$R = \sum_{hkl} |F_o - F_c| \Big/ \sum_{hkl} F_o = 0.26$$

The structures have each been refined at 1.5 Å resolution to $R = 0.18$; the refinements took different numbers of cycles to reach this point, but were terminated at the same *R* to make the standard deviations comparable. Restraints on covalent and non-covalent bond distances have been set to preserve ideality within about 0.05 Å for covalent distances and 0.1 Å for the others. Side-chain atoms involved in restrained interactions will have $\langle x^2\rangle$ values that are correlated and possibly too small. Main-chain correlations might lead to $\langle x^2\rangle$ values systematically too large. Ordered water was observed on the surface of the protein, frequently in positions identical to those found by Takano[26]. These atoms were included in the refinement, but were not constrained.

Occasionally, the displacement parameters for a few atoms were found to increase with decreasing temperature, suggesting that the average position of the atom had changed, and the position being used in the refinement was no longer correct. This

Table 1 Average side-chain displacements at 250 K

	$\langle x^2\rangle_{cv}$(Å^2)	
Residue type	Outside	Inside
Charged	0.20±0.02	0.10±0.02
Neutral polar	0.16±0.04	0.08±0.04
Nonpolar	0.07±0.02	0.05±0.01

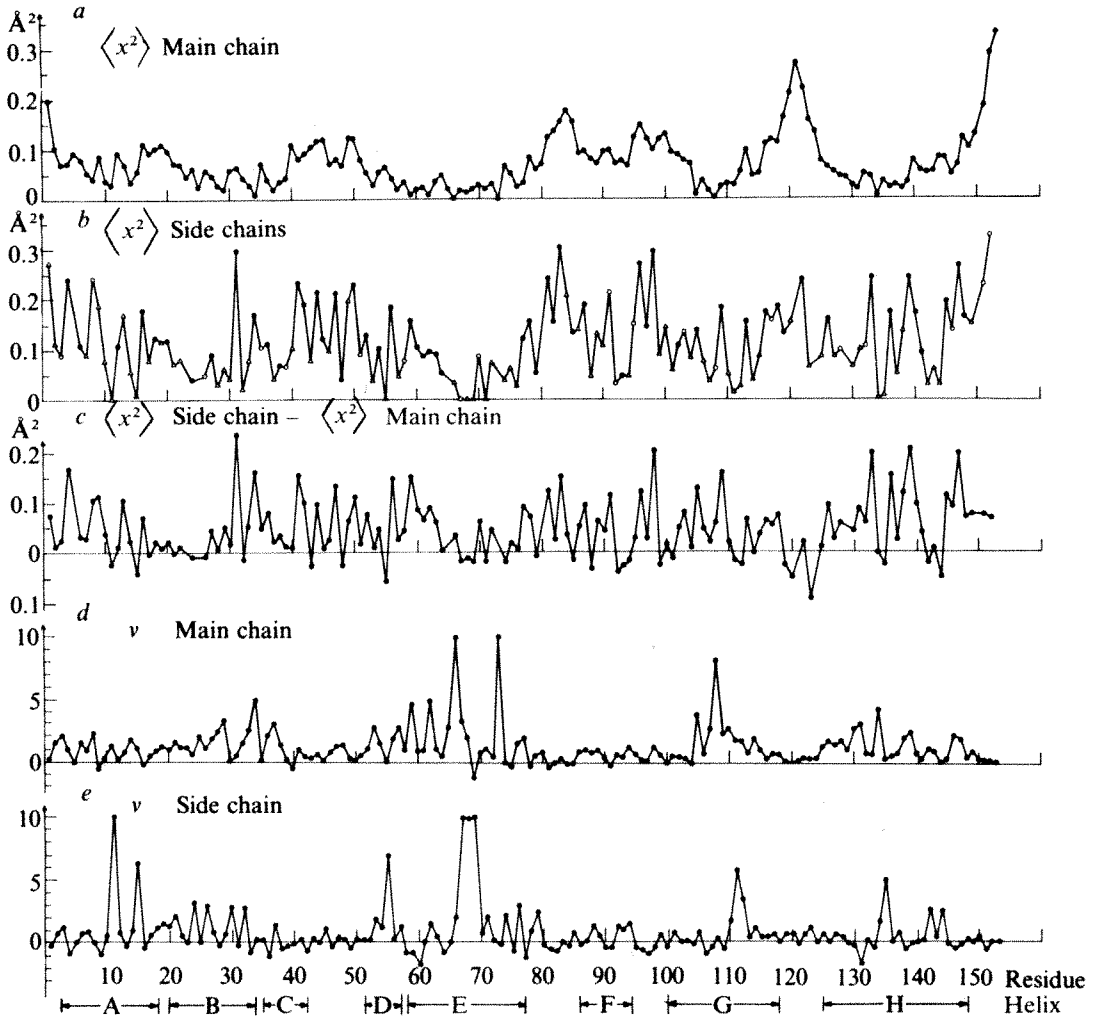


Fig. 2 *a*, The sum of the conformational and vibrational mean-square displacements, $\langle x^2\rangle_{cv} \equiv \langle x^2\rangle_c + \langle x^2\rangle_v$, averaged over the three main-chain atoms N, C^α and C, is plotted against residue number for myoglobin. *b*, The largest displacement in each side chain is plotted against residue number. ●, Hydrophilic; ○, neutral; △, hydrophobic residues. *c*, Difference between side-chain and backbone displacement. *d*, Exponent ν_b in the fit $\langle x^2(T)\rangle_{cv} = \text{const.}\ T^{2\nu}$, plotted against residue number for the main chain (backbone). *e*, Exponent ν_s for the largest side-chain displacement.

suspicion was confirmed by inspection of difference electron-density maps, which were calculated at several stages in the refinement process. Manual correction of the atomic positions led to reasonable temperature dependence in the next cycles of refinement.

In some cases, particularly for the 220 K structure, we observed the appearance of a new electron density at low temperature in positions suggestive of alternative conformations for side-chain atoms. Variable occupancy terms in the refinement may deal with this situation. Difference maps calculated at the present stage of refinement are virtually featureless for the 300 K, 275 K and 250 K structures.

The reliability of the displacement parameters has been checked by refining certain atoms, such as the iron, in a conventional, unrestrained least-squares refinement program. Over-determination was achieved by fixing the remainder of the structure. Cycles of restrained refinement have also been carried out after removal of the restraints on displacement parameters. Finally, a second set of 250 K data from a different crystal was measured and used to obtain refined parameters for comparison. From these results we estimate the standard deviation of our mean-square displacements to be 0.015 Å^2 for most atoms.

Determination of $\langle x^2\rangle_{cv}$

The evaluation described above gives $\langle x^2\rangle$ for all non-hydrogen atoms. Two assumptions underlie the standard treatment and are built into the current refinement methods: (1) $\langle x^2\rangle$ is isotropic; (2) the probability of finding a given atom a distance x from its mean position is gaussian

$$P_{1/2}(x) = P_{1/2}(0)\exp(-x^2/2\langle x^2\rangle) \quad (5)$$

For the exploratory extraction of $\langle x^2\rangle_c$, we add three more assumptions: (3) equation (1) holds; (4) diffusion can be neglected in crystals; $\langle x^2\rangle_d = 0$; (5) all atoms have the same temperature-independent value of $\langle x^2\rangle_{ld}$.

Assumptions (1) and (2) are certainly oversimplifications, but we retain them for the present. Assumption (4) is justified by the fact that the X-ray pattern is sharp even at 300 K. Assumption (5) is equivalent to considering the lattice disorder to be purely translational. Although we expect rotational disorder to be present, we have not yet assessed its magnitude. The fact that atoms on the protein surface have widely differing values of $\langle x^2\rangle$ indicates, however, that rotational disorder cannot be dominant.

Our eventual goal is the separate determination of $\langle x^2\rangle_c$ and $\langle x^2\rangle_v$. In the present study we will only determine their sum, $\langle x^2\rangle_c + \langle x^2\rangle_v \equiv \langle x^2\rangle_{cv}$. To extract $\langle x^2\rangle_{cv}$ from $\langle x^2\rangle$, we combine the results from X-ray and Mössbauer experiments and use the haem iron as calibration. Equations (1) and (4) show that $\langle x^2\rangle - \langle x^2\rangle_{M\ddot{o}}(T \gg T_r) = \langle x^2\rangle_{ld}$. Parak (ref. 27 and personal communication) has determined $\langle x^2\rangle_{M\ddot{o}}$ in metmyoglobin crystals up to 283 K, obtaining values of 0.037 Å^2, 0.061 Å^2 and 0.068 Å^2 at 250 K, 275 K and 300 K, respectively. Our X-ray data give $\langle x^2\rangle$ values of 0.091 ± 0.003 Å^2, 0.101 ± 0.003 Å^2 and 0.108 ± 0.003 Å^2, respectively, leading to $\langle x^2\rangle_{ld} = 0.045 \pm 0.006$ Å^3. Subtraction of this value from the measured X-ray results yields $\langle x^2\rangle_{cv}$ for all 1,261 non-hydrogen atoms of metmyoglobin. A table of all results at the four temperatures would be too long, and so we present in Fig. 2 only two numbers for each amino acid, the average for the backbone atoms N, C^α and C, and the value for the atom with the largest displacement in each side chain at 250 K.

As an indication of the size of $\langle x^2\rangle_v$, we note that linear extrapolation of the low-temperature Mössbauer data of Debrunner and coworkers[28] yields $\langle x^2\rangle_v \cong 0.02$ Å^2 at 300 K for the iron atom.

Structural dynamics of myoglobin

The data in Fig. 2 show that X-ray diffraction can provide an insight into the spatial distribution of the dynamic aspects of biomolecules. Phillips and coworkers have drawn similar conclusions[29,30].

Figure 2 provides evidence for conformational substates because $\langle x^2\rangle_{cv}$ reaches large values relative to typical vibrational displacements, and the large displacements are essentially temperature independent. Our Fig. 2 shows features similar to the prediction displayed in Fig. 2 of the paper by Karplus and coworkers[15]. Both prediction and experiment show very large displacements at the two terminals, the largest one occurring at the carboxyl end. To find a lower limit for the number of substates, in Fig. 2*c* the difference Δ between the values of $\langle x^2\rangle_{cv}$ for the backbone and the side chain are plotted for each residue as given in Fig. 2*a* and *b*. We arbitrarily assume that any residue with $\Delta \geq 0.025$ Å^2 has at least two conformational substates. About 95 residues satisfy this criterion, yielding $\geq 2^{95} \cong 10^{28}$ substates.

Figure 3 shows the static myoglobin structure as solid lines, areas reached by movements (at 250 K) of the backbone atoms shaded. The limiting excursion x_{lim} region is determined by setting $P_{1/2}(x_{lim})/P_{1/2}(0) = 0.01$ (equation (5)). Conformational substates still have a probability of 0.01 of being outside the shaded volume.

Figures 2 and 3 provide information on the conformational substructure of myoglobin. Figure 2*b* indicates that 44 residues have a maximum side-chain mean-square displacement larger than 0.15 Å^2. Almost all these lie on the surface of the protein. Of the 44, only 7 are hydrophobic. The observation of large values for $\langle x^2\rangle_{cv}$ on the surface implies a considerable interaction between solvent and protein. Table 1 gives the average values for the largest side-chain displacements, grouped according to type and position of the residue. Not surprisingly, displacements

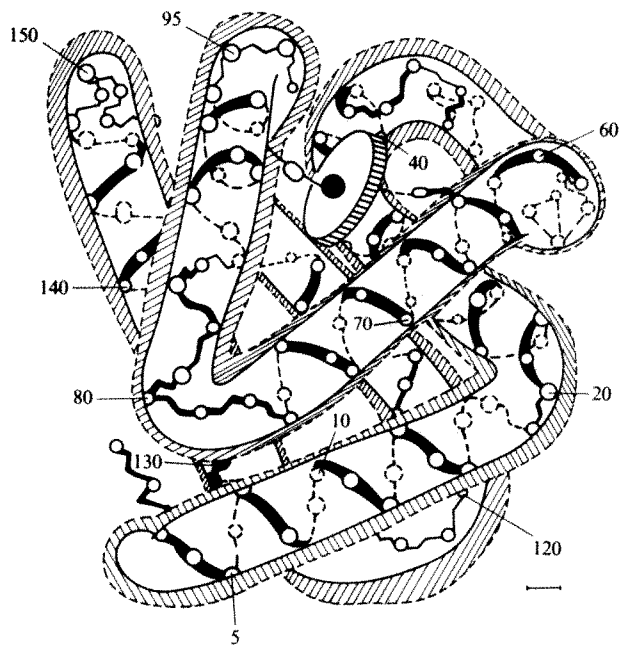


Fig. 3 Backbone (main chain) structure of myoglobin. The solid lines indicate the static structure as given in ref. 37. Circles denote the C$^\alpha$ carbons; some residue numbers are given. The shaded area gives the region reached by conformational substates with a 99% probability. Scale bar, 2 Å.

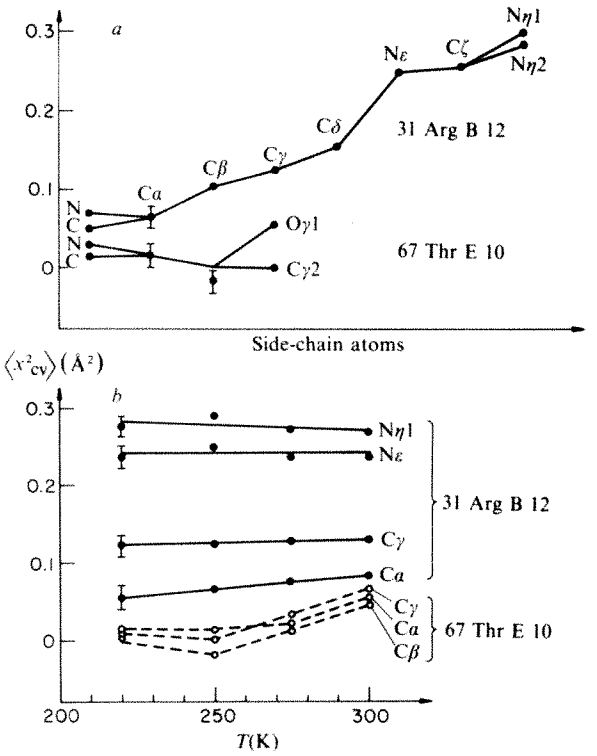


Fig. 4 Displacements $\langle x^2\rangle_{cv}$ for all non-hydrogen atoms of Arg 31 and Thr 67. *a* Shows the displacement at 250 K as a function of distance from the main chain; *b* gives the temperature dependence for some of the atoms. All points have the same estimated error; error bars are only shown on some.

are smaller inside the protein than outside. Charged and polar residues show larger displacements than the nonpolar ones, possibly because of charge fluctuations in the solvent and the hydration shell[1,2].

It is useful to follow the main chain, comparing $\langle x^2\rangle$ for both backbone and side chain with the position along the main chain. As already pointed out, the displacement is large whenever the side chain can extend into the solvent and small when other parts of the protein hinder the motion, as, for example, near residues 30, 60, 105 or 134. Even turns around the helix can be followed. Good examples are residues 10–14 in the A-helix, 25–28 in the B-helix and 73–76 in the H-helix, where the variation of $\langle x^2\rangle_{cv}$ around the helix is clearly seen. Similar periodicities occur in many places, suggesting that the helices do not move only as rigid units, but may also experience rippling or breathing modes. The accompanying article presents similar results for lysozyme[30].

The region around the haem is particularly interesting. On the distal side, near His 64, residues 39, 64, 67, 68, 71, 72 and 107 are in van der Waals contact with the haem group. The average $\langle x^2\rangle_{cv}$ at 300 K for the side chains is 0.04 Å^2, so that $x_{r.m.s.} = 0.2$ Å, comparable to values in many solids. These residues thus form a tight compartment around the binding site, guarding the access to the haem iron. Entrance to the pocket may be possible near residues 43–45, which are also on the distal side and have an average $\langle x^2\rangle_{cv} = 0.15$ Å^2. These residues are in the general region of one of the pathways found in a model calculation by Karplus and collaborators (D. A. Case and M. Karplus, in preparation). On the proximal side, near His 93, the situation is strikingly different. The average $\langle x^2\rangle_{cv}$ for the side chains in contact with the haem (42, 43, 89, 92, 97, 99, 103, 104, 138) is 0.12 Å^2. Moreover, the motion of the F-helix is also large. For haemoglobin, Perutz has suggested that this region is involved in a dynamic control mechanism[31]. The conformational displacement of the haem iron can be compared with the distribution in tunnelling distance observed in low-temperature flash photolysis[32]. If the conformational motion of Fe is perpendicular to the haem plane, $\langle x^2\rangle_{cv}(\text{Fe}) = 0.04$ Å^2 leads to $x_{r.m.s.}(\text{Fe}) = 0.35$ Å.

The tunnelling data imply an average spread in the tunnelling distance of approximately 0.3 Å in ferrous myoglobin.

Temperature dependence of $\langle x^2 \rangle_{cv}$

Additional information can be obtained from the temperature dependence of $\langle x^2 \rangle_{cv}$. We simplify the situation shown in Fig. 1 by assuming that each atom moves in a conformational potential, V_ν, indicated by dashed lines. We approximate the isotropic potential, $V_\nu(x)$, $x > 0$, by a power law

$$V_\nu(x) = \text{const.}\ x^{1/\nu} \tag{6}$$

Three different potentials are given in Fig. 1. At a temperture $T \gg T_r$, substates at distance x are populated according to a Boltzmann distribution

$$P_\nu(x : T) \propto x^2 \exp(-\text{const.}\ x^{1/\nu}/T) \tag{7}$$

The mean-square displacement for $T \gg T_r$ is given by

$$\langle x_\nu^2 \rangle = \int dx x^2 P_\nu(x; T) \Big/ \int dx P_\nu(x; T) = \text{const.}\ T^{2\nu} \tag{8}$$

where the constant depends on ν but not on T. We set $\langle x^2 \rangle_\nu = \langle x^2 \rangle_{cv}$ and obtain ν from plots of log $\langle x^2 \rangle_{cv}$ against log T. The values for the backbone (ν_b) and the side chain (ν_s) are given in Fig. 2*d* and *e*. Residues with small $\langle x^2 \rangle_{cv}$ generally have large values of ν, those with large $\langle x^2 \rangle_{cv}$ small values. Averaged over atoms with the same value of $\langle x^2 \rangle_{cv}$, the exponent ν satisfies the relation

$$\nu = \nu_0 \exp(-\xi \langle x^2 \rangle_{cv}) \tag{9}$$

where $\nu_0 = 4$, $\xi = 22$ Å^{-2} and with $\langle x^2 \rangle_{cv}$ measured at 250 K. Regions of the protein with small displacements behave like solids and have steep potential wells (V_5 in Fig. 1). In contrast, most parts with large $\langle x^2 \rangle_{cv}$ move in broad square wells (like $V_{0.05}$ in Fig. 1) and thus have many substates of about equal energy[33]. Only a few residues seem to have harmonic potentials, $\nu = 1/2$.

The behaviour of two residues, Arg 31 and Thr 67, is shown in Fig. 4. Figure 4*a* displays $\langle x^2 \rangle_{cv}$ for some atoms of the main chain and all non-hydrogen atoms of the side chains. For Arg 31, which lies on the outside of the protein, the displacements are large and increase with distance from the main chain. The displacements for Thr 67, which is in contact with the haem, are much smaller and show no clear dependence on distance. In Fig. 4*b*, $\langle x^2 \rangle_{cv}$ for some atoms of the two residues is plotted against temperature. The displacements of the atoms of the side chain of Arg 31 are essentially temperature independent ($\nu < 0.1$), whereas the atoms of Thr 67 show a pronounced temperature dependence ($\nu > 3$). The standard deviation of $\langle x^2 \rangle_{cv}$, 0.015 Å^2, is shown on some of the data points.

Figure 2*a* and *b* contains only one-fifth of our data at one temperature. Plotting all data would convey more information, but also obscure essential features. However, computer graphics provide a means of spatial representation of our results. Atoms are drawn as spheres with van der Waals radii and placed at their mean positions. Each atom is shaded according to the displacement $\langle x^2 \rangle_{cv}$. The protein is then cut in the desired plane, atoms exposed by the cut become visible, and structure and motion can then be observed at the same time. Figure 5 displays two such cuts through myoglobin at 250 K. A condensed core around the active centre, semi-liquid outer shells, and at least one pathway to the pocket on the distal side of the haem are apparent. The core can probably be likened to an aperiodic solid: the number of conformational substates per group of atoms is small, $\langle x^2 \rangle_{cv}$ is less than 0.04 Å^2 and the temperature coefficient ν is larger than 2. Residues in the semi-liquid regions have larger conformational displacements $\langle x^2 \rangle_{cv} \sim 0.04$–$0.25$ Å^2, and can probably assume many substates, and ν is generally less than 2. The term semi-liquid implies that the structure is liquid-like within volumes of a few Å^3; molecules may undergo brownian motion within the volumes accessible to them (micro-brownian motion) and the semi-liquid region may thus be very similar to glasses[34]. These features agree with the description used by McCammon, Gelin and Karplus ("fluid-like in that the local atom motions have a diffusional character")[15]. The displacement $\langle x^2 \rangle_{cv}$ increases nearly continuously from the inside to the protein surface.

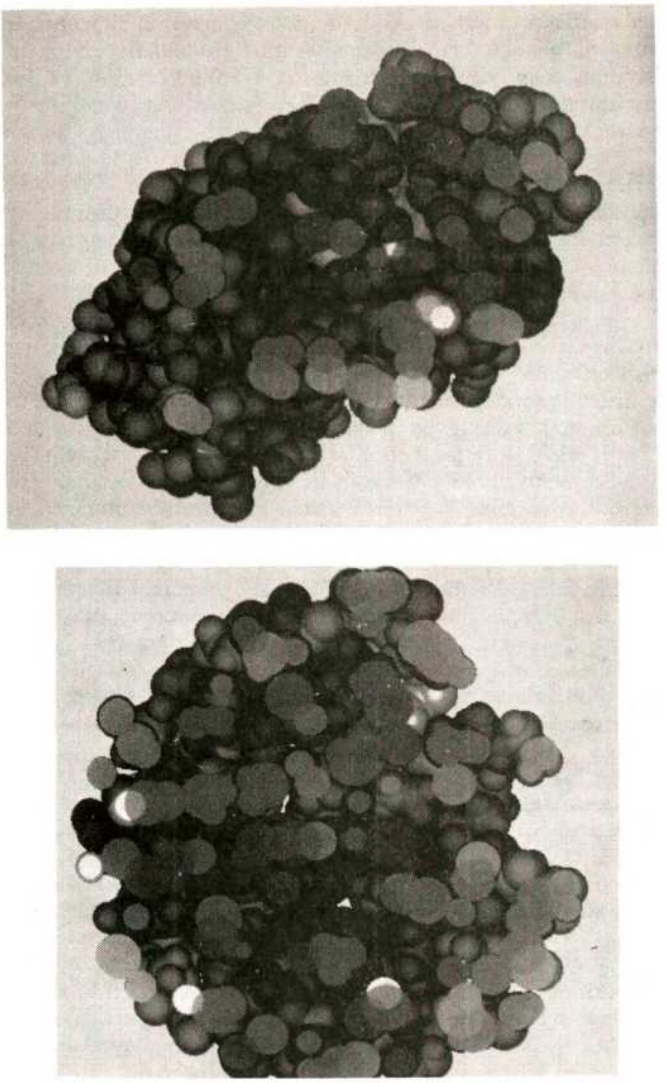


Fig. 5 Two slices through the 'dynamic' structure of myoglobin. Atoms are shown as spheres with van der Waals radii centred at their mean X-ray positions. Spheres interpenetrate when atoms are covalently bonded. The shading of each atom is proportional to the mean-square displacement $\langle x^2 \rangle_{cv}$ at 250 K; the heavier the shading of the atom, the smaller the value of $\langle x^2 \rangle_{cv}$. The upper cut is in a plane parallel to the haem plane and slightly towards the distal histidine side. The lower cut is at right angles to the haem plane and passes through residues 93 and 106; only the 'active site' area is shown. The haem plane is horizontal in the centre of the figure; Arg 45 is to the right.

Future prospects

Our results suggest that the extraction of $\langle x^2 \rangle$ from the intensity of the diffraction peaks should be carried out for non-isotropic distributions (assumption (1)) and for charge densities that are not gaussian (assumption (2))[35]. In protein structure refinement the standard method assumes correct bond lengths and bond angles, and no poor van der Waals contacts. The average structure, which fits the observed X-ray data best, will have apparent wrong bond lengths and angles and poor van der Waals contacts.

X-ray diffraction yields only information about the spatial distribution of protein motion. The temporal behaviour must be studied by other means, such as NMR, fluorescence relaxation, flash photolysis and molecular dynamics[36]. The results presen-

ted in Figs 2–5 may provide the maps to guide such work. As some of these other tools may be applied to solutions and single crystals, they may also answer the question of whether the dynamic properties of a protein are identical in a crystal and a solvent.

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Crystallographic studies of the dynamic properties of lysozyme

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The patterns of atomic displacements in the crystals of hen and human lysozyme derived from independent crystallographic refinement are broadly similar. Analysis of the pattern indicates a close correlation with molecular structure, strongly suggestive of intramolecular motion. The active site of lysozyme is located in a region of high displacement. It is concluded that protein mobility may play a significant part in biological activity and that X-ray crystallography can contribute to its analysis.

THE distribution of electron density within a molecule, which is given by X-ray analysis of single crystals, can be used to determine two major pieces of information about the atoms making up the molecule. First, the mean positional coordinates of each atom can be obtained from the maxima in the distribution, and second, the mean square amplitude of displacement of each atom from its mean position can be derived from the rate at which the electron density falls off from the corresponding maximum. A quantitative estimate of the mean square displacement can be obtained from crystallographic refinement in reciprocal space in which the electron density of each atom is modelled by $f \exp(-Bs)$, where f is the scattering factor for the atom at rest, s is a reciprocal lattice vector, and B (often called the temperature factor) is related to the mean square amplitude of harmonic diplacement (U^2) by the expression $B = 8\pi^2 U^2$. If isotropic displacement is assumed, the B-factor has a single value; however, anisotropic displacement can be modelled by splitting B into six parameters whose values define the axial lengths and orientation of an ellipsoid of displacement.

Although analysis of B-factors is routinely used in the crystallography of small molecules[1], it is much less frequently applied to protein molecules, despite the possible significance[2–4] of knowledge of atomic displacements for the study of protein dynamics, and their role in biological activity. At least part of the reason for this is that there are several experimental difficulties in obtaining accurate B values, and theoretical problems in relating observed displacements to true atomic motion, which are exacerbated in the case of very large molecules like proteins. The most vexing of the theoretical problems arises because the X-ray measurements are averaged over time and, in effect, over crystal space. Consequently, there is no time component associated with the observed displacements, and this makes it impossible to distinguish directly between true atomic motion derived from vibrational and rotational modes, and static disorder caused by different molecules in the crystal adopting different conformations, or occupying different positions and orientations in the various unit cells. A possible way of distinguishing true atomic motion from static disorder is by analysis of X-ray intensities collected at different temperatures[5]. (See accompanying paper[6] for a discussion of this, and other approaches to the problem.)

At least two other problems at this level of significance must be considered before any attempt can be made to use B factors to describe protein dynamics. First, because the values of the B factors are determined largely from the variation of the X-ray

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pattern with diffraction angle, the observed displacements are potentially very sensitive to systematic errors in the collection and correction of the X-ray measurements. Errors of this kind are particularly difficult to avoid when using large[7], liquid-containing, radiation-unstable[8] protein crystals. In addition, observable X-ray intensities from protein crystals are restricted to lower diffraction angles than those from most crystals of small molecules; this will therefore reduce the precision of the observed displacements. Second, the crystal packing may modify the atomic displacements as compared with those in free solution. Unlike small molecules, globular protein molecules are stabilised by the same weak forces that stabilise the crystals, and, although this factor may endow protein molecules with a richer variety of motions than small molecules, it may also increase the relative influence of the crystal environment on the actual displacements.

In this report we compare the displacements of the homologous hen and human lysozyme molecules, which crystallise in quite different space groups. Our results were derived from independent refinements of sets of X-ray intensities at different resolution, which were collected and corrected by different procedures. The close agreement of the displacements not only suggests that the effects of experimental error and crystal packing are not as serious as might be thought, but also that the pattern of displacements may represent a characteristic molecular property of biological significance.

Treatment of X-ray intensity data

The values of the B factors are derived from independent refinements of hen egg-white lysozyme (HEWL) at 2 Å resolution (D.E.P.G. and D.C.P., unpublished) and the homologous[9] human lysozyme (HL) at 1.5 Å resolution (P.J.A. and C.C.F.B., unpublished). The two enzymes crystallise in unrelated space groups, HEWL in $P4_32_12$ with $a = b = 79.1$ Å, $c = 37.9$ Å (ref. 10), and HL in $P2_12_12_1$ with $a = 57.1$ Å, $b = 61.0$ Å, $c = 32.9$ Å (ref. 14). In their respective unit cells the molecules are quite differently packed.

The collection and correction of the X-ray intensity data were carried out using different procedures. The X-ray intensities of HEWL[10] were collected in levels in reciprocal space on a linear diffractometer adapted to measure three reflections simultaneously[11]. More than half the reflections were measured four or more times and fewer than 5% only once. No crystal was used for longer than 20 h so that the unreduced data comprised ~30,000 measurements from 14 crystals. In contrast, the intensities of HL were measured on a computer-controlled five-circle diffractometer equipped to measure five reflections simultaneously[12]. The intensities were collected in shells in reciprocal space, each reflection being measured at least twice. Only one crystal was used for the complete data set of 50,000 reflections.

Data reduction and correction were also carried out by different methods: the net intensities of HEWL were obtained by the background–peak–background method, those of HL by profile fitting[13]. Radiation damage in HEWL was estimated from a common row of reflections measured before and after each level, and the correction was applied to each triplet level. In HL[14] it was estimated from a set of reference reflections monitored throughout the data run, and applied as a single correction to the whole data set. Absorption was corrected in HEWL by a two-dimensional[7], and in HL by a three-dimensional method[15].

Refinement of the HEWL structure was carried out by automated shifts estimated from difference syntheses[17], using the RS5D coordinates produced by the Diamond real-space refinement[16] as the starting point. Constraints on covalent bond lengths and angles, but not on non-bonded or hydrogen-bond interactions, were applied to the coordinates after one or more

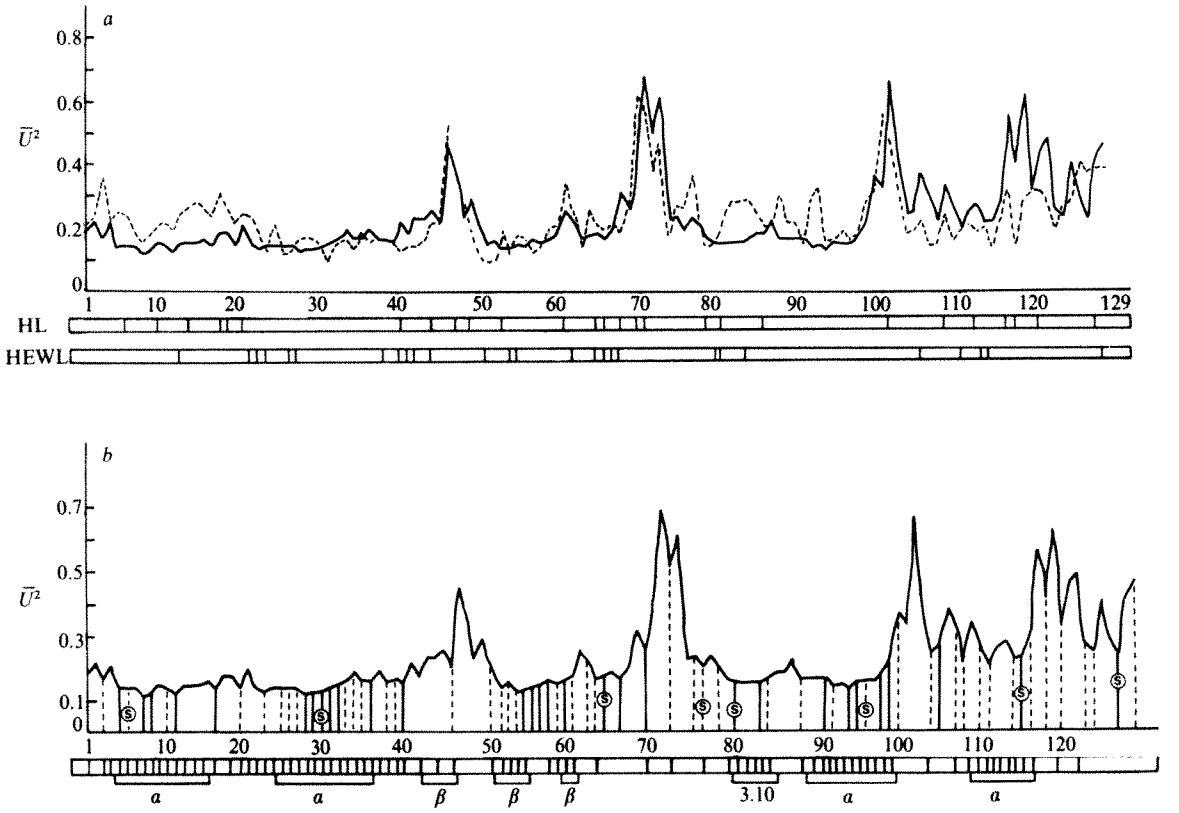


Fig. 1 *a*, A plot of mean main-chain $\overline{U^2}$ values against residue number for HL (full line) and HEWL (broken line). The numbering scheme is for HEWL; the additional residue in HL is inserted between residues 47 and 48. Residues involved in intermolecular contacts in the crystals of the two molecules are indicated in the lower rectangles. *b*, As *a* for HL. The full vertical lines indicate the location of internal residues in the lysozyme molecule, and the broken vertical lines residues lying in the molecular surface[24]. The residues involved in intramolecular hydrogen bonds are shown in the lower rectangle.

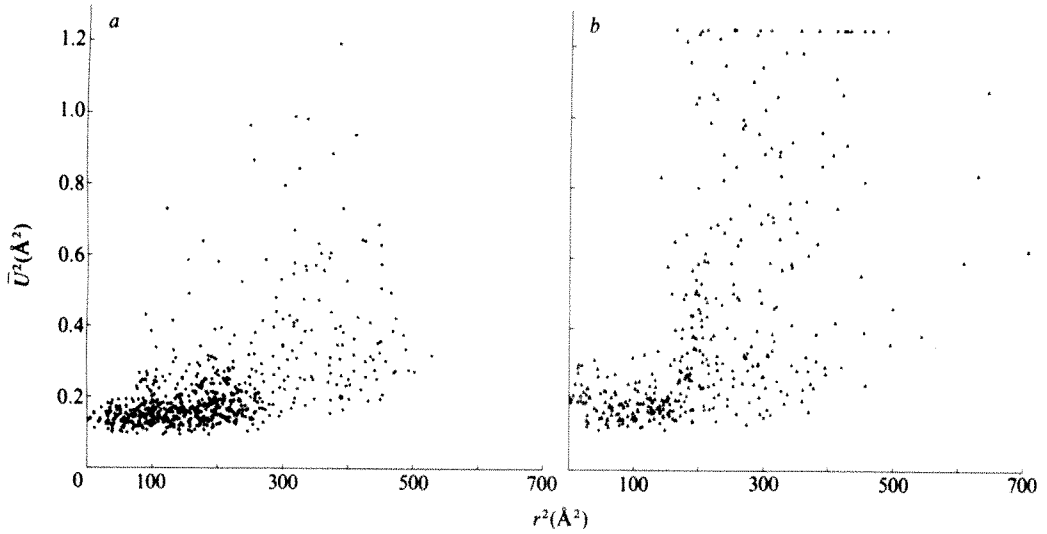


Fig. 2 A plot of atomic $\overline{U^2}$ values of HL against the square of their distance from the molecular centroid. *a*, Main-chain atoms; *b*, side-chain atoms.

cycles of shifts. Because at 2 Å resolution there are only 9,600 structure amplitudes, only two B factors were used to describe each residue, one for the four main-chain atoms and the β-carbon, and another for the side-chain atoms beyond C^β. The refinement has not been completed but the R factor is currently 0.22 for all 9,600 terms using 1,001 protein atoms and some 250 water molecules. The initial stages of the HL refinement also used difference syntheses, but the later stages were mainly carried out using the constrained least-squares method of Hendrickson and Konnert[18]. Rather tight constraints were imposed on the covalent structure of the protein: covalent bond lengths were not allowed to vary by more than 0.01 Å. However, the weights of the constraints on non-bonded interactions and hydrogen bonds were reduced to the minimum values allowed by the Konnert programme (10^{-8} of the weight of the covalent constraints), and no constraints were imposed on the B factors at any point. At intervals during the least-squares refinement, a few cycles of difference map shifts were carried out to monitor the course of the least-squares refinement. The additional resolution available for HL allowed refinement of individual B factor for each atom. The current R factor for all 19,000 terms to 1.5 Å is 0.18 (≡0.17 at 2 Å resolution) using 1,026 protein atoms and 130 water molecules.

We know that the use of tight stereochemical constraints may not always be appropriate because of the effects of libration, first discussed by Cruickshank[33]. On the other hand, because constraints are used to compensate for the lack of true atomic resolution even at 1.5 Å, relaxation of the constraints to allow for libration would be expected to result in some degree of randomisation of the atomic positions elsewhere. Preliminary consideration of this complex problem suggests that the B values of atoms positioned incorrectly, through the application of inappropriate constraints or any other factor, will become anomalously high so as to maximise the overlap between the model and the real electron density.

Comparison of the displacements

Figure 1*a* shows a plot of the mean square displacement of the main-chain atoms of HEWL and HL against residue number. For HEWL, $\overline{U^2}$ has been obtained from the common B factor for the N, C^α, C', O and C^β atoms for each residue, and for HL it has been obtained by calculating the mean B value for the equivalent five atoms in each residue. It can be seen that, to a first approximation at least, the two plots have the same characteristics: a fairly constant background on which are superposed several peaks corresponding to residues 47, 70–73, and 101 and 102. Although there are some differences, particularly around residues 117–119 and 121 and 122, which in HL have greater displacements than in HEWL, the general agrement is good, as shown by the correlation coefficient of 0.61. The distribution of $\overline{U^2}$ is much smoother for HL, which almost certainly reflects the more accurate B factors obtained from the more complete refinement of higher resolution data. As each step in the collection, reduction and correction of the X-ray data from the two molecules has been carried out differently, it is most unlikely that the parallel variations in these curves represent merely the incorporation of systematic experimental error into the B values. Similarly, because the two enzymes are packed together in their respective crystal lattices in quite different ways, the observed variations in $\overline{U^2}$ cannot in the main be due to interactions in the crystal.

These considerations suggest that the parallel variation of the displacement curves represents some particular property of the HEWL and HL molecules themselves. As there are 51 side-chain substitutions and one additional residue in the HL molecule[19,20] as compared with HEWL, it seems probable that the main chain is the major determinant of the variation. We will now consider two major possibilities: (1) that the variation is a consequence of a rigid-body displacement or simple intramolecular displacement, and (2) that it corresponds to more complex intramolecular displacements. Broadly, these two possibilities can be investigated by determining whether the displacements are related to the shape of the molecule, or to its structure, respectively.

Analysis of main-chain displacements

Various models of possible overall intermolecular and intramolecular displacement of the averaged main-chain $\overline{U^2}$ values were examined. All the models that M.J.E.S., D.E.P.G. and D.C.P.[21] describe in their analysis of HEWL were considered for both molecules, and the salient results are summarised below. The best parameters to describe each model were found by a least-squares fit to the observed $\overline{U^2}$ values, and the quality of fit by a parameter R^2, expressed by

$$R^2 = \sum_{a=1}^{N} [(\overline{U^2}_a)_{obs} - (\overline{U^2}_a)_{calc}]^2 / \sum_{a=1}^{N} [(\overline{U^2}_a)_{obs} - \overline{U^2}_{mean}]^2$$

where

$$\overline{U^2}_{mean} = \sum_{a=1}^{N} (\overline{U^2}_a)_{obs}/N$$

and N is the number of residues. $R_2 = 0$ indicates total agreement and $R^2 = 1$ the worst possible agreement. The models described are as follows.

Model A: a rigid body mode, consisting of a uniform translational displacement together with independent librational displacements about three principal axes that intersect at the centroid (the 'TL' model of Cruickshank[22]).

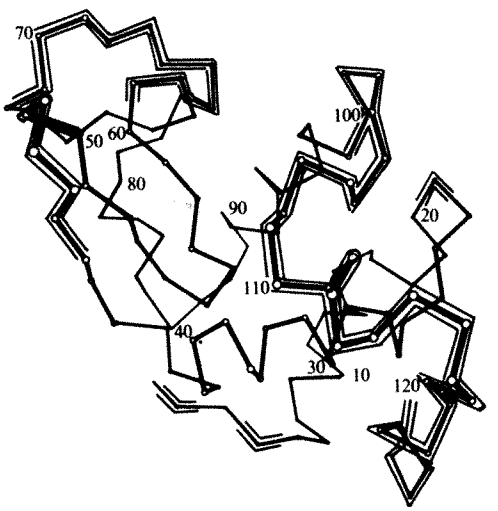


Fig. 3 A perspective drawing of the lysozyme molecule with the residues having displacement >0.2 Å^2, in HL outlined by the parallel lines. The active site cleft runs almost vertically down the front of the drawing.

Model B: a general 'breathing' mode, which is a reinterpretation of model A as intramolecular displacements consisting of a uniform component together with displacements that increase with distance from the molecular centroid, and have independent magnitudes along the three principal axes.

Model C: an intramolecular 'hinge bending' mode, in which displacements corresponding to a variation in the separation of the two lobes of the molecule that enclose the active site cleft, are superimposed on a uniform displacement. This mode is analogous to that studied by Karplus and coworkers[23] using energy calculations of molecular dynamics.

In neither molecule did hinge bending account for much of the variation [R^2(HEWL) = 0.79; R^2(HL) = 0.79], even though, as shown below, the lips of the active-site cleft correspond to regions of high displacement. Rigid-body displacement (model A) or the equivalent breathing mode (model B) gave better agreement [R^2(HEWL) = 0.54; R^2(HL) = 0.49]. The magnitudes of the libration or breathing for each molecule were significantly, and similarly, non-uniform along the three principal axes. However, the directions of the axes of principal displacement in HEWL and HL differed by 35°. Certain residues, including those marking the peaks in Fig. 1*a*, had markedly higher observed U^2 values than were accounted for by models A and B, and these residues probably have additional intramolecular displacements. Models A and B were therefore amended to include and determine the additional motion of each of these residues, increasing the agreement of these models to R^2(HEWL) = 0.17 and R^2(HL) = 0.13. Without anisotropic B values it is difficult to distinguish between the alternatives of rigid-body displacement and intramolecular breathing.

Given that even for the rigid-body model considerable allowance had to be made for intramolecular displacement, we have examined independently the case for intramolecular displacement by considering the relationship between the U^2 values and the molecular fold in terms of regular secondary structure and the relative exposure of the side chains[24]. Figure 1*b* shows the plot of this relationship, which indicates that segments of regular secondary structure, both α and β, correspond quite closely to the relatively smooth minima in the U^2 distribution. Closer examination reveals some correlation with the finer details of structure; for example, there are cyclic variations along the 4–16 and 108–116 helices that correspond to greater displacements of residues located on the exposed surface of these helices, and the most exposed strand, residues 42–46, of the small three-stranded β-sheet in lysozyme has a consistently higher displacement than the other two. There is also a good correlation between local minima in the U^2 plot with internal or surface residues. This is particularly striking in regions where there is little or no regular secondary structure; for example, in the C-terminal region, from residue 100 onwards, each downward trend in the sawtooth distribution of displacement corresponds to an internal or surface residue, and two of the deepest troughs correspond to the two disulphide links in this region, residues 115 and 127. Thus, the regions of high displacement correspond to main-chain segments that are not involved in regular secondary structure and whose side chains are external. These close correlations of displacement with structure reinforce the conclusion that we are observing a characteristic molecular property, and not some artefact of the crystallography.

If the regions of high displacement ($\overline{U^2} > 0.2$ Å^2) shown in Fig. 1*a* for HL are located on the folded lysozyme molecule (Fig. 3), it can be seen that most of them will lie on a continuous strip on the enzyme surface that begins at the exposed strand of the β-sheet, runs along to the 'top' of the molecule, down one side of the active-site cleft and along to the C-terminus. It is intriguing that this region of high displacement includes both lips of the active-site cleft[31], which is also the principal antigenic determinant of lysozyme[32], and also most of the main-chain segments which undergo conformational change when ligands are bound[31].

Analysis of side-chain displacements

Figure 2*b* shows a plot of $\overline{U^2}$ against r^2, where r is the distance from the molecular centroid for the side chains of human lysozyme. The striking feature of this plot is the clear discontinuity in the U^2 distribution at an r^2 value of about 180 Å^2: below this value the displacements are closely grouped between 0.1 and 0.3 Å^2, but above this value the displacements vary widely, with several displacements up to 1.25 Å^2 corresponding to the arbitrary allowed maximum for B factor of 100 Å^2. The lysozyme molecule is ellipsoidal, with dimensions of about 30 Å × 30 Å × 45 Å (ref. 10). Thus, the value of r at the discontinuity of about 14 Å corresponds quite closely to the minimum radius of the molecule. This shows that the internal residues, those with $r^2 < 180$ Å^2, are subject to quite small displacements of the same order as the main-chain displacement in this region (Fig. 2*a*), whereas those side chains with $r^2 >$ 180 Å, which are mostly external, have widely varying displacements, some of which are very large. To analyse the pattern of side-chain displacements more closely we have listed the mean difference between the side-chain and main-chain displacement for each type of amino acid residue (Table 1). With the exception of the smallest of the hydrophobic residues (Ala) all types of side chains are displaced more than their local main

Table 1 Incremental displacements of the side chains of human lysozyme

Type	No.	$\langle\Delta\overline{U^2}\rangle$	Type	No.	$\langle\Delta\overline{U^2}\rangle$
Ala	14	−0.006 ± 0.020	Ser	6	0.024 ± 0.052
Cys	8	0.008 ± 0.028	Thr	5	0.013 ± 0.055
Val	9	0.012 ± 0.057	Pro	2	0.145 ± 0.173
Ile	5	0.023 ± 0.019	Asp	8	0.152 ± 0.079
Leu	8	0.069 ± 0.043	Asn	10	0.208 ± 0.207
Met	2	0.051 ± 0.030	His	1	0.404
Phe	2	0.051 ± 0.046	Tyr	6	0.108 ± 0.157
Trp	5	0.049 ± 0.027	Glu	3	0,380 ± 0.351
			Gln	6	0.387 ± 0.280
			Lys	5	0.251 ± 0.230
			Arg	14	0.380 ± 0.276

$\langle\Delta\overline{U^2}\rangle = (\sum_{a=1}^{n}\langle\overline{U^2}_a\rangle$ side-chain atoms $-\langle\overline{U^2}_a\rangle$ main-chain atoms$)/n$, where n is the number of side chains of a given type. In the calculation the β-carbon is considered to be part of the main chain: relaxation of this condition is required to calculate the incremental displacement for alanines. The increase of the spread of the values with increasing $\langle\Delta\overline{U^2}\rangle$ shows that the side chains with largest incremental displacements also show the largest variations.

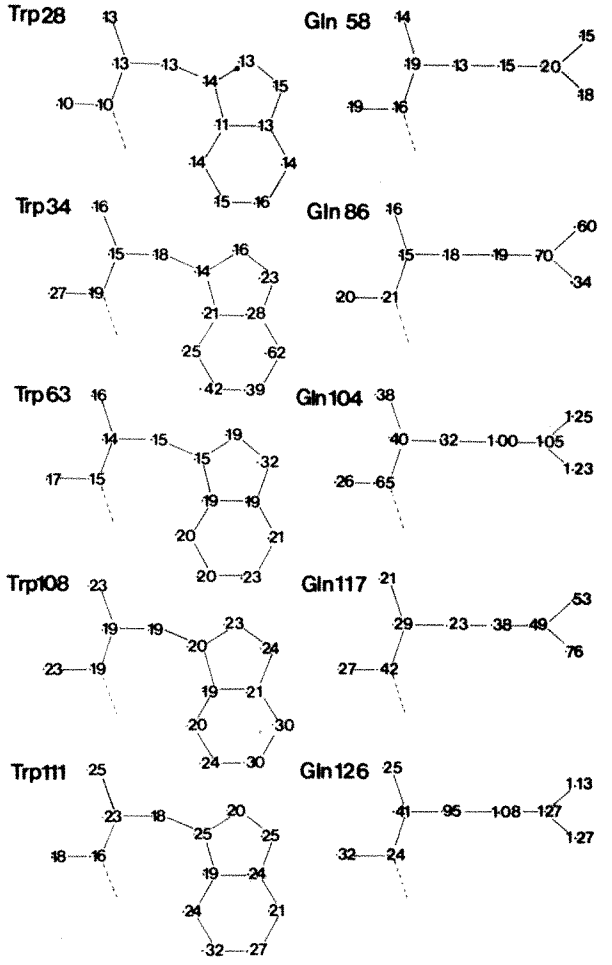


Fig. 4 The five tryptophan residues, and five of the six glutamine residues of human lysozyme showing the individual mean-square displacements of their atoms in Å^2. Trp 28 is the only residue of this group that is totally buried in the core of the molecule, and its atoms show consistently low displacements. In Trp 34, 108 and 111 it can be seen that the atoms of the six-membered part of the indole ring have larger displacements than the other atoms, consistent with some degreee of 'flapping' displacement about their α–β or β–γ bonds. In contrast to the tryptophan residues, the glutamine residues, which with the exception of Gln 58 are external, show increasingly large displacements along their length. Gln 58 lies in the surface of the enzyme with its amide group hydrogen bonded to the main chain, and the displacements of its atoms are lower and do not increase along the length of the side chain. The pattern of displacements in other types of side chain are fully consistent with these examples.

chains. The positive increment is quite small for all hydrophobic residues, but increases sharply with length for the hydrophilic side chains. This analysis reinforces the above conclusion, and suggests that the displacement of side chains is governed largely by their environment and their length, or perhaps, more precisely, by the number of bonds about which free rotation is possible. Figure 4 shows the atomic displacements of the tryptophan and glutamine residues. We have also made a preliminary analysis of anisotropic displacements of certain side chains. Figure 5 shows the results for Trp 63 which suggest that the indole ring of this residue undergoes greatest displacement in the plane of the ring.

Discussion

This study suggests that X-ray crystallography can be used to describe the atomic displacements in a protein molecule. Preliminary analyses indicate that the displacements must have an intramolecular component superimposed on either a rigid-body motion or a general breathing mode. The close correlation between the displacements and the molecular structure suggests that the observed pattern of displacements is characteristic of the lysozyme molecule. Possibly the most significant finding is that the lips of the active-site cleft, and the region of the enzyme that undergoes conformational change on ligand binding to the active site, are included in the regions that have the greatest displacements.

Unfortunately, the X-ray data described here do not allow us to interpret these displacements directly in terms of motion. There is, however, both theoretical and experimental evidence of intramolecular motion in proteins. Scheringer[25] has shown that even in molecules much smaller than lysozyme, the contribution of intramolecular motion increases with the number of atoms in the molecule. His analysis gives a rough estimate of the mean-square amplitude of intramolecular motion in lysozyme as 0.2 Å^2, which compares remarkably well with mean-square displacement amplitude of 0.23 Å^2 that we observe for the main-chain atoms of both HEWL and HL. A study of the dynamic motion of the pancreatic trypsin inhibitor[4] by energy calculations has estimated an r.m.s. displacement for all non-hydrogen atoms of 0.9 Å, the main chain moving less than the side chains. Nuclear magnetic resonance studies of HEWL in solution[26–28] have shown that Tyr and Phe residues 'flip' by 180° about their β–γ bonds; this requires considerable movement in neighbouring atoms and that external side chains undergo a rapid waving motion. Hydrogen–isotope exchange data for proteins have been interpreted as indicating breathing motions in proteins[29], and the similarity in the rates of hydrogen–tritium exchange of HEWL in solution and in the crystal[30] implies that the motion is not reduced by crystallisation.

This evidence of motion in proteins, which is consistent in a qualitative sense with our observations, suggests that it is reasonable to allocate some part of the observed displacement to molecular motion. However, there must be further investigation of methods, such as the analysis of the temperature dependence of the displacements, that may help in apportioning the relative contributions of static disorder and motion to the observed displacements. The analysis of the motion in terms of the contributions from rigid-body and intramolecular modes would be much improved if a complete set of anisotropic B factors could be calculated; this may be possible at 1.5 Å resolution or better. It is also evident that thermal diffuse scattering can be observed from proteins using synchrotron radiation (L. N. Johnson, personal communication), which could provide evidence of dominant libration axes[1]. The importance of anharmonic vibrations[21] might also be assessed, for example, by reformulation of the temperature factors in the manner proposed by Dawson[34] and Johnson[35]. Finally, in view of the location of some parts of the active site of lysozyme in regions of high displacement, if would be very interesting to discover the effect of binding substrate-like ligands on the pattern of displacements.

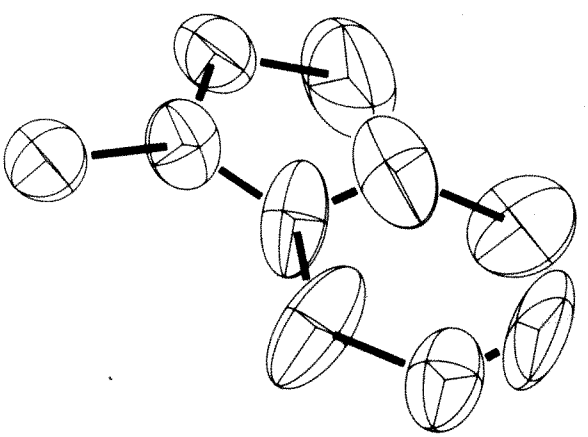


Fig. 5 A conventional drawing of the displacement ellipsoids of Trp 63 obtained from a preliminary analysis of anisotropic B values of the tryptophan residue of human lysozyme.

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letters

Soft X-ray emission from the vicinity of MV Lyr

EVIDENCE is presented for the existence of an X-ray source in the range 0.18–0.43 keV at a location consistent with the position of the 'nova-like' variable star MV Lyr. The source, designated H1901+43, was observed by the HEAO-1 A2 experiment[1] between 18 and 22 October 1977.

We have analysed data from the smallest field of view of the A2 experiment 0.18–2.5 keV detectors. This has dimensions of 1.55° FWHM in the scan direction (ecliptic latitude) and 3.1° FWHM in the perpendicular direction (ecliptic longitude). The total geometric area of the detector with this field of view is 175 cm^2. Figure 1 shows the 0.18–0.43 keV scan data in the vicinity of MV Lyr summed over the time interval specified above. A 5σ count excess above background is observed at the latitude of MV Lyr. The solid curve depicts the expected point source response of the detector collimator superposed on a parabolic background function. This combination has been fitted to the data in such a way as to minimise the χ^2 statistic, allowing the strength and position of the source to be free parameters, together with the background terms. Adding higher order terms to the background function does not improve the quality of the fit. The best fit source intensity in the 0.18–0.43 keV band is found to be 1.5 ± 0.3 counts s^{-1}.

No evidence is found for 0.5–2.5 keV emission from H1901+43. However, the data in this band are contaminated by emission from a second harder source, H1918+43, probably associated with the hard X-ray emitter 4U1919+44[2](2A1919+435[3]), which is separated from H1901+43 by ~1° in ecliptic latitude. We can conservatively adopt the measured flux of H1918+43 between 18 and 22 October, 1.0 counts s^{-1}, as an upper limit to 0.5–2.5 keV emission from H1901+43. The constraint thus imposed on the count ratio in the 0.18–0.43 and 0.5–2.5 keV bands limits the temperature of the H1901+43 source to be less than 1.2×10^7 K, assuming an optically thin bremsstrahlung spectrum. A lower limit to the temperature of $\sim 10^5$ K is implied by the low energy cut-off of the detector. Assuming a spectral temperature of 3×10^5 K, which is consistent with a detection in the 0.18–0.43 keV band only, the observed count rate corresponds to an energy flux of 4×10^{-12} erg cm^{-2} s^{-1} between 0.18 and 0.43 keV.

Figure 2 depicts the two-dimensional error box for H1901+43. The positional bounds in ecliptic latitude have been obtained by fitting the collimator response to the scan data, and correspond to a confidence level of 90%. The source location in ecliptic longitude is defined by the extent of the collimator in this direction (3.1° FWHM) and the daily motion of the satellite as it tracks the Sun. H1901+43 was observed in data taken on several consecutive days and must lie within the common area observed by the collimator on those days. The dimensions of the error box in this direction are therefore less than the full width at base of the collimator and define absolute limits on the position of the source in ecliptic longitude. The error boxes for the 2–10 keV sources 4U1919+46 and 2A1919+438 are also shown in Fig. 2. The positions of these medium energy sources are inconsistent with that of H1901+43.

It can be seen from Fig. 2 that MV Lyr is well contained within the error box of H1901+43. A cursory search revealed no other obvious candidates for the source of the X-ray emission. This, coupled with the fact that several other cataclysmic variables, notably AM Her[4], U Gem[5] and SS Cyg[6] have been found to emit soft X-ray radiation, leads us to suggest MV Lyr as a candidate for identification with H1901+43. Note, however, that the possibility of chance positional coincidence cannot be discounted because of the large positional uncertainty involved. MV Lyr was within the ±3.1° longitudinal extent of the detector collimator between 16 and 30 October. H1901+43 was detected in scan data summed between 18 and 22 October, when the mean distance of MV Lyr from the scan path was ~1°. If the identification with the star is correct, the failure to detect X-ray emission after 22 October may imply variability. This would not be surprising in view of the variable nature of the X-ray sources associated with other cataclysmic variables[4,5].

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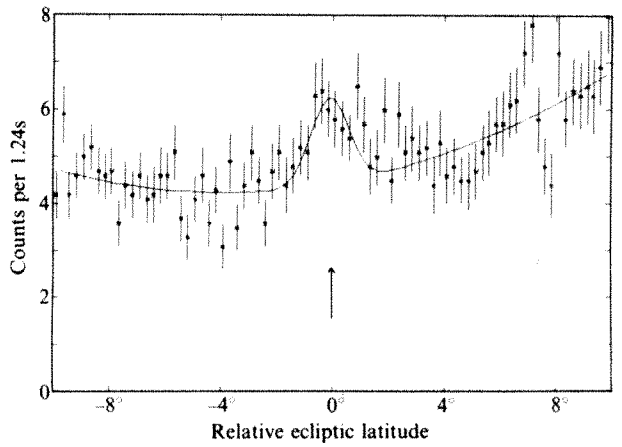


Fig. 1 Summed scan data in the 0.18–0.43 keV band plotted relative to the expected position of MV Lyr (arrow). The solid curve is the best fit collimator response superposed on a parabolic background function.

MV Lyr can be classed among the nova-like stars which have as their prototype UX UMa[7–9]. It exhibits rapid light variations[8,10] and a hydrogen emission spectrum which may be superposed on broad Balmer absorption[8,10–12]. There is evidence that MV Lyr is a binary system with an orbital period of ~2 h (ref. 11). The spectrum, luminosity and temporal characteristics of the UX UMa stars are similar to those of the dwarf novae at maximum light, and they may be essentially the same kind of system, but in an extended outburst[7–9]. Further to this suggestion, we note that if the proposed X-ray identification is correct, the ratio of soft X-ray to optical luminosity for MV Lyr is within a factor of two that observed from U Gem during outburst[5]. The range of absolute magnitude inferred for the UX UMa stars as a class ($3 \leqslant M_v \leqslant 6$)[7] together with the observed visual magnitude of MV Lyr[7], suggests that this star is at a distance of between ~300 pc and ~1,200 pc, corresponding to an X-ray luminosity range of $(4–60) \times 10^{31}$ erg s^{-1}. The distance of the star is consistent with its detection at ~1/4 keV given its relatively high galactic latitude ($b^{II} = 16°$).

The tentative identification of MV Lyr as a soft X-ray emitter may provide an important clue to the relationship between various types of cataclysmic variable stars. We note that the likely optical counterpart for the X-ray source 2A0526–328 may also be a non-erupting cataclysmic variable[13]. It will be important to seek confirmation of these results, and to search for X-ray emission from other stars in the UX UMa category.

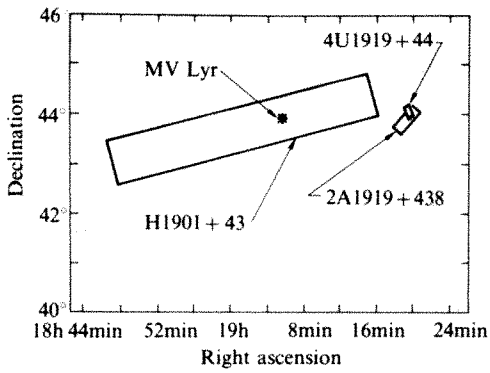


Fig. 2 Error box for H1901+43. The confidence level is 90% in the narrow (scan) direction. In the perpendicular direction, the positional uncertainty is defined by the dimensions of the collimator, and the time interval over which the source was detected.

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Parity nonconservation and stellar collapse

ACCORDING to the current theory, the evolution of sufficiently massive stars leads eventually to gravitational instability and collapse. After thermonuclear burning has stopped, dense stellar cores become unstable and collapse to form neutron stars (pulsars) or black holes. In both cases energy of the order of $0.1\,Mc^2$ is released, mostly in the form of neutrinos and gravitational waves. As the radius of the core decreases, its magnetic field grows (magnetic flux is conserved) and can reach huge values of order 10^{12}–10^{13} G. Even greater azimuthal fields ($\sim 3 \times 10^{15}$ G) can be generated by differential rotation of the core[1]. It will be shown here that neutrino emission in the collapse is asymmetric, as a result of parity nonconservation in weak interactions. Neutrinos and antineutrinos are emitted preferentially in the direction parallel to the local magnetic field of the collapsing core.

The basic physical effect here is the generation of neutrino energy flux by magnetic fields in the presence of electrons (and positrons). The magnetic field polarises the electrons, and neutrinos scattering on electrons come off with their momenta correlated with the magnetic field. Clearly, this effect violates reflectional symmetry, as the energy flux is a polar vector and the magnetic field is an axial vector. The neutrino energy flux depends on the details of neutrino–electron scattering and its calculation requires a fully fledged transport theory. However, a crude estimation can be made if we take into account the following general features of electron–neutrino interaction. Neutrinos and antineutrinos are coupled to left-handed electrons and right-handed positrons more strongly than to right-handed electrons and left-handed positrons, the difference in the coupling constant being of the order of the coupling constant itself (assuming that electrons are relativistic, that is $T \geqslant 10^{10}$ K). The neutrino–electron cross-section is a rapidly growing function of energy, so that the probability of head-on collision is greater than that in the case of a neutrino running after an electron. Assuming for clarity that the magnetic field is directed upwards, it is easily seen that left-handed electrons and right-handed positrons move preferentially upwards, and thus neutrinos and antineutrinos moving down scatter more

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frequently than those moving up. (In this order-of-magnitude estimation we disregard the motion of neutrinos in the transverse plane.) The ratio of the corresponding mean free times is of the order of $\tau_\downarrow/\tau_\uparrow \sim n_\uparrow/n_\downarrow$ where $n_\uparrow$ and $n_\downarrow$ are the densities of electrons with spin up and with spin down, respectively. If $\nu_\uparrow$ and $\nu_\downarrow$ are the densities of neutrinos moving up and moving down, respectively, then in a stationary flux $\nu_\uparrow/\tau_\uparrow \sim \nu_\downarrow/\tau_\downarrow$. Assuming for simplicity that the neutrino chemical potential is smaller than the temperature T, the neutrino momentum density $\mathbf{S}$ is of the order of $n_\nu T(n_\uparrow - n_\downarrow)/(n_\uparrow + n_\downarrow)$. Here $n_\nu = \nu_\uparrow + \nu_\downarrow \sim T^3$ is the total neutrino density and the system of units in which $c = \hbar = k = 1$ is used here. In the presence of scatterers other than electrons (such as neutrons or nuclei) the expression for $\mathbf{S}$ has to be multiplied by $\alpha \sim \tau_{\rm tot}/\tau_{\nu e}$, where $\tau_{\nu e}$ and $\tau_{\rm tot}$ are neutrino–electron and total neutrino mean free time, respectively. The ratio $(n_\uparrow - n_\downarrow)/(n_\uparrow + n_\downarrow)$ is of the order of $\mu B/\varepsilon$, where ε is the characteristic electron energy, $\varepsilon \sim T$ or $\varepsilon \sim \zeta_e$ (whichever is greater), $\mathbf{B}$ is the magnetic field, ζ_e is the chemical potential of electrons, and μ is the effective electron magnetic moment, $\mu = e/2\varepsilon$. (The latter equation follows from the expression[2] for electron energy levels in a constant magnetic field, $\varepsilon = (\varepsilon_0^2 \pm eB)^{1/2}$, where ε_0 is the spin-independent part of the energy.) The neutrino energy flux can now be written as

$$\mathbf{S} \sim \alpha n_\nu T \varepsilon^{-1} \mu \mathbf{B} \qquad (1)$$

This equation implies that the average momentum per neutrino is of the order of $\alpha\mu\mathbf{B}T/\varepsilon$. Note that neutrino and antineutrino currents in magnetic field have the same direction. A detailed derivation of equation (1) from the Boltzmann equation will be given elsewhere.

To estimate the role of parity-violating effects in stellar collapse, I shall use the results of numerical calculations for conventional supernovae models[3,4]. These calculations show that at the neutrino photosphere $T < \zeta_e \sim 13$ MeV, and thus $\varepsilon = \zeta_e \sim 13$ MeV. The neutrino transport is dominated by coherent scattering on nuclei, so that $\tau_{\rm tot} \sim 0.1\ \tau_{\nu e}$ and $\alpha \sim 0.1$. The total momentum carried away by neutrinos is of the order of

$$\mathbf{P} \sim \alpha N_\nu T \zeta_e^{-1} \mu \mathbf{B}_{\rm av} \sim \alpha E_\nu \zeta_e^{-1} \mu \mathbf{B}_{\rm av} \qquad (2)$$

where $N_\nu \sim E_\nu/T$ is the total number of emitted neutrinos, E_ν is the energy lost in the form of neutrinos, and $\mathbf{B}_{\rm av}$ is the magnetic field averaged over the neutrino photosphere (the quantities α, ζ_e and $\mathbf{B}_{\rm av}$ have also to be averaged over the whole period of intensive neutrino emission). The corresponding recoil velocity of the core is

$$\mathbf{v} \sim -\alpha\mu\mathbf{B}_{\rm av}E_\nu/M\zeta_e$$

For the characteristic values $B_{\rm av} \sim 5 \times 10^{12}$ G, $E_\nu/Mc^2 \sim 0.1$ we get $v \sim 5 \times 10^4$ cm s^{-1}. The actual velocities of pulsars are of the order of 10^7 cm s^{-1}. We see that the effect of asymmetric neutrino emission can account only for a small fraction of this value. The effect can be more important in collapse of magnetic stars where one can expect greater values of the magnetic field.

As already mentioned, the azimuthal magnetic field B_φ can be much greater than $B_{\rm av}$. Numerical calculations show[1] that the spatial distribution of the azimuthal field can be rather complicated. B_φ reaches its maximum value ($\sim 10^{15}$ G) in relatively small localised regions of the core. Neutrino fluxes generated by azithmuthal magnetic fields can have a role in a redistribution of the angular momentum of the collapsing core or of a hot neutron star. It can be shown that the angular momentum flux at distance r from the rotation axis is of the order of $\mathscr{L}_{\rm B} \sim \alpha n_\nu T \mu \zeta_e^{-1} l_\nu r \nabla B_\varphi$, where l_ν is the neutrino mean free path and it is assumed that the characteristic length scale of B_φ is smaller than r but greater than l_ν. An estimation shows that $\mathscr{L}_{\rm B}$ can be of a comparable magnitude to that of the neutrino angular momentum flux induced by rotation[5], $\mathscr{L}_{\rm R}$. However, the directions and thus the effects of the two fluxes are quite different. $\mathscr{L}_{\rm R}$ transports angular momentum from the inner to the outer parts of the core. $\mathscr{L}_{\rm B}$ tends to slow down the rotation in the regions of most intensive azimuthal field if the rotational velocity v_φ has the same sign as B_φ and tends to accelerate the rotation if $v_\varphi B_\varphi < 0$. If, for clarity the angular momentum of the core is directed upwards, then B_φ tends to have opposite signs in upper and lower hemispheres, and thus the flux $\mathscr{L}_{\rm B}$ develops an asymmetry in the angular velocity distribution.

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Solar radio spectrum extended to 10 km wavelength

KNOWLEDGE of the range of solar radio burst flux densities is important for understanding the physical processes generating these bursts. However, past summaries of the solar radio spectrum[1,2], compiled from ground-based observations, have not extended to wavelengths much below 10m because the ionosphere of the Earth is opaque to longer wavelengths. Consequently, radio waves generated at altitudes above 5–10 solar radii in the outer corona can be observed only from above the ionosphere. The spectrum presented here covers wavelengths of 1 mm to 10 km; the observations between wavelengths of 100 m and 10 km were made by Earth satellites.

At hectometric and kilometric wavelengths thermal radiation from the Sun is typically five orders of magnitude weaker than the experiment threshold sensitivity, determined by local plasma noise and cosmic background noise. Nevertheless, satellites have detected hundreds of thousands of hectometric and kilometric solar radio bursts of nonthermal emission in the past decade. Type III solar radio bursts are by far the most common variety observed at these long wavelengths. According to our present knowledge[3], type III bursts are generated when energetic (10–100 keV) electrons are ejected from the Sun and travel outwards along open magnetic field lines. These energetic electrons excite emission at the second harmonic of the local solar wind electron plasma frequency. As the exciter electrons move away from the Sun into regions of lower density, the radiation occurs at progressively longer wavelengths and later times. Average type III burst emission wavelengths are 1 km at 0.3 AU and 5 km at 1 AU. A few type II bursts at wavelengths longer than 100 m have also been observed, occuring at both the fundamental and second harmonic of the plasma frequency in association with a propagating interplanetary shock[4,5].

Figure 1 includes long wavelength data from the Interplanetary Monitoring Platform IMP6 and the Radio Astronomy Explorer RAE1 Earth orbiters. Radio astronomy experiments on these satellites use long wire antennas, 91 m and 229 m respectively, connected to multi-channel radiometers in the frequency range of 25 kHz to 10 MHz. The IMP6 orbital altitude ranged from 2 to 33 Earth radii (the experimental arrangement has been described by Brown[6]). RAE1 operated in a circular orbit at an altitude of one Earth radius (its experiment systems have been discussed in detail elsewhere[7]).

Flux density measurements for very intense bursts are shown in Fig. 1. Smaller bursts are much more numerous. The distribution of burst maximum intensities at a given wavelength follows a power law, $n = A(\lambda)S^{-\beta}$, where n is the occurrence rate (bursts per day per unit flux density), S is the observed flux density, and $A(\lambda)$ is a constant for each wavelength. Observations demonstrate that $\beta = 1.5$ over a wavelength range of 0.1–300 m [8–10]. Accordingly, for a given wavelength, there are

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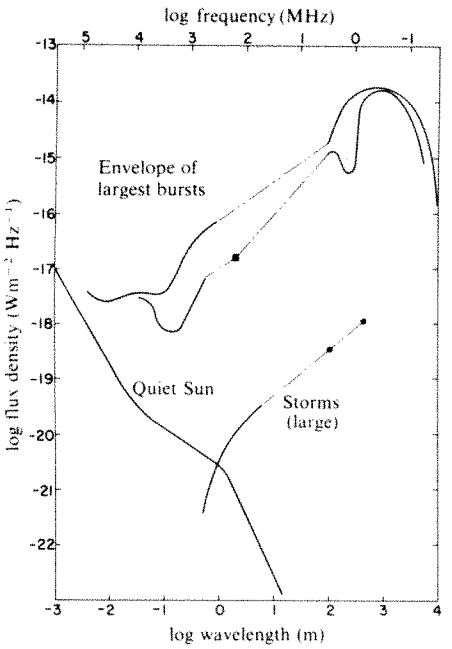


Fig. 1 Solar radio spectrum showing the envelope of flux levels reached by the largest reported bursts, one very large type III burst on 4 August 1972 (■), large noise storms, and the quiet Sun background. ●, RAE 1 measurements for a type III storm in August 1968.

approximately 3.2 times as many bursts with maximum flux density in the interval $S, S+\Delta S$ as in the interval $10S$, $10(S+\Delta S)$.

Evans *et al.*[11] reported RAE 1 measurements at 107 and 430 m for a type III storm in August 1968; these data are indicated in Fig. 1. A wide variety of spectra for many individual type III bursts observed by IMP 6 have been presented elsewhere[12,13]. Half of these bursts had spectral peaks occurring between 330 and 1,200 m; nearly all peaked within the 100 m–10 km observing range. The envelope of the largest long wavelength type III bursts, shown in Fig. 1, is derived from these IMP 6 data. This envelope should be considered as a lower limit for strongest bursts, because the IMP 6 observations cover only 500 days. The envelope of largest bursts at shorter wavelengths was assembled by Shimabukuro[2] from burst maxima observed between 1954 and 1972 by Badillo and Salcedo[14] and Castelli *et al.*[15]. Taken together, the envelopes of largest bursts indicate that the maximum observed flux density continues to increase with increasing wavelength up to a spectral peak near 500 m.

Figure 1 also contains the spectrum for a particularly intense burst associated with a large proton flare on 4 August 1972. This burst was reported by Castelli *et al.*[15] in the 3–60 cm range, by Solar Geophysical Data at 1.5 m and by me[12] in the 100 m–10 km range. Maximum flux density for the burst is observed between 330 and 1,500 m, wavelengths emitted at coronal levels of ~25–85 solar radii.

The quiet Sun spectrum is taken from Erickson *et al.*[16], Aubier *et al.*[17] and Linsky[18]. The spectrum for metric storms is from Wild *et al.*[1].

Solar radio burst flux densities have been observed far above quiet Sun flux levels, extending over seven decades of wavelength. These bursts are important indicators of solar activity. They are often associated with high-energy wave and particle emissions which influence the magnetosphere and upper atmosphere of the Earth. The long wavelength data contributed here show flux densities sometimes exceeding 10^{-14} Wm^{-2} Hz^{-1} in large bursts, with the most intense radio burst emission observed at wavelengths near 500 m and generated at heliocentric distances of 20–65 solar radii.

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Chemical modification of a titanium (IV) oxide electrode to give stable dye sensitisation without a supersensitiser

SEMICONDUCTING electrodes stable in aqueous solution during the photoelectrochemical oxidation of water have generally been large-bandgap oxides, and their photosensitisation to visible light is of continuing interest[1]. In particular, n-type TiO_2 (rutile, with a bandgap of 3.0 eV) is a stable photoanode for the photoelectrolysis of water by uv light[2], and considerable effort has been devoted to its photosensitisation to sunlight[1]. One approach to photosensitisation is the use of a dye that, on excitation by visible light, transfers an electron to the conduction band of the solid and subsequently returns to its reduced ground state by the oxidation of water. The three major problems to be avoided are (1) irreversible degradative oxidation of the dye[3], (2) failure of the oxidised form of the dye to oxidise water, reduction being carried out by a supersensitiser, such as hydroquinone, that is consumed by the reaction[4,5], and (3) inefficient

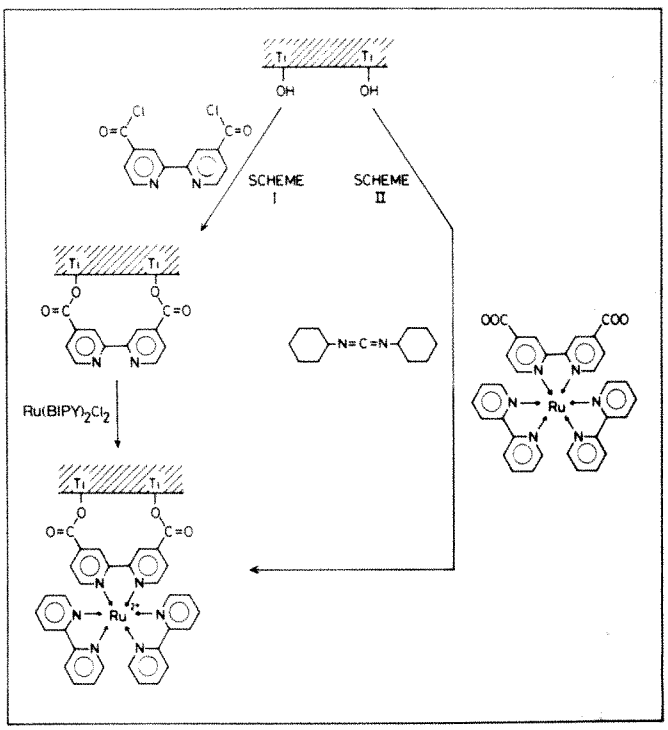


Fig. 1 Two reaction schemes for the derivatisation of TiO_2 with a monolayer of a $[Ru(BIPY)_3]^{2+}$ derivative.

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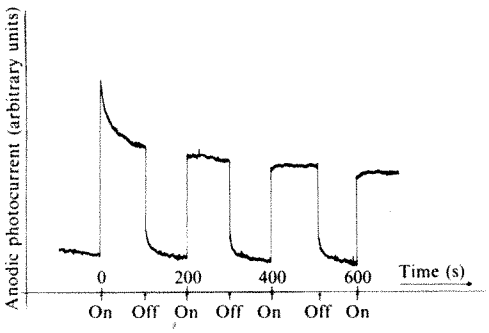


Fig. 2 Photocurrent–time characteristics for a derivatised TiO_2 electrode held at 1.2 V (versus SSE) and illuminated with light from a 150-W Xe lamp passing through a 450-nm interference filter. Arrows represent time of light switching on and off. Steady-state current was 70 nA cm^{-2}.

electron transfer from the dye to the solid[6]. To circumvent the first two of these problems, we have selected an inorganic complex as the dye, a derivative of $[Ru(BIPY)_3]^{2+}$ (BIPY = 2,2′-bipyridine). $[Ru(BIPY)_3]^{3+}$ is known to oxidise water in suitable *p*H conditions evolving about 80% of the theoretical yield of oxygen[7]. The last problem was avoided by chemically attaching the complex to the electrode surface, a procedure recently demonstrated for organic dyes (such as rhodamine-B) that require the use of a supersensitiser[4,5]. We report here that a single-crystal n-type TiO_2 electrode, chemically modified by the attachment of a monolayer of a derivative of $[Ru(BIPY)_3]^{2+}$, will produce significant anodic photocurrents when irradiated with visible light in the absence of a supersensitiser.

Two preparative routes (represented schematically in Fig. 1) were used to chemically modify single-crystal n-type TiO_2 electrodes. Both rely on the presence of electrode-surface sites that react like hydroxyl groups. Scheme I is a two-step process: derivatisation of the electrode surface with 2,2′-bipyridine-4,4′-di(carbonyl chloride), BPCC, followed by reaction with a solution of bis(2,2′-bipyridine)dichlororuthenium(II). Although BPCC has been prepared *in situ*[8], we report[9] its isolation and characterisation (by microanalysis, IR, NMR and mass spectrometry) for the first time. It was prepared by the reaction between 2,2′-bipyridine-4,4′-dicarboxylic acid, BPCA–H_2, and thionyl chloride. Scheme II represents a one-step derivatisation reaction between bis(2,2′-bipyridine)(2,2′-bipyridine-4,4′-dicarboxylate)ruthenium(II), $[Ru(BIPY)_2(BPCA)]$, and dicyclohexylcarbodiimide (DCC). Electrodes modified by both these routes showed similar electrochemical behaviour, and we assume that the product in each case is a monolayer of a stable ruthenium complex attached to the electrode by an ester linkage.

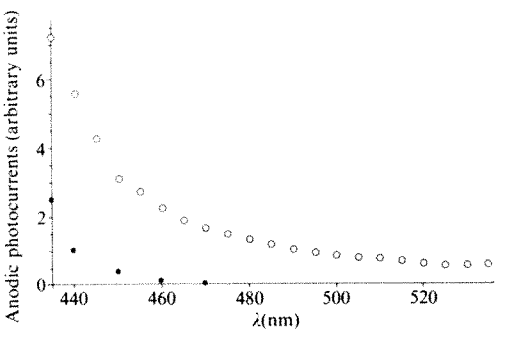


Fig. 3 Photocurrent–wavelength characteristics ('action spectrum') of a derivatised (○) and underivatised (●) TiO_2 electrode held at 1.0 V (versus SSE) (corrected for the lamp profile).

The photoelectrochemical responses of both modified and unmodified electrodes were studied with a.c. and d.c. techniques capable of detecting very small currents[10]. Their response to light from a xenon lamp filtered to remove all radiation with $\lambda < 450$ nm, a wavelength close to the absorption maximum of aqueous solutions of $[Ru(BIPY)_3]^{2+}$ (ref. 11), was measured. As the absorption edge of TiO_2 is 420 nm, any observed photocurrent cannot be due to an electron excitation across the semiconductor bandgap. Indeed, neither the unmodified electrodes nor those modified only by the first step of Scheme I (Fig. 1) showed any significant photoresponse, whereas the fully modified electrode exhibited the response illustrated in Fig. 2, which clearly indicates the critical role of the ruthenium(II) moiety. The d.c. photocurrent is anodic and falls initially to a steady value; it does not exhibit the slow-rise-time cathodic component that has been observed[10] with unmodified electrodes sensitised by $[Ru(BIPY)_3]^{2+}$ in solution. The steady-state current may be maintained for many hours, with no obvious signs of diminution. From these observations we conclude that light absorbed by the complex excites electrons into the conduction band of TiO_2, and the ruthenium(III) complex left on the surface is reduced back to ruthenium(II) by the capture of an electron from the solution. As no conventional supersensitiser is present in the aqueous electrolyte (0.5 M sulphuric acid) in contact with the electrode, we thus demonstrate the direct catalytic photo-oxidation of water with visible light. The oxidation occurs at a rate faster than that observed for the chemical oxidation of water by $[Ru(BIPY)_3]^{3+}$

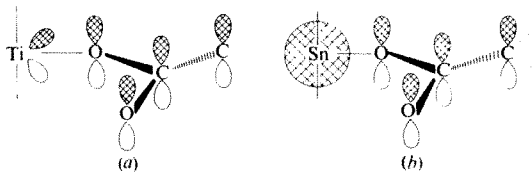


Fig. 4 Schematic representation of the orbitals of (*a*) esterified TiO_2 and (*b*) esterified SnO_2. Crosshatching represents positive lobes, no crosshatching represents negative lobes.

in homogeneous solution[12], as might be anticipated for a redox couple in a charged monolayer. Moreover, the initial decrease in photocurrent may be attributed to an oxidation of surface states at energies above the ruthenium(III) level in addition to the oxidation of water. These would remain empty in the dark at the +1.0 V bias [versus standard silver electrode (SSE), which itself is +0.22 V versus standard hydrogen electrode] of measurement, and indeed the steady-state photocurrent returns immediately after a dark-cycle. On the other hand, the surface-states would be refilled if the bias voltage is cycled to −0.1 V during the dark period, and in fact such a programme recovers the initial photocurrent when the light is again switched on.

An intriguing feature of the photoresponse of the modified electrode is the absence of a peak near 450 nm in the action spectrum (illustrated in Fig. 3). Such a peak would be expected if electron transfer followed dye excitation, and peaks characteristic of dye absorption have been observed with isostructural SnO_2 electrodes modified by both chemically attached rhodamine-B[4,5] and surfactant derivatives of $[Ru(BIPY)_3]^{2+}$ deposited by a Langmuir–Blodgett technique[12,13]. However, rhodamine-B attached to TiO_2 also gives a featureless action spectrum, similar to that shown in Fig. 3 (ref. 14), which suggests that, at TiO_2, the dye electron is excited directly into the conduction band. In TiO_2, the conduction band has a t_2-orbital parentage, which may bond strongly with the π^* system of the ruthenium–bipyridine complex through the ester linkage (see Fig 4); the 5s conduction band of SnO_2, on the other hand, is orthogonal to this π^* system. These observations suggest that the rate of electron transfer to the conduction band is much faster for TiO_2 than for SnO_2 modified electrodes. Experiments are in progress to further investigate and characterise these systems.

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Odd and even numbered hydrogen ion clusters

Since the suggestion that hydrogen ion clusters of the type H_n^+ may be present in gaseous nebulae[1,2] and interstellar space[3,4] and the early experiments of Clampitt and Gowland[5], who discovered such clusters for $3 \leq n$ (odd) ≤ 99 in low temperature (3K) mass spectrometer experiments, it has sometimes been assumed that only odd-numbered clusters exist (ref. 6, and refs therein). There have been extensive studies in the past decade on the binding energy and the geometry of these ion-induced-dipole clusters. Massa *et al.*[7] have carried out *ab initio* calculations on H_n^+ for odd values of n ranging from 3 to 15. Weak binding energies of the order of 44 meV against dissociation according to

$$H_n^+ \rightarrow H_{n-2}^+ + H_2 \qquad 5 \leq n \text{ (odd)} \leq 15$$

were found. The geometrical shape of these clusters was calculated to be dominated by a triangular nucleating centre H_3^+ with the relatively high proton binding energy of 4 eV. Salmon and Poshusta[8] have conducted polarised-orbital valence-bond calculations on the ground state properties of H_5^+. Most recently, the molecular structure of H_3^+ has been experimentally determined by means of the beam-foil technique[9]. We describe here an ion-trap experiment in which we studied collisional processes of electrons with hydrogenic trapped ions of mass numbers, 1, 2, 3, 5, 7, 10, 14, and 20. Since these are the only ions observed, up to a mass limit of 40 AMU, there are serious implications for the assumption by some theoretical chemists that H_n^+ exists only for odd values of n.

The data were obtained in the hollow beam positive ion trap[10] (nonspecific in mass) with concentric electron beam collision facility; this has previously been used for the study of ionisation of atomic ions by electrons[11] and the temperature dependence of dissociative recombination and electron-impact dissociation of molecular ions[12,13].

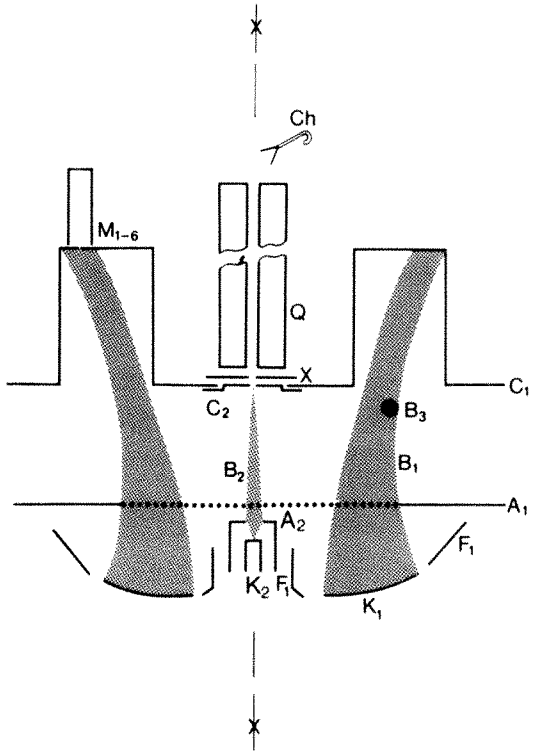


Fig. 1 Scale diagram of cylindrically symmetrical concentric electron beam device. Shaded beams: B_1, hollow electron beam; B_2, axial electron beam; B_3, molecular beam injected tangentially into hollow beam; K_1, toroidal cathode; K_2, rhenium strip filament; A_1, A_2, anodes; F_1, focusing electrode; C_1, hollow beam collector; C_2, axial beam collector; M_{1-6}, beam profile monitoring collectors; X, ion extraction electrode; Q, quadrupole mass spectrometer; Ch, channel electron multiplier.

The apparatus, shown in Fig. 1, consists of an indirectly-heated toroidal cathode which produces an intense electron beam; the potential configuration of the electron space charge produces a trap for positively-charged particles, the depth of which is of the order of 1 V. Advantage is taken of the capability of the hot nickel cathode to emit the species H_5^+, from which other hydrogen cluster species are derived by electron–ion and ion–ion collisions. These processes dominate within the hollow beam, which is collisionally pumped to an ultrahigh vacuum ($\sim 10^{-8}$ torr). A molecular beam is injected at B_3 (Fig. 1) whilst the hollow electron beam B_1 is in operation. A quadrupole mass spectrometer sampling system detects the resulting ionic products.

The hollow beam is operated at a perveance of 15 $\mu A\, V^{-\frac{3}{2}}$, with anode potentials V_{HB}, as shown in Fig. 2. It can safely be assumed that mass numbers 1, 2 and 3 correspond to the well-known H^+, H_2^+, and H_3^+ species. The definitive evidence of the assignment of mass 5 as H_5^+ is its dissociation[13] by axial beam electron impact into $H_3^+ + H_2$ and also $H^+ + 2H_2$. An upper limit of 10^{-13} A can be placed on mass 4 ion current so that the well-known HeH^+ can be ruled out. Alternative assignments for the other mass numbers might be possible: HF^+, 20; N^+, 14; Li^+, N^{2+}, 7; B^+, 10; but each presents difficulties such as the absence of N_2^+, 28; F^+, 19; Li^+, 6, or boron halide ions. In view of the absence of any other ion species in the trap (up to a mass limit of 40 AMU), the most likely conclusion is that all the ions in the trap are hydrogenic.

The intensities of ions of mass numbers 2, 3, 5, 7, 10, 14, and 20 are found to be independent of gas pressure over the range 10^{-8} to 10^{-5} torr, with slight increase in intensities in the region between 1×10^{-5} and 6×10^{-5} torr. The intensity of H^+ rises from 5×10^{-11} A at 2×10^{-7} torr to 1×10^{-7} A at 10^{-4} torr.

These cluster ions have been detected for the first time at laboratory temperatures because the hollow beam trap with surface-emitted H_5^+ ions successfully creates a situation in which

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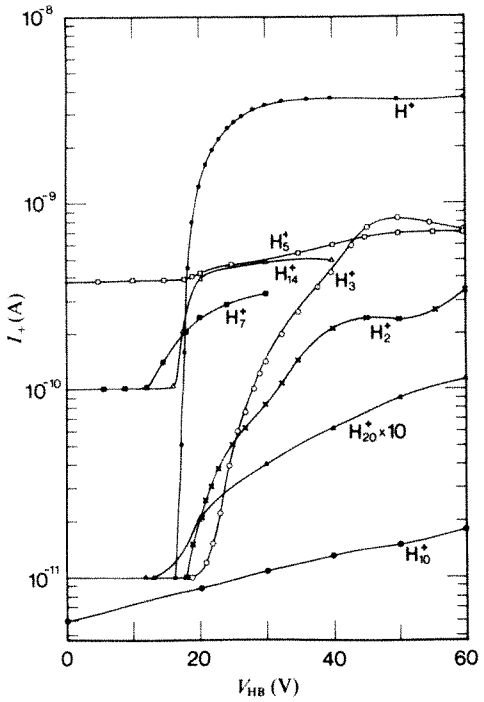


Fig. 2 Observed abundances of hydrogen ion clusters as functions of hollow beam energy V_{HB}.

ion–neutral collisions, which would destroy clusters and complexes, contribute only marginally. It can be speculated that ion–ion collisions of two types are responsible for cluster formation in our device. First, there is the possibility of three-body collisions in which the third body is an electron:

$$H_5^+ + H_5^+ + e \rightarrow H_{10}^+$$

H_5^+ is released from the nickel cathode surface, and is therefore independent of gas pressure and only marginally dependent on V_{HB}; therefore, if this process dominates H_{10}^+ should exhibit similar behaviour. The data in Fig. 2 indicate that H_{10}^+ does indeed persist at the lowest hollow-beam anode voltage. Similar arguments can be applied to the reaction:

$$H_{10}^+ + H_{10}^+ + e \rightarrow H_{20}^+$$

Second, it is possible that the observed ions are produced in the following types of process:

$$H_5^+ + H_5^+ \rightarrow H_7^+ + H_3^+$$
$$H_5^+ + H_7^+ \rightarrow H_{10}^+ + H_2^+$$
$$H_5^+ + H_3^+ \rightarrow H_7^+ + H^+$$
$$H_5^+ + H_{10}^+ \rightarrow H_{14}^+ + H^+$$

But why should odd-numbered clusters persist only up to $n = 7$, H_9^+ not being observed, although H_{10}^+ is stable? Conceivably in the low n odd-numbered clusters a proton orbits about H_2 molecules arranged orthogonally in one, two or three dimensions, so that a further extension of this principle beyond H_7^+ would not be possible.

No quantitative discussion of the cluster formation processes can be presented at this stage, but our data do indicate the need for theoretical chemists to initiate investigations of the hitherto unexplored problem of even-numbered H_n^+ clusters.

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Processes controlling the stanol/stenol ratio in Black Sea seawater and sediments

STEROIDAL ALCOHOLS[1–10], ketones[11] and hydrocarbons[12,13] have been used as tracers for the transformation processes of biogenic organic matter in Recent sediments. Several of these studies have been concerned with one of the sedimentary transformation reactions of steroidal alcohols, the reduction of stenols to stanols. However, little attention has been directed towards the role of the overlying water column in controlling sedimentary stenol/stanol distributions. The input of stanols from surface-water organisms coupled with relatively different degradation rates of stenols and stanols[9,14] could also contribute to observed sediment distributions. To evaluate further the relative importance of these processes in controlling stanol/stenol distributions in anoxic water and surface sediments, we undertook a cruise to the Black Sea. Because water below 125 m is anoxic in the two central 2,000-m basins of the Black Sea, it is an ideal environment for observing any microbially or chemically mediated reduction reactions in the water column or surface sediments[15,16]. In addition, since the surface sediments are anoxic, the effects of macrobenthos on these reactions are minimal. Indeed, sterol transformation reactions have been postulated to be microbially mediated in sediments[1,2,7–9,11,13], and in seawater[17]. However, very few, if any, bacteria are thought to biosynthesise desmethyl stenols[17,18]. If stenol reduction reactions are chemically mediated, they should also be observed in this highly reducing (low E_H) environment. We discuss here the major processes controlling a specific transformation reaction (stenol to stanol conversion) of a class of biogenic organic compounds. These conclusions are based on results obtained by sampling both the sediments and the overlying water column from two stations in the Black Sea.

Seawater and sediment samples were collected on RV Chain Cruise 120, Leg 1 in April 1975. Core 7 was taken at hydrostation 1355, 42°50.0′ N, 33°00.0′ E, Core 23 was taken at 43°00.0′ N, 34°00.0′ E and hydrostation 1356 was taken at 42°41.3′ N, 30°65.5′ E. The procedure for collection of the water samples[17] and cores[19] and the interpretation of the hydrographic data[17] are reported elsewhere. The presence of high concentrations of hydrogen sulphide as well as the absence of dissolved oxygen in the sediments and within the anoxic zone of the water column restrict life primarily to various species of microorganisms[15,16]. Investigation of Recent sediments near Station 1355 has shown this environment to be of uniform deposition[20]. The sediment analysed contained approximately 4 cm of a loose black floc above a grey unconsolidated organic-rich material. Karl[16] found using scanning electron microscopy that the sediment contained coccoliths in various stages of dissolution and occasional diatom frustule fragments. In addition to these calcareous and siliceous detrital fragments, he observed many colonies of bacteria. A more detailed description of the sediment is published elsewhere[19]. The methods for the extraction, isolation, and quantification of the sterols are given in detail for seawater by R.B.G. and F.H.[17], and for sediments by C.L. *et al.*[5,6]. Structural assignments were based on high-resolution glass-capillary gas-chromatographic retention times of authentic sterol acetates in conjunction with glass capillary gas chromatography-mass spectrometry as described earlier[6,17].

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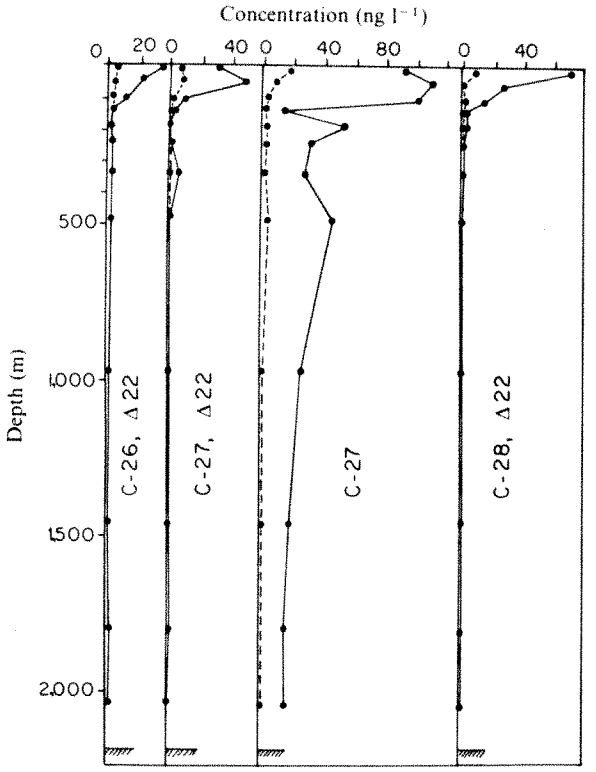


Fig. 1 Concentrations for four stanol (dashed lines), stenol (solid lines) pairs (I, II; III, IV; V, VI; VII, VIII) as a function of depth in the water column from Station 1355. See text for systematic names.

Sixteen stanols and stenols were resolved in the surface water samples from Stations 1355 and 1356 (ref. 17). Over 30 stenols were found in the surface sediments from Cores 7 and 23 (ref. 19). However, for the purpose of this report, we will limit our discussion to four clearly separable stanol/stenol pairs for both the seawater and sediment samples: 24-nor-5α-cholest-22-en-3β-ol(I)/24-norcholesta-5,22-dien-3β-ol(II); 5α-cholest-22(E)-en-3β-ol(III)/cholesta-5,22(E)-dien-3β-ol(IV); 5α-cholestan-3β-ol(V)/cholest-5-en-3β-ol(VI); and 24-methyl- 5α-cholest-22-en-3β-ol-(VII) / 24-methylcholesta-5,22-dien- 3β-ol(VIII). The concentrations of these stanol/stenol pairs as a function of depth in the water column for Station 1355 are shown in Fig. 1. Cholest-5-en-3β-ol and 5α-cholestan-3β-ol are the only sterols detected above blank levels in waters deeper than the O_2/H_2S interface (located at 110–130 m). Similar results were found for two other stations in the Black Sea[17]. Although stenol Δ5 bonds can be reduced chemically and microbiologically to both 5α and 5β stanols, only the 5α isomer of cholestanol was detected. In the upper 100 m of the water column, all eight sterols were found in concentrations ranging from 5 to 93 ng l^{-1}. The surface water stanol/stenol ratios for compounds I–VIII are 0.15, 0.16, 0.18 and 0.10 for the I/II, III/IV, V/VI, VII/VIII pairs respectively. These four sterol pairs were also found at the four depths sampled from Core 23 (Fig. 2) and the two depths sampled from Core 7. The 0–4-cm stanol/stenol ratios for the four respective pairs were found to be 0.28, 0.42, 1.17 and 0.45 for Core 23.

Since there is no evidence for sediment transport by turbidity currents at these locations and benthic sterol production is unlikely in the strongly anoxic water and sediments, the sedimentary sterols we observed were probably deposited by transport on large particles originating in the oxic zone but not captured by our seawater samplers. Recent studies using large-volume *in-situ* filtration systems[21], and moored[22,23] and free-floating[24] sediment traps indicate that the percentage of surface productivity rapidly reaching the sea floor directly below is much higher than previously thought. The large particles being transported potentially contain labile organic compounds since they reach the sea floor in a relatively short time (days).

The stanol/stenol ratio for the four corresponding pairs in the surface waters range from 0.10 to 0.18 (Fig. 1). These values are lower than those reported from surface sediments[2,7,10]. They probably reflect the input from stanol-containing organisms (jellyfish[25], red algae[26], echinoderms[27] and sponge[18]) since the bacterial conversion of stenol to stanol would be expected to be slow in the oxic surface waters[9,14]. From these comparatively high surface values, the concentrations of all the stanols decrease quickly with depth (faster than the corresponding stenols) to values near zero at the O_2/H_2S interface.

All of the stanol concentrations in the deep anoxic waters are below 5 ng l^{-1}. The only stanol/stenol pair that can be observed from the surface to the deep anoxic waters is the 5α-cholestan-3β-ol/cholest-5-en-3β-ol pair (Fig. 3). The ratio drops from surface values of 0.15–0.20 to a ratio of about 0.05 below 100 m. The high value at 140 m depth from Station 1355 is due to an extremely low cholest-5-en-3β-ol concentration rather than to an increase in 5α-cholestan-3β-ol (see Fig. 1). These results clearly show that no major chemical or microbial stenol → stanol conversion is occurring despite the highly favourable conditions at the interface and in the deep anoxic waters. The fact that the inflections of the stenol depth profiles are not mirrored by the corresponding stanol profiles[17] (Fig. 1) also indicates very strongly that the stanol/stenol ratio in the oxic zone (upper 100 m) is primarily determined by varying inputs from specific source organisms.

Sediment ratios are quite different than those in the water column. In both Cores 7 and 23 the stanol/stenol ratio is 1.18, decreasing to about 1.0 with depth. Several processes may be responsible for this large difference between seawater and sediment ratios. First, as mentioned above, large particles probably transport sterols from the surface waters to the sediment surface. Stenol → stanol conversion could take place as these large particles transit to the sea floor. However, this conversion was not observed on the small particles collected by our water samplers. Since the small particles have a longer time for reaction due to their longer residence time in the water column, it is unlikely that this conversion occurs on the large particles. This reasoning assumes that the organic and microbiological compositions on the large particles are the same as on the small particles. This may not be true since the larger particles will have had much less time to degrade in the water column and may be a hormonal or more nutritional substrate. Plankton and other surface organisms are sources of the large particles. However, they usually do not contain more than 10% stanols relative to stenols[8,9]. On the other hand, faecal pellets produced by these organisms are another source of large particulate matter[21,22,24], and may contain larger quantities of stanols formed by gut bacterial activity.

A second potential process for the increase in the stanol/stenol ratio in the surface sediments is selective degradation of stenols relative to stanols at the sediment/water interface. Enough stanols are produced in the surface waters to account for those we observed in the 0–4-cm sediment layer[9,28] which represents about 500 years' accumulation[19,20]. A selective degradation process is probably not occurring for two reasons.

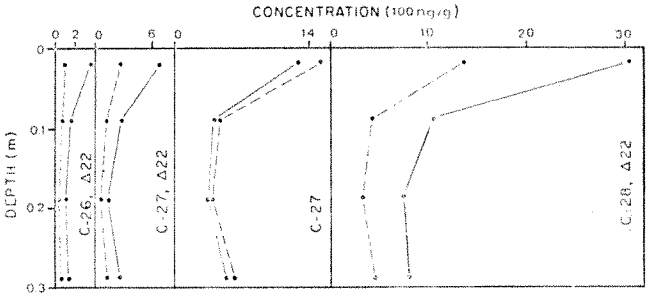


Fig. 2 Concentrations for four stanol (dashed lines), stenol (solid lines) pairs (I, II; III, IV; V, VI; VII, VIII) as a function of depth for Core 23. See text for systematic names.

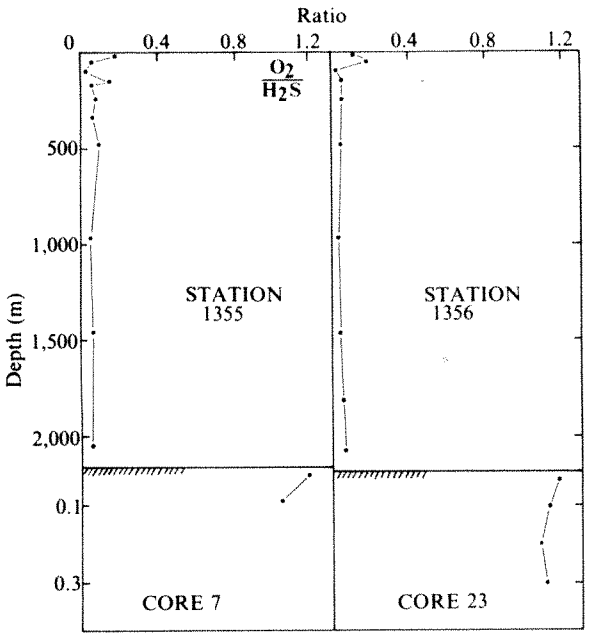


Fig. 3 Ratio of 5α-cholestan-3β-ol to cholest-5-en-3β-ol as a function of depth in the water column from Stations 1355 and 1356 and in Cores 7 and 23.

First, although stenols degrade faster than stanols in oxic lacustrine sediments, and the stenol → stanol conversion is slow, the case in microbially active anoxic sediments is different. It seems that stenol degradation is suppressed and the reduction of stenols to stanols occurs rapidly, causing a marked increase in the stanol/stenol ratio with depth[1,2,9,14]. Although this increase was observed in the surface sediments of the Black Sea, an increase with depth in the core was not found. However, the distribution of bacteria with depth and the sedimentation rate in the Black Sea can be expected to be quite different from those of lacustrine environments[1,7,16]. Second, if stenols were selectively being degraded relative to stanols, we might have expected the 5α-Δ22 stanols to degrade faster than 5α-cholestan-3β-ol. This does not appear to be the case, as shown in Figs 1 and 2. 5α-cholestan-3β-ol(V) is over twice the concentration of 24-methyl-5α-cholest-22-en-3β-ol(VII) in the surface waters. However, the concentration of VII is about equal to V in the surface sediment. The same argument applies for the stability of I and III relative to V.

The third possible process controlling stanol/stenol ratios in the surface sediments is direct stenol → stanol conversion at the sediment/water interface. Since microbiological hydrogenation of stenols is well known[29-31], this pathway has been postulated as a major source of stanols in anoxic lacustrine sediments[1,2,9,14]. The Black Sea sediments in this study have high microbial biomass and activity[16]. Hence, microbial transformation is the most likely control of stanol/stenol distributions in Black Sea surface sediments. However, input from large particles produced in the overlying water cannot be ruled out as a source of stanols. It is clear that in order to define the role large particles have in controlling surface sediment stanol/stenol distributions, sediment trap data is needed. We hope to collect such data in this most interesting anoxic environment in the near future.

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Analysis of aliphatic and aromatic hydrocarbons in Antarctic marine sediment layers

THE presence of both paraffinic and polycyclic aromatic hydrocarbons (PAH) in sediments and soils from the Northern Hemisphere has been well documented[1-5]. Paraffinic hydrocarbons are generally assumed to be derived predominantly from land plants[6] but the sources of PAH are still uncertain[7]. However, the general consensus seems to be that they are from the fallout of airborne combustion products[3]. Industrialised areas have higher PAH loadings[5] whilst dated cores from areas with a history of increasing industrialisation show a concomitant increase in total PAH loads but no great change in the relative proportions of individual components. Considerable interest is also vested in the fate of hydrocarbons once deposited in sediments[6]. With mineral resources being depleted in more accessible areas, those of remote regions (including polar ones) are being considered. But little is yet known of the effects that near-zero temperatures may have on the rate of biochemical or chemical alteration of hydrocarbons such as those found in petroleum. We report here our attempts to test the 'combustion source hypothesis' and to investigate the fate of hydrocarbons in sediments from a remote sub-Antarctic island with a well-documented history of localised industrial pollution[8] spanning the period 1904–65. Only very minor local inputs of pollutant hydrocarbons are likely to have occurred on either side of these dates.

South Georgia (54 °S, 37 °W) became an important seal hunting ground during the first half of the nineteenth century. But with the virtual extinction of the local seals it was then little used until 1904, when a whale processing factory was built at King Edward Cove. Thereafter, fossil fuels (first coal and then, increasingly from about 1925, fuel oil) were transported to the factory to fire cookers and catchers. Fossil fuel contamination of the bay occurred especially when the tanks of the transporters were cleaned before reloading with whale oil. In addition, large quantities of organic material entered the bay as a result of

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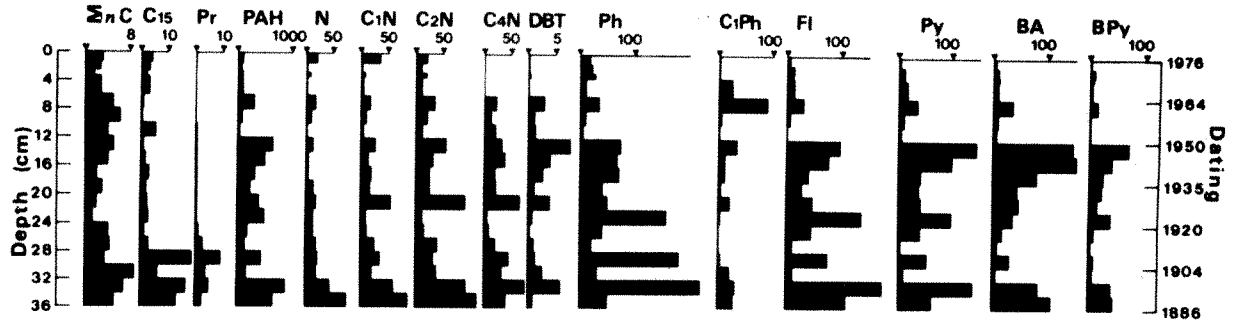


Fig. 1 Depth distribution of selected hydrocarbons in a marine sediment core from South Georgia (units are ng per g dry wt except for *n*-C and Pr (μg per g) and C_{15} (% of Σ*n*-C)). For abbreviations see Table 1.

processing the whales, most markedly before 1916 when only the blubber was utilised. The factory closed in 1965 and the site subsequently returned to light use, with a small scientific station being visited by occasional research and fishing vessels.

In 1976, divers collected cores from 18 m in the deepest part of the bay. Sections of a 2.2-cm diameter, 36-cm long core were extracted[9] and the alkane and PAH fractions isolated according to the method of Giger and Schaffner[10]. The quantitative alkane data were obtained by glass capillary GC[11]. Analyses of the PAHs were also performed on a glass capillary column but used a mass spectrometer, operated in the multiple ion mode, as a detector[11]. Identifications are based primarily on retention indices with random peaks scanned to confirm identities. A minimum of two injections of a standard mixture were made daily to confirm both retention time and spectrometer response. The parent ion was used for quantitation on all components except for the C1 and C2 substituted naphthalenes for which the M-1 and M-15 ions respectively were used.

The sediment core results are shown in Fig. 1 and the South Georgia whale oil (grade 2) and fuel oil (from abandoned storage tanks) data are given in Table 1. Figure 1 also shows a putative dating based on inferences made from the hydrocarbon and organic carbon profiles of the cores and from sedimentation experiments[12]. The estimate of the 1975–76 annual sedimentation rate based on sediment trap data agreed well with the implications of assuming that the unusual carbon profile reflected the increased organic input to the sediment during the whaling years. As this input of waste may have altered the sediment accumulation rate it is not easy to state the reliability of the precise dating between 1904 and 1965. However, all the available evidence seems to be consistent with the conclusion that the sediment at 30 cm depth (in 1976) was laid down in 1904 and that the average annual sedimentation rate thereafter was of the order of 2.8×10^3 g dry wt m^{-2}.

Present evidence suggests bioturbation could effectively backdate any dating by about 10 yr (any material layed down in 1 yr would be mixed with sediment laid down up to 10 yr previously). The macrobenthic fauna in the area[13] consists predominantly of burrowing polychaetes in the upper 2–4 cm aerobic layers. However, organic inputs from the whaling operations may have either altered the benthic fauna so as to increase bioturbation or reduce it and so decrease such mixing. Evidence from other areas subjected to massive organic pollution suggest the macrofauna would have been eliminated[14,15] so a large dating error due to macrobenthic activity between 1904 and 1965 is unlikely. Nevertheless, it is still possible that a persistent meiofaunal population could have survived to cause some mixing[16].

The upper sections of the core can be considered free of local pollution: most of the aliphatic components are entirely consistent with their being locally derived, from marine organisms and land plants[17]. However, as biogenic PAH synthesis is considered unlikely[18], the aromatic components would seem to be the result of a world-wide dissemination of natural or anthropogenic combustion effluents and/or petroleum. The low ratio of parent-to-alkylated phenanthrenes effectively rules out petroleum as a source, petroleum being low in unsubstituted species. This leaves combustion products from continental land masses as the prime source, as evidenced by the roughly equal proportions of fluoranthene to pyrene and the predominance of parent tricyclic over alkylated tricyclics[3]. Thus, the results from South Georgia support the hypothesis that sedimentary PAH are combustion derived.

The mean PAH concentration in the top 3.5 cm of the King Edward Cove mud (100 parts per 10^9 (p.p.b.) dry wt) was only eight times less than that found in the fine sands of Buzzards Bay[7], a shallow (17 m) site near the heavily industrialised northeastern seaboard of the US and just slightly less than the 160 p.p.b. figure for the (presumably) finer grained surficial sediments at 130 m depth 94 km west of Boston (Massachusetts)[4]. Therefore, despite the fact that PAH concentration is a function of sediment type as well as relative input, the concentrations found at South Georgia still seem to be surprisingly high for such an isolated location, indicating that global contamination reaches even remote parts of the world in relatively undiminished quantities.

The apparent presence of small amounts of PAH in the whale oil deserves comment. Table 1 shows that the relative proportions of the component PAHs bear a similarity to those of the fuel oil so that it seems that the whale oil probably became contaminated by petroleum at some stage.

Anthropogenic combustion of fossil fuels has been continuously increasing during this century[7], so that the greater hydrocarbon concentrations deeper in this core could only have resulted from the local industry. Indeed, the abrupt increase in many components at the 6–8 cm depth seems to coincide with the cessation of industrial activity. The obvious discontinuities in both amounts and distribution must reflect local contamination

Table 1 Hydrocarbon composition of whale oil and fuel oil from South Georgia

Component	Whale oil	Fuel oil
Total *n*-alkanes C_{15}–C_{33} (Σ*n*-C) (μg per g)	8.9	4.9×10^4
Normal pentadecane (C_{15}) (% of Σ*n*-C)	59.6	8.1
Pristane (Pr) (μg per g)	322.7	1550.9
Total identified PAHs (ng per g)	86.2	3.3×10^6
Naphthalene (N) (ng per g)	12.4	0.2×10^6
Methylnaphthalene (C1N) (ng per g)	14.3	0.7×10^6
Dimethylnaphthalene (C2N) (ng per g)	17.0	2.2×10^6
Tetramethylnaphthalene (C4N) (ng per g)	ND	0.4×10^6
Dibenzthiophene (DBT) (ng per g)	2.0	9.6×10^4
Phenanthrene (Ph) (ng per g)	5.8	5.5×10^4
Methylphenanthrene/anthracene (C1Ph) (ng per g)	26.1	10.8×10^4
Fluoranthene (F1) (ng per g)	0.6	ND
Pyrene (Py) (ng per g)	1.8	0.5×10^4
Benzanthracene/phenanthrene (BA) (ng per g)	6.6	3.6×10^4
Benzo(*a*)pyrene (BPy) (ng per g)	1.6	0.4×10^4

ND, not detected.

as all other sources should in principle give relatively homogeneous distributions. These discontinuities further imply that neither diffusion nor sediment reworking was a major factor during these years. In general, the qualitative distribution of the PAH below 8 cm is again consistent with combustion being the source, with inputs from the whaling station far exceeding allochthonous inputs.

The total *n*-alkanes are most abundant in the 6–14 cm and 30–36 cm regions. The lower peak is a legacy of the wasteful early years of whaling, as illustrated by the *n*-pentadecane and pristane data (both major constituents of whale oil). The upper alkane peak coincides with increasing post-1945 use of fuel oil, indicated by reduced carbon preference index (CPI) values[19] and enhanced phytane concentrations: CPI of 1.15 in 8–12 cm section compared with a normal value of 1.65 and phytane concentration of 0.3 μg per g in 10–14 cm section compared with a normal value of 0.1 μg per g.

However, the various indicators of the presence of petroleum are scattered but not coincidental throughout the sections dated 1945–69 (3.5–14 cm). Thus, if the phytane values[19] are examined, the greatest concentration of petroleum is from 1945 to 1955, if the ratio of parent to substituted tricyclics[20] is used, oil is present mainly in the 1959–69 regions whilst the amount and distribution of alkanes[19] suggest that 1955–60 are the years of major fuel oil input. No reasonable explanation is evident for this phenomenon, which must await a closer understanding of the fate of petroleum hydrocarbons in cold benthic ecosystems. Clearly these results represent evidence for the long-term stabilisation of petroleum components in these conditions, at least 20 yr—apparently the longest so far reported.

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Ordovician conglomerates and the evolution of the Midland Valley

ORDOVICIAN and Silurian marine clastic sequences containing boulder bearing conglomerates overlie the Ballantrae ophiolite complex in South-west Ayrshire, Scotland. These conglomerates are thought to have been emplaced by submarine slides[1] or less viscous marine currents[2]. The Ordovician conglomerates accumulated at the toes of contemporary submarine faults downthrowing to the south[3] and in both their stratigraphy and palaeocurrents there is substantial evidence for a northerly source. The conglomerates contain clasts of the underlying ophiolite, metamorphic rocks, acid and intermediate volcanic and hypabyssal rocks and clasts of undeformed pink granite some of which are >80 cm in largest dimension. Dewey[4] and many subsequent workers[5] envisage these sediments as having accumulated on the southern margin of a continent under which oceanic plate was being consumed in a northerly dipping subduction zone. The time span of this subduction is subject to much speculation[6] but is generally thought to have ceased by Devonian time. We report here our investigations of the clasts in the southern Ayrshire conglomerates to elucidate (1) the nature and extent of plutonic activity in the area to the north of the subduction zone, and (2) the nature of the crust into which the plutons were emplaced.

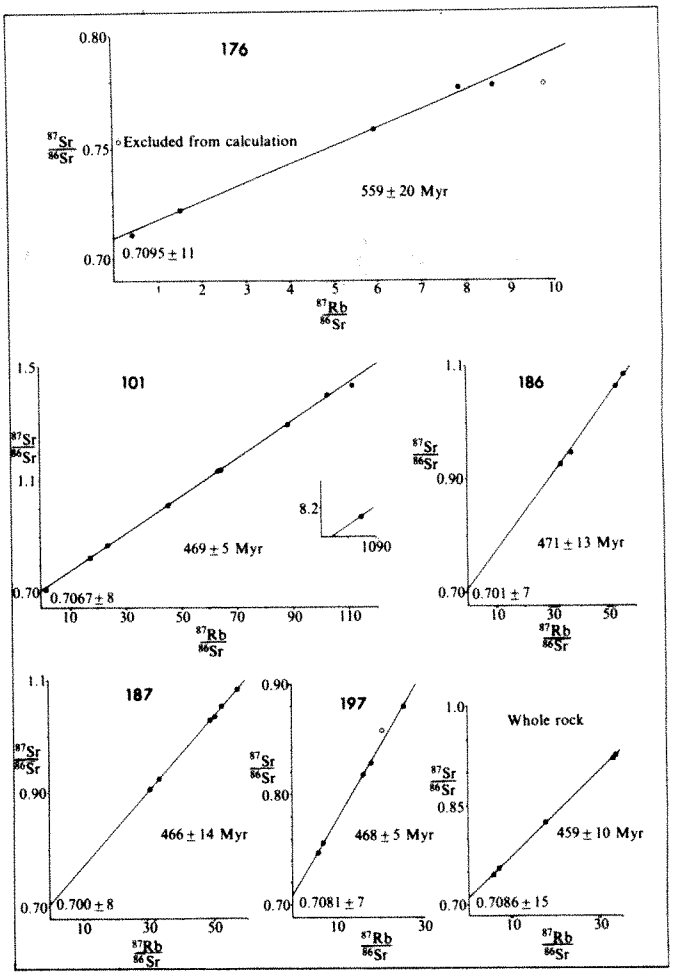


Fig. 1 Mineral isochrons of five different granite clasts and one composite whole rock isochron.

Rb–Sr isotope dilution analyses of these clasts follow the analytical methods described by Blaxland *et al.*[7]. Rb–Sr ages have been calculated, or recalculated, using $\lambda^{87}Rb = 1.42 \times 10^{-11}$ yr^{-1}. Analytical uncertainties at the 1σ level for $^{87}Rb/^{86}Sr$ and $^{87}Sr/^{86}Sr$ isotope ratios are ±0.7% and ±0.03% respectively. Seven analyses of the NBS 607 potassium feldspar standard yield average $^{87}Rb/^{86}Sr$ and $^{87}Sr/^{86}Sr$ values of 24.21 ± 11 (1σ) and 1.2006 ± 6 (1σ) respectively. The average $^{87}Sr/^{86}Sr$ value for NBS 987 standard $SrCO_3$ is 0.71020 ± 5 (1σ). Rb–Sr isochron ages were calculated using the least squares method of York[8]. All isochrons are concordant (MSWD < 2.5) or near concordant suggesting the observed scatter to be analytical rather than geological.

Rb–Sr analytical data are shown in Table 1. Mineral isochrons determined for five different granite clasts (minimum of 30 cm in longest dimension) and one composite whole rock isochron are plotted in Fig. 1.

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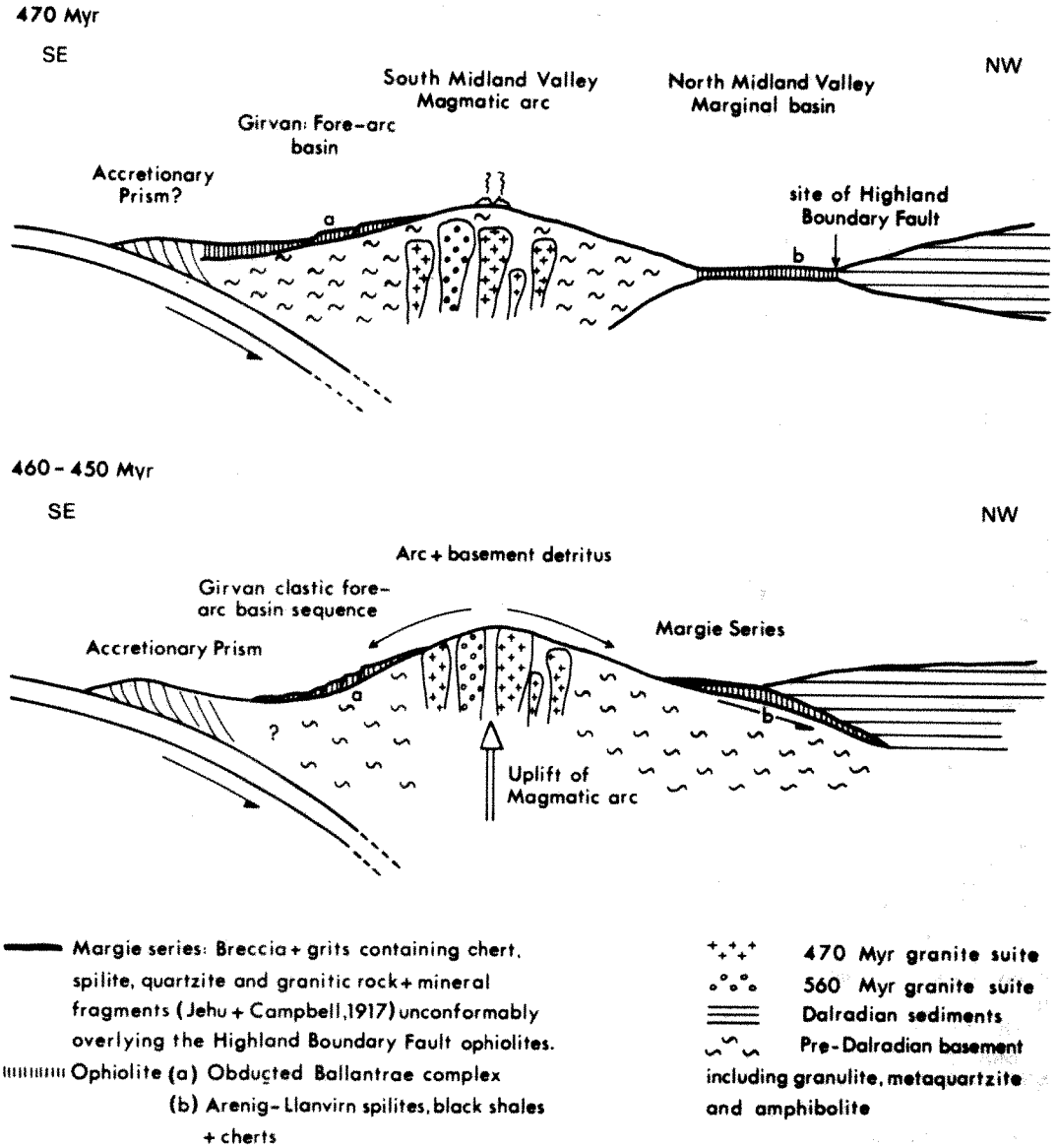


Fig. 2 Reconstruction of the Midland Valley during the Ordovician.

The age values obtained suggest a two-fold division defining one event at ~560 Myr and another at ~470 Myr. Sample 176, an undeformed non-perthitic biotite–chlorite bearing subsolvus granodiorite[9] yields an age of 559 ± 20 Myr. The other four clasts, 101, 196, 187 and 197 yield ages of 469 ± 5, 471 ± 13, 466 ± 14 and 468 ± 5 Myr respectively. These samples are less mafic, commonly microperthite-bearing subsolvus granites. The four whole rock points from the samples defining the ~470 Myr event were plotted along with another whole rock point from a chemically and petrographically similar clast (Fig. 1). These define a tentative isochron of 459 ± 10 Myr.

The initial $^{87}Sr/^{86}Sr$ ratios of three younger samples agree within experimental error (those for 186 and 187 were excluded as being ill defined and anomalously low) ranging from 0.7067 ± 8 to 0.7087 ± 15 and giving a mean of 0.7078 ± 10. The initial $^{87}Sr/^{86}Sr$ ratio for sample 176 is 0.7095 ± 11.

The age and stratigraphic position of the granite clasts places constraints on the Ordovician time scale. The dated clasts come from the Benan conglomerates of Llandeilo age. Petrographically similar clasts are, however, found in conglomerates ranging through to Llandovey in age[10]. Presently observed rates of uplift and erosion[11] suggest that surface exposure of the granites is possible in <10 Myr. This gives a maximum age for the base of the Llandeilo of about 460 Myr. This figure is in reasonable agreement with some published Ordovician time scales[12,13] based largely on K–Ar and Rb–Sr ages, but is irreconcilable with older ages based on fission track data[14].

Consideration of dispersal of the conglomerates and the size of the granite clasts indicate a provenance to the immediate north, in the south-west margin of the Midland Valley. The granite clasts have a similar petrography and age to the exposed pre and late tectonic granites in the Highlands. Ages of ~450 Myr are reported for many of the smaller granites of North-east Scotland[15] whereas the Carn Chuinneg–Inchbae granite is dated at 560 ± 10 Myr[16,17]. The known pre and late tectonic plutonic activity which preceded the ubiquitous ~400 Myr granites, relating to the final closure of the Iapetus Ocean, is therefore extended south of the Highlands.

The initial $^{87}Sr/^{86}Sr$ ratio of the Carn Chuinneg granite (0.710 ± 2)[16] is similar to that of sample 176—unless the 559 ± 20 Myr age for this rock is a cooling age and the high $^{87}Sr/^{86}Sr$ ratio reflects Sr isotopic equilibration on a mineral scale. In contrast, the initial $^{87}Sr/^{86}Sr$ ratios of around 0.708 for the ~470 Myr suite are significantly lower than the values of 0.714–0.717 for the ~450 Myr suite of Aberdeenshire granites[15]. Taken together, the 470–450 Myr Midland Valley–Aberdeenshire granites show a northerly increase in initial $^{87}Sr/^{86}Sr$ ratios. This pattern together with the high normative corundum and presence of muscovites in the northern granites[18] can be explained by increasing crustal involvement in the northern source. Although this evidence does not prove northward subduction the data are consistent with $^{87}Sr/^{86}Sr$ isotope ratio patterns of Mesozoic and Cenozoic subduction related granites in the western US[19,20] and such a model accords with

Table 1 Analytical data

Sample	Phase	Rb (p.p.m.)	Sr (p.p.m.)	$^{87}Rb/^{86}Sr$	$^{87}Sr/^{86}Sr$
17600	Whole rock	94.9	177.8	1.5640	0.72212
17607	Plagioclase	57.8	374.8	0.4465	0.71274
17613	Alkali feldspar	264.0	130.0	5.9044	0.75801
17616		287.7	106.9	7.8362	0.77133
17617		296.1	100.1	8.6205	0.77687
17618		312.3	92.5	9.8451	0.78417
10100	Whole rock	163.8	27.1	17.6832	0.82536
10107	Perthite	35.2	86.0	1.1833	0.71460
10113		450.6	21.5	63.1951	1.13128
10115		591.6	17.7	103.374	1.39660
10116	Muscovite	1049.1	4.8	1084.75	8.17221
10104*	Perthite	284.3	35.0	23.8428	0.86476
10108*		389.5	25.6	45.3412	1.00714
10111*		445.5	21.0	64.1603	1.13346
10113*		521.6	18.1	88.2863	1.29343
10114*		607.6	16.9	111.411	1.43777
18600	Whole rock	112.0	10.5	33.8337	0.92777
18605	Perthite	173.3	14.0	36.7596	0.94866
18612		244.5	13.6	53.9222	1.06083
18613		258.9	13.7	56.7424	1.08367
18700	Whole rock	127.1	11.1	33.9507	0.92320
18705	Perthite	142.4	13.7	30.6406	0.90431
18709		227.1	13.7	49.2947	1.02998
18711		226.9	13.4	50.5927	1.03354
18713		235.9	13.4	52.6500	1.05118
18714		258.0	13.4	57.8238	1.08135
19700	Whole rock	117.2	48.1	7.0899	0.75569
19704	Alkali feldspar	133.2	65.4	5.9144	0.74731
19706		256.6	45.8	16.4009	0.81789
19708		280.2	45.4	18.0603	0.82799
19709		306.0	43.5	20.6541	0.85765
19710		364.9	41.7	25.7471	0.87975
19400	Whole rock	117.4	61.2	5.5704	0.74441

All mineral separates are of a size fraction 210–175 μm except those marked * which are 500–210 μm.

many views on plate configuration in this region at that time[21].

The discovery of granite boulders of this age, together with consideration of the type and distribution of clasts in other Midland Valley conglomerates helps to refine existing models. Boulders and large cobbles of granite, quartz–porphyry, rhyolite and andesite present in Ordovician and Silurian conglomerates, suggest major intrusive and extrusive activity in the source area. The plutonic activity is now dated, and Kelling[22] in recording an influx of andesite fragments in Llandeilo greywackes has suggested volcanic fields in this region at the same general time as the intrusion of the younger granites of this study. This evidence is consistent with the development of an island arc in the southern margin of the Midland Valley reaching maturity in Lower Ordovician time. The existence of an island arc associated with the northward subduction has been previously postulated[4,23,24] but hitherto no evidence for its existence has been available.

Cobbles and boulders of metaquartzite, amphibolite and sheared granite occur in the northerly derived Ordovician conglomerates discussed here and the southerly derived Silurian conglomerates of the southern Midland Valley[25]. Metaquartzite thus formed part of the provenance in the Midland Valley at this time. However, as the 560 Myr granite is unfoliated it seems likely that the event which created the metamorphic assemblage pre-dated the early igneous episode. Furthermore, the presence of metaquartzite suggests ensialic deposition. Thus the evidence points to the existence of Precambrian basement under the Midland Valley, in accord with the original interpretation placed on granulite clasts of intermediate composition found in Carboniferous vents[26]. Mineral chemistry of these clasts indicate pressures and temperatures of metamorphism exceeding 11 kbar and 850 °C (ref. 27) and recent Sm–Nd isotopic work indicates a maximum Grenville age[28]. This evidence for late Precambrian continental basement under the Midland Valley agrees well with the LISPB north–south seismic profile across Scotland[29] and possibly supports both Kennedy[30] and George[31] who found evidence of a southerly source for the Upper Dalradian sediments.

The temporal and spatial setting of the probable post-Grenville quartzite deposition and its metamorphism in the late Precambrian, possibly very early Cambrian time, is critical to the evaluation of the early history of the Midland Valley block. It would, nevertheless, seem likely (also with evidence for Grenville basement under the Dalradian[32,33]) that the Midland Valley began its separate development after the rifting associated with the extrusion of the Tayvallich volcanics[34] which initiated the southward derived sediments of the Upper Dalradian.

The Tayvallich volcanism has been correlated with similar basic volcanism in Newfoundland (dated at 605 ± 10 Myr[35]) which has been attributed to the opening of Iapetus[36]. Our evidence, however, suggests that this rifting event was related to the formation of the first marginal basin along the southern margin of the North American Continent[23].

The serpentinite, spilite, radiolarian chert and black shale assemblages along the Highland Boundary Fault (HBF) are probably of Arenig–Llanvirn age[37]. The new data make it likely that these rocks were deposited behind the magmatic arc, in a marginal basin. The later closure of this basin, by subduction related under thrusting, then resulted in the preservation of a thin ophiolitic sliver along the HBF (see Fig. 2). This closure is likely to have occurred in Caradocian time, immediately after the deposition of the arenaceous 'molasse' of the Margie series, also preserved along the HBF. The closure resulted in the Highland Border rocks having a structural grain similar to those of the adjacent Dalradian, thus giving a spurious impression of structural unity in them both.

The evidence from the Tayvallich volcanics, the HBF suite and also the Ballantrae Complex[38] indicate that the southern margin of the North American continent comprised a series of marginal basins and associated microcontinents. It therefore seems improbable that the Grampian Orogeny resulted from a large-scale continent–continent collision. Consequently we conclude that the landmass which supplied the sediments of the Upper Dalradian was emplaced beneath the latter (see Fig. 2) resulting in the symmetrical folding and mushrooming of the Grampian orogeny and the dramatic uplift along the HBF[39].

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Fly photoreceptor fluorescence is related to UV sensitivity

THE predominant photoreceptor type in flies, R1-6, contains a rhodopsin which absorbs maximally at around 480 nm. Light converts rhodopsin into a thermostable metarhodopsin peaking at around 580 nm (refs. 1–8). A curious discrepancy is the finding that the electrophysiological sensitivity is highest in the UV[5,9]—completely at variance with known rhodopsin absorption curves. Several possible explanations of the high UV-peak, including the presence of a UV-rhodopsin and waveguide effects, have recently been excluded[5–10]. Kirschfeld and co-workers have presented an alternative explanation[6,11], that R1-6 also contains a photostable pigment that absorbs UV light and converts the photopigment by energy transfer. This hypothesised sensitising pigment would most probably be a carotenoid, since vitamin A deprivation preferentially suppresses UV sensitivity[8,12,13]. Of course, vitamin A deprivation also lowers sensitivity by reducing the photopigment level[14,15]. Here we present further evidence for the existence of a vitamin A-dependent non-photopigment substance in fly photoreceptors. We measured fluorescence while simultaneously monitoring photopigment conversions and argue that the fluorescence is probably related to the UV-sensitivity.

This and previous studies on fly photoreceptors exploited the phenomenon of the deep pseudopupil. The deep pseudopupil is a superposition of the magnified virtual images of the distal photoreceptor endings located in several ommatidia (depending on the numerical aperture of the objective). It is observed by focusing at the centre of curvature of the eye[16–18]. Fluorescent deep pseudopupils from white-eyed fruit flies (mutants *cn bw Drosophila melanogaster*) under UV excitation are shown in Fig. 1. The six bright spots in Fig. 1*a* (high vitamin A reared fly) demonstrate that a fluorescing substance is located in the rhabdomeres, that is, the visual pigment containing organelles of the photoreceptors, of cells R1-6. This fluorescing substance is clearly vitamin A dependent, as the fluorescence is absent in flies deprived of vitamin A (Fig. 1*b*).

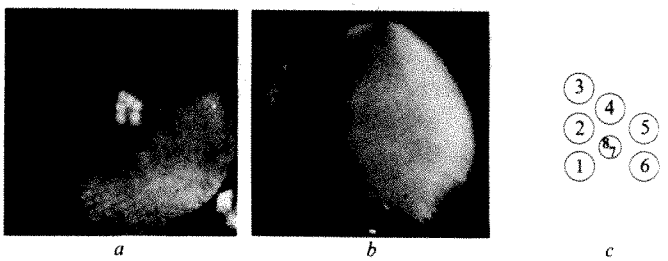


Fig. 1 UV-induced fluorescence photographed in the deep pseudopupil of the compound eye of completely intact and living white-eyed *Drosophila* (mutants *cinnabar* (*cn*) and *brown* (*bw*)), reared on diets high (*a*) and low (*b*) in vitamin A. In flies the rhabdomeres, which are the photopigment containing structures of the photoreceptors, are organised in a characteristic pattern (Fig. 1*c*). Only the rhabdomeres of cells R1-6 of high vitamin A flies (*a*) give an observable fluorescence to UV excitation in the deep pseudopupil. The vitamin A-dependent rhabdomere emission is pink (>550 nm). However, when superimposed on the blue autofluorescence of the eye, which is not vitamin A dependent, the fluorescent deep pseudopupil in vitamin A enriched flies has a whitish appearance. No distinct fluorescence is observed from the deep pseudopupil of vitamin A deprived flies (*b*).

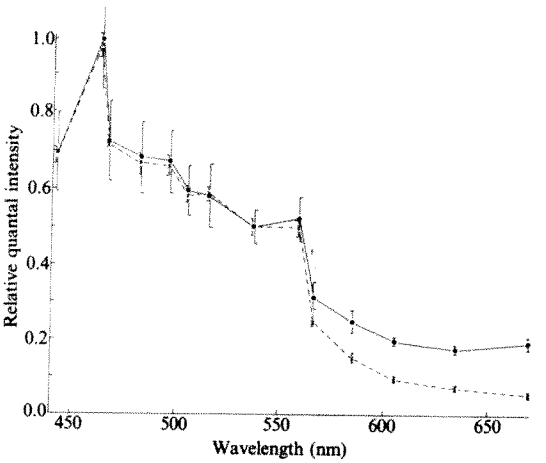


Fig. 2 Emission spectrum for UV excited fluorescence from the deep pseudopupil of *Drosophila*; exciting wavelength 365 nm. The relative quantal emission as a function of wavelength is plotted for high (●) and low (×) vitamin A reared *Drosophila* ($n = 4$ of each type, standard errors are calculated among different preparations).

The emission spectrum under UV excitation from the deep pseudopupil of flies fed on diets high and low in vitamin A was measured (Fig. 2). The difference, representing the emission spectrum of the vitamin A-dependent substance, is substantial only above 560 nm. Measurements were performed in a Leitz Dialux microscope equipped with incident fluorescence excitation and an EMI 9659 QB photomultiplier. An aperture delimited the area of all six photoreceptor images in the deep pseudopupil (≈80 μm diameter on the eye). For technical reasons we were unable to determine the emission spectrum above 670 nm. However, we suppose that the emission spectrum peaks in the red. In the experiments presented below (Figs 2 and 3), we measured fluorescence at >570 nm to accentuate the vitamin A-dependent proportion of the emission.

The magnitude of the emission is virtually identical whether the visual pigment molecules are either predominantly in the rhodopsin or in the metarhodopsin state (Fig. 3). Photopigment conversions were measured by antidromic transmission, that is, illuminating the head capsule, which is rather transparent in *Drosophila*, from the back, and then measuring the light leaving the distal part of the rhabdomeres[19]. Visual pigment conversion was monitored at 578 nm, near the peak of the metarhodopsin absorption spectrum ($\lambda_{max} \simeq 580$ nm for *Drosophila*)[4,5,8,13,20]. At the beginning of the experiment shown in Fig. 3 a high rhodopsin content was produced by a preceding 578 nm light. The transmission at 578 nm then is initially high, but it drops when the eye is irradiated by UV (365 nm) or blue (437 nm) light, due to conversion of rhodopsin into metarhodopsin. On the other hand, however, the fluorescence signal above 570 nm induced by the identical UV and blue irradiation intensities remains constant. This also proves to be the case in a comparable experiment performed on the white-eyed mutant *chalky* of the blowfly *Calliphora erythrocephala* (Fig. 3).

Since fluorescence was independent of whether the photopigment was in the rhodopsin or metarhodopsin state, it is likely that the vitamin A-dependent substance which fluoresces on UV excitation is related to the photoreceptor's UV sensitivity. To characterise this substance its excitation spectrum was found by

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determining the light intensity needed to evoke a criterion emission >570 nm as a function of excitation wavelength. The spectral characteristics of the vitamin A-dependent substance were factored from background measuring from the deep pseudopupil of both high and low vitamin A reared *Drosophila*. A spectrum peaking in the UV with a tail in the blue was obtained (Fig. 4). (This tail could result from the long wavelength fluorescence which has been characterised for R7, another photoreceptor type, under blue excitation[21,22].) The nature of the fluorescing substance, of course, is still enigmatic, except for its vitamin A dependence. Nevertheless, in principle it qualifies well for the hypothesised sensitising pigment which functions in the visual process by transferring energy to both rhodopsin and metarhodopsin[5,6,8,11,21].

Note that a distinct red fluorescence under blue excitation was observed in R1-6 receptors of the housefly *Musca* by Franceschini[21,22], which was interpreted to be related to the amount of metarhodopsin. The data shown in Fig. 2 argue against this interpretation since fluorescence does not change during a monitored rhodopsin to metarhodopsin conversion. We have further shown that in *Drosophila* red fluorescence is only induced with blue excitation intensities 2–3 log units above those applied in the experiment of Fig. 3 (as well as for Fig. 4). Such extremely intense blue irradiation ($\simeq 10^{18}$ quanta $cm^{-2} s^{-1}$) also causes photolysis of the visual pigment. However, Franceschini has categorised different photoreceptor types by observations of this bright light induced fluorescence and we do not challenge his classification scheme[21,22].

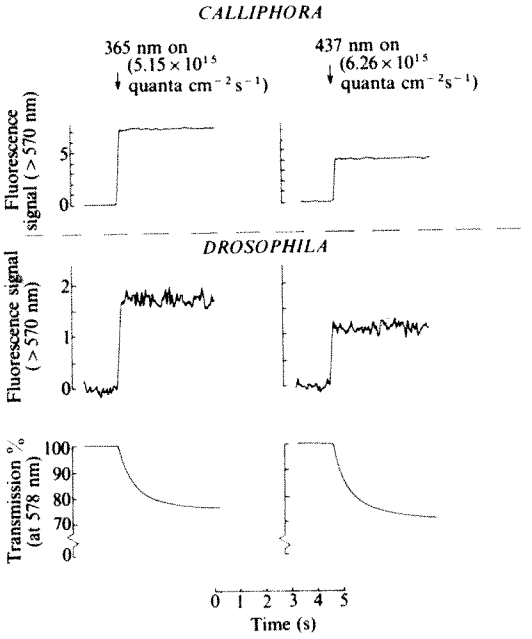


Fig. 3 Fluorescence and antidromic transmission measurements from the fly deep pseudopupil. Rhabdomere transmission was measured by illuminating the back of the rather transparent head of a *Drosophila* (that is antidromically) and determining the relative light intensity leaving the distal rhabdomere tips as visualised in the deep pseudopupil[19]. Yellow light of 578 nm wavelength was used to detect conversion of blue absorbing rhodopsin into yellow absorbing metarhodopsin induced by irradiating the eye with UV (365 nm) and blue (437 nm) light. (Initially a photoequilibrium with a high rhodopsin fraction was established by prolonged 578 nm light.) The indicated intensities were selected to induce conversion taking of the order of seconds. The fluorescence induced by these same intensities stays constant during this time period, and thus is independent from the state of the visual pigment molecules. The identical conclusion is obtained in the case of a white-eyed mutant (*chalky*) of the blowfly *Calliphora*. Vitamin A-enriched flies were used. From measurements on vitamin A deprived *Drosophila* it follows that background fluorescence due to eye tissue amounts to about 0.9 divisions in both cases of irradiation.

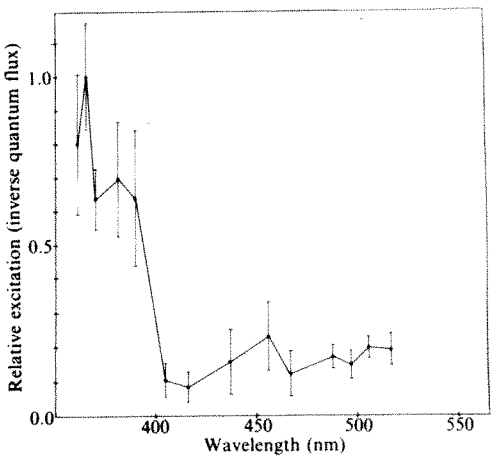


Fig. 4 Excitation spectrum of the vitamin A-dependent photostable substance of *Drosophila* photoreceptors. The intensities $I_h(\lambda)$ and $I_l(\lambda)$ needed to evoke a criterion signal c of emitted light >570 nm were measured in the case of high and low vitamin A reared flies respectively. (The identical aperture around the centre of the deep pseudopupil, enclosing all photoreceptor types, was applied throughout the experiments.) Let the spectral excitation of the vitamin A-dependent substance and that of the background be given by ε_s and ε_b respectively. Then it follows from $c = I_h(\varepsilon_s + \varepsilon_b)$ and $c = I_l\varepsilon_b$ that $\varepsilon_s \propto (I_h^{-1} - I_l^{-1})$. The assumed linearity of fluorescence signal with intensity was tested and verified. The data are from four high and four low vitamin A preparations with standard errors of difference shown.

It was claimed recently[23] that changes in the antidromic transmission caused by high intensities ($\simeq 5 \times 10^{15}$ quanta $cm^{-2} s^{-1}$) of blue light are too slow (minimum time constant >40 ms) to reflect visual pigment conversions. This is contrary to our interpretation of the Fig. 2 data. However, an extensive body of literature[2,3,5,18,24,25] is entirely consistent with the interpretation that these transmission changes are mediated exclusively by photopigment conversions. Moreover, we did not confirm the slow change in transmission[23] in our measurements of antidromic transmission from the deep pseudopupil of completely intact and living *Drosophila*. The time constant for the transmission change reflecting the speed of rhodopsin to metarhodopsin conversion was directly measured by applying light from a pulsed blue dye laser. The time constant is $\simeq 90$ μs, confirming predictions made on the basis of low temperature camera-flash experiments[26].

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Saccadic suppression by corollary discharge in the locust

DURING rapid eye movements, saccades, human subjects do not perceive movement of their visual surroundings, and their sensitivity to light flashes and moving objects is reduced. This phenomenon is known as saccadic suppression. The physiological basis of this suppression is not well understood. The necessity for a mechanism which allows discrimination between retinal image movement generated by the object and that generated by the subject was recognised by Helmholtz[1]. Sperry[2] proposed that along with the eye movement, the central nervous system generates a 'corollary discharge' into the visual centres that compensates for displacement of the retinal image, and von Holst and Mittelstaedt[3] independently postulated a similar 'efference copy' mechanism. Functionally significant efferent control has been demonstrated in several non-visual sensory pathways[4,5]. Such a mechanism could account for experimentally produced effects in human subjects[6,7]. The prevailing view[8–12], however, is that saccadic suppression in higher mammals is caused not by corollary discharge but instead by inhibition derived from the retinal image motion generated by eye movement. This view has been extended to include saccadic suppression of the visual movement detector system in crickets and locusts[13–15], in which the neural circuit performing the inhibitory function has been identified[15,16]. The experiments leading to this conclusion have all used passive movement of the animal with respect to the visual environment, or the reverse, and not true saccades. We have now investigated the effects of head movements by the locust on its visual responses, and report that movement detector neurones are strongly inhibited during the fast phase of optokinetic nystagmus and during voluntary saccades by a central nervous mechanism of the corollary discharge type. The effect of the inhibitory mechanisms of retinal origin are negligible by comparison.

In the locust movement detector system, one can study the neurophysiology of saccadic suppression in identified neurones that have well understood feature-detection properties. The lobula giant movement detector neurone and its tightly coupled postsynaptic cell, the descending contralateral movement detector (DCMD) neurone (the identified neurones of the locust movement detector system) are optimally responsive to novel, small-field stimuli in their hemispherical visual fields[14–18]. The DCMD neurones provide synaptic input to identified metathoracic motorneurones used for escape jumping[19,20] that can be provoked by novel changes in contrast. Locusts do not have moveable eyes and perform saccadic movements by turning their heads rapidly[21]. The changes in visual input thereby produced could inappropriately excite the DCMD neurones. This possibility is greatly reduced by the inhibition reported here that accompanies the fast phase of optokinetic nystagmus and voluntary saccades.

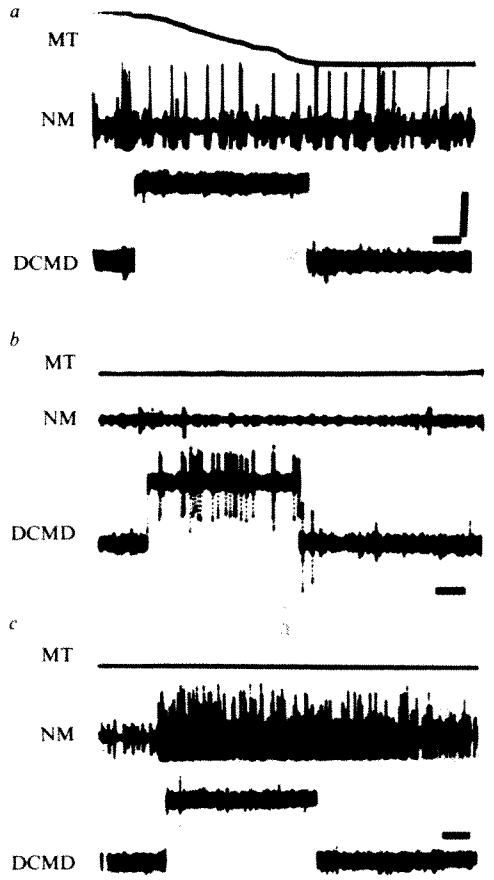


Fig. 1 Inhibition of the response of the DCMD to a moving spot stimulus during the fast phase of optokinetic nystagmus. *a*, A fast flick-back saccade of the locust's head is indicated by the abrupt decrease in the level of the signal produced by a capacitative movement transducer (MT)[34]. The onset of a burst of spikes of an M51 fast unit (NM) precedes the onset of the saccade by a brief interval. The high rate of firing of the M51 fast unit, triggers the stimulus, a moving spot of light produced by an oscilloscope beam, visible to the locust through a 15° window in the striped drum. The duration of the moving spot stimulus is indicated by the voltage step superimposed on the DCMD record (bottom trace). The DCMD neurone recording shows that there is no response to the moving spot stimulus during a fast flick-back. *b*, Thirty seconds after each saccade-triggered moving spot, a control stimulus, a second moving spot of light, similar to the experimental stimulus, excites the movement detector system in the absence of fast head turning, during the slow (following) phase of optokinetic nystagmus. The fast M51 unit (MN) is silent, and the DCMD responds vigorously to a moving spot stimulus. *c*, The locust's head is rigidly clamped to its prothorax, giving a constant value of the MT signal. The high frequency firing of M51 (NM) triggers a moving spot stimulus. The DCMD response is strongly suppressed during the M51 burst. Movement transducer calibration (vertical bar): 20°. Time scale (horizontal bar), 100 ms.

In the absence of movement of the visual field, locusts produce voluntary saccades, but they do so rarely. The fast phase of optokinetic nystagmus and voluntary saccades follow the same amplitude–duration relationship in locusts[21], and they are regarded as identical in higher mammals[22]. In order to make a large number of observations, therefore, saccadic movements were evoked in our experiments by tethering locusts inside a rotating striped drum, whereupon they readily perform optokinetic nystagmus, consisting of a slow (following) phase and a succeeding fast (flick-back) phase. Locusts were suspended by their pronotum, given a polystyrene ball to hold with their legs and left otherwise free to move. Single unit action potentials were recorded from muscle 51 (M51) of the neck, one of the muscles which turns the head[23], and from the ventral nerve cord axon of the DCMD by means of chronically implanted electrodes made from 100-μm insulated silver wire.

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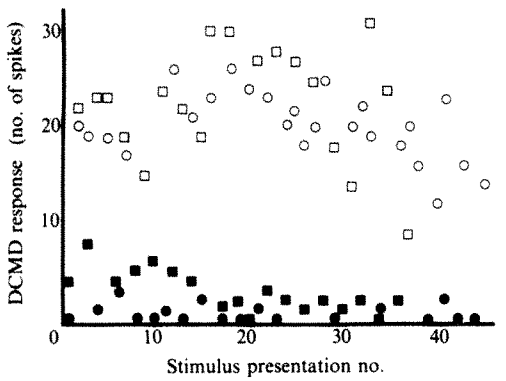


Fig. 2 The responses of the DCMD to two sequences of moving spot stimuli, recorded from (1) an open-loop condition locust (squares) and (2) from a closed-loop condition locust (circles). In both sequences experimental stimuli (solid symbols), triggered by fast-flick back motor commands, are followed 30 s later by control stimuli (open symbols), during which there is no flick-back command. Precautions were taken throughout all stimulus presentations to insure that a reduction of the DCMD response during saccades is a consequence of inhibition and not one of habituation of the response of the DCMD to repetitive stimuli[24]. A gating circuit effected a long minimal interval, 1 min, between successively triggered moving spot stimuli, and the control stimulus followed the experimental stimulus, rather than preceded it. The target stimulus was moved on the face of the oscilloscope by two independent, free-running waveform generators of slightly different frequency applied to the x and y deflection plates, so that it rarely traversed the same path twice.

The response of the DCMD to small moving stimuli is easily recognised as a burst of large single-unit action potentials delayed with respect to the stimulus by 50–75 ms (refs. 14, 17). During the fast phase of optokinetic nystagmus or during spontaneous saccades when the drum is stationary, the response of the DCMD is strongly inhibited; it responds not at all or with just a few acton potentials (Figs 1*a*, 2). During the slow phase of optokinetic nystagmus or if the locusts's head is stationary, the DCMD responds vigorously to optimal stimuli (Figs 1*b*, 2).

Activity of the fast and slow motor axons which excite M51 can be identified in extracellular recordings from the fibres of M51 as large (fast units) and small (slow units) spikes, respectively[23]. We find that inhibition of the DCMD is strongly correlated with high frequency burst activity of the fast motor units of M51 (Fig. 1*a*, c), and that the DCMD is not inhibited during low frequency tonic activity of either fast or slow M51 motor units (Fig. 1*b*). DCMD inhibition does not terminate sharply with the end of M51 fast motor unit bursts, but may continue afterwards for a duration of 200–300 ms. High frequency antidromic stimulation of the motorneurones to M51 does not produce inhibition of the DCMD. Inhibition of the DCMD is thus correlated with commands for fast turning of the head and not simply with activity of fast M51 motor units.

Relative movement of the eye and a complex visual background can suppress the response of the locust movement detector system[24,25] by mechanisms that are visual and not efferent in origin[15,16,18]. The inhibitory influence of the rotating striped drum in these experiments can be discounted. The contrast frequency, 0.02 Hz, produced by rotation of the striped drum alone is far too low to produce inhibitory effects of visual origin[15]. However, visual mechanisms for the inhibition of the locust movement detector system might well be activated and cause the inhibition we have described during the fast phase of optokinetic nystagmus when the locust's head turns with angular velocity as high as 300 deg s^{-1}. This possibility is excluded by two lines of evidence. First, the DCMD is strongly inhibited during spontaneous commands for voluntary saccades in a homogeneous white background. And second, after the head has been rigidly secured to the body (open-loop condition), the unilateral high frequency burst activity of the fast motor units of M51 which would normally cause rapid head movement still coincides with a period of strong inhibition of the DCMD response (Figs 1*c*, 2). This experiment also precludes proprioceptive feedback from head movement, via cervical and proprioceptive hairs[26,27] as a source of inhibition. (Elimination of proprioceptive feedback by securing the locusts head to its body or by elimination of neck hairs[21] reduces the frequency of occurrence of fast flick-backs but does not abolish them, contrary to Shepheard's[23] interpretation.

Further experiments, in which the nerves supplying the neck muscles are cut and motor unit activity recorded from the cut ends of the motor axons to M51, exclude proprioceptive feedback from neck muscle stretch receptors (should these exist) as a source of inhibition of the locust movement detector system during the fast phase of optokinetic nystagmus and voluntary saccades. The DCMD is as strongly inhibited in these conditions as it is when the neck muscles can contract. A command for rapid head turning is therefore a sufficient condition for inhibition of the locust movement detector neurones. Neither change in visual contrast, nor proprioceptive information produced by change in position of the head or muscle contraction, nor the visual signal produced by the rotating striped drum used to induce optokinetic nystagmus contribute significantly to this inhibition, which must therefore be efferent in origin and distinct from the previously described[15,16,18] visual inhibitory mechanisms of the locust movement detector system.

Relative movement of the eye and environment thus inhibits the locust movement detector neurones by either central or peripheral visual mechanisms. Presumably the same is true for the cricket movement detector system which is very similar to that of the locust[13,14]. These mechanisms do not effect inhibition of the entire locust visual system. For example, visual interneurones that are excited by relative movement of eye and background are not suppressed during optokinetic nystagmus[28]. The movement detector neurones, however, are inhibited predominantly by corollary discharge during saccadic movements, but visual reafference could function alone when movement is otherwise produced as in flight or locomotion. Central inhibitory mechanisms may be active here, as well.

Saccadic suppression in mammalian visual systems may involve mechanisms of both central and peripheral origin. However, there has been no neurophysiological demonstration of a functionally significant corollary discharge mechanism for saccadic suppression in any vertebrate, such as we have shown occurs in the locust. The similarities between the fast phase of optokinetic nystagmus in the locust and mammalian saccades[29,30], the former a continuous movement with a small amount of jitter, duration typically 200–300 ms and angular velocity maximally 300 deg s^{-1} (ref. 21), suggest that corollary discharges may at least participate in saccadic suppression of mammalian visual systems. A striking example of inhibition produced by corollary discharges associated with saccadic movements is found in the visual neurones of the superficial layer of the superior colliculus of the rhesus monkey[31,32,35]. Although they are in themselves poor sensory analysers, anatomical pathways exist[33] by which the suppression of the activity of the neurones of the superior colliculus could affect perceptual function through modification of the responses of the feature detector units of striate and extra-striate visual cortex. As in the locust, visual perception in higher mammals could thus be inhibited by corollary discharges that accompany saccadic movements as well as by retinal image motion[8–10,12].

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Nuclear transplantation in teleost *Misgurnus fossilis* L.

ALTHOUGH the transplantation of somatic cell nuclei into eggs has been achieved in amphibian species[1,2], *Drosophila*[3] and some mammals[4,5], it is important to use further groups of animals because experience with amphibia has shown that the subsequent development of normal animals depends largely on the species used. We report here successful transplantation of nuclei from middle or late blastula into the eggs of teleost fishes. The eggs of teleosts have several distinctive features of structure, cleavage and early development which make nuclear transplantation more difficult than with amphibia. Teleost eggs are telolecithal, with the yolk segregated from the active cytoplasm. We used eggs and embryos of *Misgurnus fossilis* L. (loach). In this fish, 30–60 min after natural or artificial activation of an egg, part of the cytoplasm migrates to the animal pole, forming a small polar cap where the nucleus is situated, completing meiotic division[6]. Thus, nuclei must be introduced into the animal pole region which occupies not more than one-tenth of the total egg.

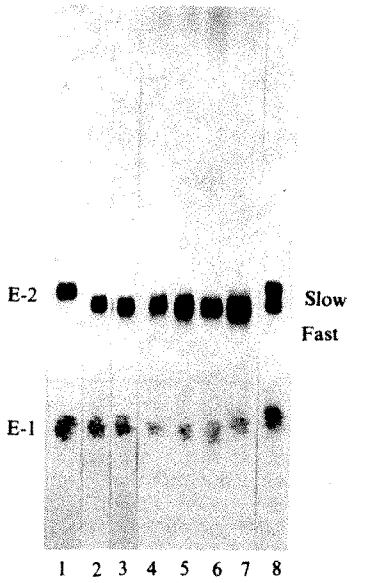


Fig. 1 Esterases of larvae obtained from eggs supplemented with blastulae nuclei. Individual larvae 120 h old were homogenised in a 1% solution of Triton X-100 in distilled water. Each homogenate was centrifuged (2,000*g* per 10 min) and aliquots of supernatant were subjected to polyacrylamide gel disc electrophoresis in 1-mm diameter tube. Esterase bands were identified using a slightly modified method of Sims[10]. The substrate was α-naphthylacetate. The start is at the top and the anode is at the bottom. Two zones of esterase activity are revealed, the more cathodal being E-2 carboxylesterase. Gels 1 and 2 show the mobility of two allelic variants of E-2 slow and fast isoenzymes in homozygous larvae, respectively. Gel 8 results from a control mixture made from the homogenate analysed in gel 3 and from a standard slow E-2 preparation.

The procedure was basically similar to that of Briggs and King[1]. Eggs of *M. fossilis L.* were obtained by injection of females with chorionic gonadotropic hormone (100 mequiv. per 100 g body weight). They were activated by placing them in Petri dishes with tap water; activated eggs became attached by their vegetal poles to the bottom of the dish. After 30 min, when the polar cap had formed, the water was replaced by Niu–Twitty solution, which served as a medium for further experiments. A blastodisk of mid-blastula stage was removed from the yolk and placed in Niu–Twitty solution without Ca^{2+} and Mg^{2+}. Dissociation was complete after 5–10 min. A single cell ($\sim$50 μm in diameter) was sucked into a micropipette of inner diameter about 35 μm. The end of the pipette was inserted into the egg at

Table 1 Nuclear transplantations in *Misgurnus fossilis* L.

Type of transfer	Total no. of transfers	Cleavages yielding blastulae	No. of blastulae under observation	No. of embryos that reached				
				Gastrula	Blastopore closing	Heartbeat	Prelarvae	Feeding larvae
From mid-blastula to enucleated (by X-irradiation, 15.20 rads) eggs	791	349†	89(*a*)	52	29	16	13	10
			146(*b*)	88			13	
From mid-blastula to enucleated (by X-irradiation, 38–49.40 rads) eggs	72	30	16	9	4	2	2	2
From mid-blastula to non-enucleated eggs	320	156†	57(*a*)	30	11	9	7	3
			51(*b*)	21			6	
Control*	1,000	0	0	0	0	0	0	0

* Irradiated (by X rays) or non-irradiated eggs; both have been activated with tap water.
† Some blastulae were used for chromosome analysis and the rest were used for further observation (*a*) or to provide prelarvae for esterase analysis (*b*) (Fig. 1).

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Table 2 Ploidy estimation in blastulae and gastrulae obtained

Nuclear transfers from mid-blastulae	Stage	Embryos analysed: Number	No. of embryos: Anucleate subhaploid	Haploid	Diploid	Triploid	Tetraploid
To enucleated eggs	Blastula	30	11	0	16	0	2
	Gastrula	9	0	0	9	0	0
To non-enucleated eggs	Blastula	19	3	2	11	1	2
	Gastrula	6	0	0	5*	1	0
Control	Blastula	7	0	0	7	0	0

Blastodisks isolated from the yolk were fixed in a mixture of alcohol and acetic acid in the ratio 1:3 for 24 h, washed with 70% at 5 °C and stained with acetorcein. Stained blastodisks were squashed in a drop of 45% acetic acid. In each blastodisk preparation 8 to 10 metaphase plates were chosen to calculate chromosome number. The diploid set in *M. fossilis L.* consists of 100 chromosomes[8]. The number of chromosomes in preparations of metaphase plates was counted to an accuracy of ± 10%, as shown by the analysis of untreated diploid embryos. We therefore took as diploids all treated embryos in which we counted 100 ± 10 chromosomes. Triploid and tetraploid embryos were those with 150 ± 15 or 200 ± 20 chromosomes, respectively. Embryos were termed anucleate or subhaploid if most of the cells had no nuclei and if the few cells which contained nuclei were subhaploids. In one of two blastulae presented above as haploids we counted 50 chromosomes in each metaphase plate analysed and in another 50 ± 5 chromosomes. * Some metaphase plates in these embryos contained a subhaploid (< 20) or polyploid (> 200) number of chromosomes.

equatorial level and the contents were injected into the polar cap region. The operation was carried out within 60 min of activation. Operated eggs were incubated in the same medium at 16–18 °C for 10 days, sufficient for the formation of actively feeding larva.

Two series of experiments were carried out, with and without enucleation of eggs. Enucleation was achieved by X-ray irradiation in an appropriate dose to inactivate the nuclei of loach eggs[7]. As our results show (Table 1) irradiation produced almost complete inactivation of the female pronucleus, while the cytoplasm remained able to support normal development at least to the larval stage. We used 791 enucleated and 320 non-enucleated eggs, and into each of them we transplanted a single somatic cell nucleus (together with cytoplasm) from the mid-blastula. Of the 200 gastrulae thus obtained, 41 developed to the prelarval stage or into actively feeding larvae. The development of most embryos stopped at some point before these stages and in many cases there were evident abnormalities. Some embryos were lost after the feeding larva stage, but others were quite normal and were either used for analysis of enzymes or fixed as total preparations. Unfortunately, we have not been able to maintain the development of loach larvae beyond the stage of actively feeding larva in laboratory conditions.

To investigate whether this embryonic development was determined by the transplanted nuclei, we looked at the karyotypes and carboxylesterase (EC 3.1.1.1.) isoenzymes of the embryos. Table 2 shows that most of the blastulae were diploid, although some were tetraploid. This was possible only if the transplanted nuclei had participated in the development of the embryos. During transplantation some cytoplasm, including the centriole, is transferred from the somatic cell into the egg together with the nucleus. Thus, it might be said that the centriole provokes 'parthenogenetic' cleavage; however, if this were so, only blastulae with an abnormal chromosome set should be formed. Indeed, when enucleated eggs are fertilised with irradiated sperm and are supplemented with sperm centrioles, they begin to cleave but yield only embryos with abnormal chromosome sets, developing no further than the blastula stage.

Transplantation into non-enucleated eggs yielded about the same proportion (~50%) of cleaving embryos which formed blastulae, and about 10% developed into normal larvae. This could not have happened without the transplanted nuclei because unfertilised and artificially activated eggs of *M. fossilis L.* never cleave. Again, it might be said that cleavage was provoked by the introduction of the somatic cell's centriole, but if so, the embryos would have been haploid, for when fertilised with irradiated sperm, loach eggs cleave but spontaneous diploidisation of a female pronucleus does not usually occur and only haploids are formed[7]. Table 2 shows the tentative estimation of the ploidy of blastula cells. We expected that they would be either mainly haploid (if parthenogenetic development was induced by the introduced centriole) or triploid due to fusion of the transplanted nucleus with the female pronucleus. Most embryos seemed to be diploids, with only a small part of the blastulae having cells with 1*n* chromosomes (or even fewer).

Two alleles for carboxylesterase are known in *M. fossilis L.*[9], coding for two distinct forms of the enzyme (E-2), electrophoretically fast and slow (Fig. 1, gels 1 and 2). Maternal E-2 synthesised at oogenesis has been shown to disappear within 120 h, after which only the esterase controlled by the larval genome is found[9]. When nuclei of blastulae homozygous for fast E-2 were transplanted into enucleated eggs from females homozygous for slow E-2, only fast E-2 was found in the larvae that developed (Fig. 1, gel 3). When nuclei from blastulae homozygous for fast E-2 were transplanted into non-enucleated eggs from blastulae homozygous for slow E-2, five larvae out of six obtained had only fast E-2 (Fig. 1, gels 4–6); in one larva there was a trace of slow E-2 in addition to fast E-2 (Fig. 1, gel 7), indicating partial function of E-2 genes characteristic of the female pronucleus. Probably, either the whole pronucleus or the chromosome carrying the relevant gene was preserved in this larva. Only the blastulae and gastrulae were karyotyped and most of them seemed to be diploid, although there were polyploid individuals among them. Based on these data we consider that our larvae were mainly diploids, and that in the experiments with non-enucleated eggs the female pronucleus was eliminated in the embryos, although it may have been preserved in some cases, yielding triploids, as reported for amphibia[11]. The possibility that we measured some maternal enzyme activity can be discounted because maternal E-2 esterase synthesised at oogenesis disappears within 120 h (ref. 9) and our analyses were made on larvae older than 120 h. Therefore, we applied an enzyme analysis to indicate that there was little or no activity of the genes of recipient eggs in the resultant larvae.

This cannot be attributed to the fact that cytoplasm of the blastula cell which contained some amounts of donor-like enzyme was introduced to the recipient egg because this cytoplasm constituted less than 0.1% of the egg cap cytoplasm[12] and the exogenous enzyme was further diluted during cell multiplication.

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Expression of genetic resistance to an oncogenic herpesvirus at the target cell level

THE oncogenic potential of herpesviruses has been demonstrated in several species and herpesviruses have been strongly implicated as aetiological agents of various human neoplastic diseases[1,2]. The extreme heterogeneity of human populations makes genetic analysis of mechanisms of host resistance to neoplastic disease in man a difficult task. Another herpesvirus, Marek's disease virus (MDV), is the aetiological agent of a naturally occurring lymphoma of chickens, Marek's disease (MD) (for review see ref. 3). MD is the animal model for Burkitt's lymphoma of man in which the Epstein–Barr virus (EBV) has been strongly implicated[4]. At least two loci are associated with resistance to MD: the major histocompatibility complex (MHC)[5,6] and a non-MHC locus[7]. The mechanism of MHC-associated resistance may involve an active rejection of MD tumour cells[5]. We now report data which strongly suggest that non-MHC-associated genetic resistance is expressed at the level of the target cell for malignant transformation.

The target cell for neoplastic transformation by MDV is generally thought to be a T lymphocyte[8]. The tentative association of the *Ly-4* locus, which codes for alloantigenic markers present on T cells, with the degree of susceptibility to MD[7] led us

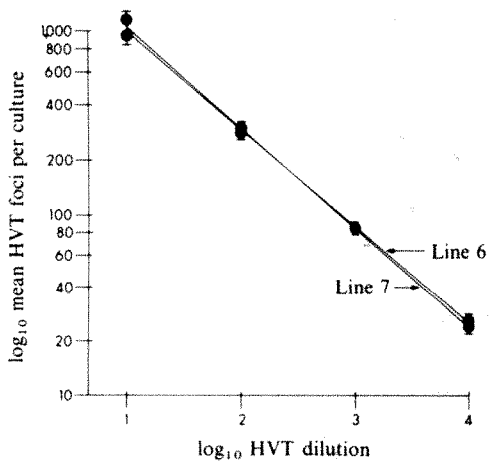


Fig. 1 Secondary chick embryo fibroblasts (CEF) were prepared from 10-day line-6 and line-7 embryos and propagated in Linbro multi-well trays. Lyophilised FC126 strain HVT vaccine was reconstituted with medium and centrifuged at 500g for 15 min to remove large cell debris. Four replicate wells were infected with each serial 10-fold dilution of this preparation, the highest concentration of which had yielded 1.25×10^4 focus-forming units per well when assayed previously on Hy-line SC CEF. HVT foci were stained with Giemsa stain and counted at 64 h post-infection. Error bars indicate 1 s.d. from the mean.

Table 1 Infectious centres induced by spleen cells exposed to HVT *in vitro* or MDV *in vivo*

Spleen cell source	HVT lesions per 1×10^7 cultured spleen cells	MDV lesions per 5×10^5 injected spleen cells
Line 6	33.5 ± 25.5	9.4 ± 6.5
Line 7	223.9 ± 38.0	38.6 ± 3.6

For determination of infectious centres after *in vitro* exposure to HVT, aliquots of the spleen cells used for the HVT adsorption studies of Fig. 2 were washed three times in medium and cultured in RPMI-1640 with 10% fetal calf serum for 24 h at 37 °C at a density of 5×10^6 spleen cells per ml. Cells were then washed twice in medium and infectious centres were assayed *in ovo* according to the method described previously[9]. For determination of infectious centres after *in vivo* exposure to MDV, groups of five Hy-line SC (B^2/B^2) third-party chicks were given 500R of γ-rays on days 3 and 7 post-hatching and reconstituted with 1×10^8 spleen cells obtained from 6-week-old line-6 or line-7 donors. Following a 7-d period of natural exposure of the recipients to MDV, spleens from each group were pooled and the number of infectious centres was assayed *in ovo*[9] in B^2/B^2 eggs histocompatible with the donor MHC. Background host-specific infectious centres in unreconstituted, γ-irradiated, MDV-exposed chicks formed less than 15% of the observed totals and this value was subtracted from the data to obtain donor-specific infectious centres. Error values are 1 s.d. from the mean.

to postulate that non-MHC-associated resistance could be due to differential infectivity, transformability or proliferation of the target cell for MDV. Susceptible line-7 birds display a much higher leukocyte-associated titre of MDV than resistant line-6 birds following natural exposure to MDV[7]. Although this observation is consistent with the target-cell hypothesis, it is explained equally well by immune surveillance mechanisms. However, differential infectivity is also demonstrable in line-6 and line-7 embryos[9] at stages of development during which immune mechanisms are unlikely to be involved. This finding supports the target cell hypothesis rather than classical immunological surveillance mechanisms. Chickens which are genetically resistant to MD nevertheless become infected with and shed virulent MDV. Furthermore our own evidence shows that embryonic fibroblasts from lines 6 and 7 are equally permissive to *in vitro* infection with a related herpesvirus, the herpesvirus of turkeys (HVT, Fig. 1). Similar observations have been made for MDV infection of adult kidney cells. However, one cannot assume from this that genetic resistance to lymphoma development could not be due to differential infectivity at the level of the target cell. Productive infection of fibroblasts or epithelial cells by MDV, a process which may reflect a capacity for infection at the level of the whole animal, is not equivalent to infection and neoplastic transformation of T lymphocytes and, thus, may not be subject to the same genetic restrictions. To determine whether line-6 and line-7 lymphocytes display differences in viral adsorption or infectivity demonstrable *in vitro*, spleen cells from these lines were incubated with HVT, pelleted by centrifugation, and the amount of infectious virus remaining in the supernatant was assayed on chick embryo fibroblast monolayers. HVT was used in these studies because it is much more stable in cell-free form than MDV, but it may be assumed that these two very similar herpesviruses use the same cellular receptors.

Spleen cells from line-7 (susceptible) birds adsorbed HVT in a dose-dependent fashion but line-6 spleen cells adsorbed less virus at the concentrations tested (Fig. 2). This difference is highly reproducible: this experiment has been repeated six times with the same result. In parallel experiments not presented here, line-7 thymocytes also adsorbed significantly more HVT than did line-6 thymocytes[10]. However, the difference in adsorptive capacity of spleen cells was greater than that observed for thymocytes, as might be expected if a mature T lymphocyte were the target for MDV.

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Table 2 Marek's disease mortality in chickens which received thymus transplants

	Group				
	A Line 7 N→Line 6 TX	*B* Line 7 γR→Line 6 TX	*C* Line 6 N→Line 6 TX	*D* Line 6 TX	*E* Line 6 Sham TX
Total	38/72	10/24	6/36	12/46	3/17
%	52.8	41.7	16.7	26.0	17.6

Newly hatched chicks were thymectomised or sham-operated as described previously[19]. For thymus transplants, thymus lobes were cut into two or three pieces and immediately transplanted subcutaneously lateral to the oesophagus in areas which had been vacated by the host's thymus lobes. At least one entire thymus equivalent (14 lobes) was transplanted per recipient. On day 2 post-hatching chicks were contact exposed to MDV by housing them next to 4–6-week-old chickens which had been inoculated with 10,000 *in ovo* lesion-forming units[9] of the BC-1 isolate of MDV[20]. The incidence of MD-specific deaths was recorded for 14 weeks at the end of which significant losses due to MD had ceased. N = normal, non-irradiated donor thymus; TX = thymectomised; γR = 1,000R γ-ray irradiated donor thymus. Using Fischer's calculation of exact probability, $P = 0.00046$ for a two-tailed comparison of groups *A* and *C* and $P = 0.0033$ for a one-tailed comparison of groups *B* and *C*.

Following viral adsorption, the number of infected cells of each type was estimated after the cells had been washed and incubated for 24 h. Line-7 spleen cells induced 6.7 times more infectious centres than did the line-6 spleen cells (Table 1). To confirm this observation for MDV *in vivo*, line-6 and line-7 spleen cells were adoptively transferred into separate groups of irradiated, histocompatible, third-party recipients which were then naturally exposed to MDV. Following this procedure, over four times more line-7 spleen cells than line-6 spleen cells were infected with MDV (Table 1).

These experiments suggest that it might be possible to make a resistant (line-6) bird more susceptible to MD by providing a source of susceptible (line-7) target cells. For this purpose, two sources of line-7 cells were used for transplantation into line-6 recipients. First, we prepared classical haematopoietic chimaeras following the intravenous injection of line-7 embryonic spleen cells into line-6 embryos and found that this procedure did not result in a significant increase in MD mortality[10]. Thus, the mere presence of line-7 haematopoietic cells apparently did not result in an increased mortality due to MD. To provide a more direct source of T lymphocytes, the putative target cells for transformation, we transplanted line-7 thymuses into newly hatched, thymectomised line-6 recipients. The pooled data from six experiments (Table 2) show that this procedure rendered the line-6 birds much more susceptible to MD. We observed no significant differences in MD mortality between thymectomised line-6 birds, sham-thymectomised line-6 birds and thymectomised line-6 birds which received a line-6 thymus transplant. This finding serves to control for the possibility that the line-7→line-6 transplanted birds showed a higher mortality simply because the donor thymus failed to reconstitute a depleted T-cell compartment. In addition, there have been no reports that thymectomy *per se* has an effect on MD mortality, although neonatal thymectomy combined with irradiation[11] or anti-T cell serum treatment[12] can affect MD mortality and virus titre.

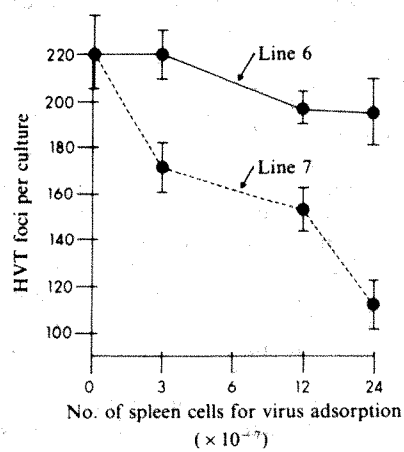


Fig. 2 HVT was prepared as described in Fig. 1. Cell suspensions from spleens of 3–4-week-old donors were prepared by collagenase digestion, washed three times in medium and mixed with aliquots of HVT. Only those cells having morphologies characteristic of lymphocytes were counted and included in the calculation of spleen cell number for data shown in Fig. 2 and Table 1. Virus adsorption was carried out at room temperature for 1 h and the cells were then pelleted by centrifugation. The amount of infectious HVT remaining in the supernatant was estimated by focus formation on replicate cultures of CEF of Hy-line SC origin. Error bars indicate 1 s.d. from the mean.

The finding that transplantation of susceptible thymuses made the resistant birds more susceptible but that the presence of haematopoietic cells from the susceptible line did not, could mean that (1) thymus transplants simply provide more susceptible target cells at the appropriate stage of differentiation than a more primitive population of embryonic spleen cells, or (2) the genotype of the thymic micro-environment may influence the target-cell phenotype, as stem cells can be induced to undergo differentiation into mature T cells in the thymus gland. In support of the latter possibility, we have found that transplanted, irradiated (1,000R) line-7 thymuses increased the susceptibility of line-6 chicks (Table 2). Further experiments will be necessary to determine whether line-6 stem cells undergo a phenotypic change under the influence of line-7 thymus glands. One prediction would be that unlike the adoptive transfer of embryonic spleen cells, adoptive transfer of line-7 adult spleen cells, a portion of which have presumably migrated through a susceptible thymic microenvironment, should be sufficient to increase susceptibility to MD. These experiments are in progress.

Thus, we have presented data which strongly suggest that genetic resistance to an oncogenic herpesvirus can be manifested at the level of the target cell. Genetic resistance to C-type RNA viruses at the cell level has been shown in several systems[13–16] but we believe that our studies represent the first demonstration of cellular resistance to an animal DNA virus. We transplanted susceptible thymuses into resistant animals and made them much more susceptible to oncogenesis. Conversely, Shieh and Sevoian[12] reported no significant effect of transplantation of a susceptible thymus into a thymectomised resistant host in lines of chickens in which resistance was associated with the MHC. Our data provide strong evidence that the target cell for MDV-induced transformation is a T lymphocyte. The mechanism of resistance at the target T-lymphocyte level remains unknown but our viral adsorption studies suggest that resistant birds may have target cells with fewer virus receptors, receptors of lower affinity for virus, or fewer target cells with identical receptors. That differences may exist between line 6 and line 7 in the size of T-cell subpopulations is suggested by other studies which show that line-7 birds possess three times as many allo-reactive T cells as line-6 birds[17] and that susceptible T cells may proliferate at a greater rate than resistant T cells[18]. In view of this, this, the observed differences in MDV infectious centres assayed *in ovo* are indicative of target cell phenotype, but may reflect the capacity of inoculated cells to divide and/or

spread in the embryo. A role for such post-adsorptive mechanisms can only be ruled out by the careful use of serological and physical cell markers.

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Feedback inhibition by human primed T-helper cells

STUDIES in animal models, in particular that of the murine system, have revealed that the sequence of events leading to an antibody response is similar to other complex biological processes in that they are controlled by interrelated regulatory mechanisms[1,2]. In man helper activity which regulates the antigen-induced antibody response *in vitro* is initiated by T lymphocytes bearing receptors for the Fc fragment of IgM ($T\mu^+$ cells), whereas suppressive activity can be induced in T cells capable of binding the Fc fragment of IgG ($T\gamma^+$ cells)[3]. We present here data which indicate that $T\mu^+$ cells, apart from providing help in the human antigen-driven process of B-cell activation, are also capable, in certain conditions, of inducing a subpopulation of unprimed T cells to suppress an antigen-induced antibody response.

As has been reported[4], the *in vitro* induction of suppressor activity by $T\gamma^+$ cells requires the presence of a relatively high dose of antigen in the culture, whereas helper activity of $T\mu^+$ cells prevails over suppressor activity if a relatively low dose of antigen is applied to a culture of unselected human peripheral T and B cells[5]. In an attempt to induce and measure helper activity without simultaneous activation of $T\gamma^+$ cells, $T\mu^+$ cells were isolated from the unselected T cells, added to purified B cells and stimulated by a dose of antigen known to induce a suppressor activity in cultures containing unselected T cells.

As can be seen in Table 1 (and ref. 3), considerable numbers of plaque-forming cells (PFC) were obtained, indicating that effective T-helper activity is generated if a high dose of antigen is used. Moreover, when isolated $T\mu^+$ cells were primed by preculturing the cells with a high dose of sheep erythrocytes (SE) for 6 days and subsequently added to purified B cells, a PFC response is obtained that exceeds the number of PFC of B-cell cultures to which only unprimed $T\mu^+$ cells were added (see Table 1). When, however, primed $T\mu^+$ lymphocytes were added to a suspension of B cells supplemented with unselected, unprimed T cells, a low PFC response was observed, indicating that a negative regulatory activity had been generated by the subsequent addition of unselected, unprimed T cells. Figure 1 also shows that as the number of primed T-helper cells, added to a culture of unselected, unprimed T cells and B cells, increases, suppression also increases; this meets the definition of a feedback type of suppression.

Shore *et al.*[6], who induced optimal helper activity by using 24-h primed $T\mu^+$ cells, did not observe any sign of a feedback inhibition of the antibody response when the cells were cultured with unprimed T cells and B cells. On the contrary, these cells reacted as adequate primed T-helper cells.

Our results lead us to suggest that during the 6×24 h priming period some differentiation and/or education of $T\mu^+$ cells occurred which resulted in their capacity to suppress the B-cell response. However, our data do not allow us to state definitely that a feedback signal is provided by $T\mu^+$ helper cells as such. Within the $T\mu^+$ subset, cells could be present which do not express antigen-specific help, but which are programmed exclusively to induce a feedback type of suppression. Cantor *et al.*[7], however, when describing a similar system for the mouse, reported that the primed cell which is capable of delivering the feedback signal in the murine system, is a cell residing in the Lyt 1^+, Qa 1^+ subpopulation. It appears that the Lyt 1^+, Qa 1^+ cell is a T-helper cell capable of synergising with a Lyt 1^+, Qa 1^- T cell to generate optimal helper activity. We cannot yet substantiate our hypothesis further because we are unable to visualise membrane structures that will allow the recognition of possible subsets of $T\mu^+$ cells.

Table 1 Regulation by SE-primed T helper cells

Primed T cells	Assay system*	PFC per 10^6 Lyt
None	$+3.5\ T\mu^+$	2,333 (1,514–2,922)
$3.5\ T\mu^+$	None	3,804 (2,581–4,201)
$1.0\ T\mu^+$	$+2.5\ T^\dagger$	178 (0–364)
None	$+2.5\ T^\dagger + 1.0\ T\mu^+$	1,875 (900–2,224)

* Assay system: 1.5 B cells plus 5.0 SE (cell numbers $\times 10^6$) plus indicated T-cell fractions.
† Unselected, unprimed T cells.

For the induction of PFC to SE, cultures were set up in 17 × 100 mm tissue culture tubes (Falcon) containing 1.5×10^6 B cells in 10 ml of culture medium consisting of RPMI-1640, 25 μg Fungizone, 100 μg penicillin, 100 IU streptomycin, 2-mercaptoethanol (0,02 M), L-glutamine (2 mM) and supplemented with 10% serum derived from AB^+ donors (AB serum) which had been preabsorbed with SE to prevent artefactual plaque formation[11]. To these cultures various T-cell fractions were added: unprimed, unselected T cells ($T^\dagger$) and/or $T\mu^+$ lymphocytes, together with 5×10^6 SE as antigen. Cultures were kept in a humidified atmosphere of 5% CO_2 in air. After culturing the cells for 6 d, cells were collected, washed twice in RPMI-1640 at 4 °C and assayed for PFC according to Dosch and Gelfand[12]. The data shown are expressed as the arithmetical mean of values obtained in five individual experiments. Within each experiment determinations were done in sixfold. Values in parentheses represent the ranges. *Priming procedures*: $15 \times 10^6\ T\mu^+$ cells, which were isolated as described in Table 2 legend, were cultured with 15×10^7 SE in the inner tube of a Marbrook–Diener type of culture tube. The inner tube contained 3 ml of culture medium supplemented with 15% SE-absorbed AB serum. The outer tube contained the same medium as the inner tube with the exception that the medium was supplemented with 5% fetal calf serum instead of 15% AB serum. The Marbrook–Diener cultures were kept for 6 d in a humidified atmosphere of 5% CO_2 in air. After the culture period the primed cells were freed of the SE by NH_4Cl-induced lysis. The cells were then washed twice with RPMI-1640 containing 5% AB serum. Viability of the cells was tested for by Trypan Blue dye exclusion which always exceeded 90%.

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Table 2 Unprimed T-cell subsets mediating feedback inhibition

Primed $T\mu^+$ cells	Assay system*	PFC per 10^6 Lyt
3.5	+None	4,857
1.0	+2.5 T^+	186
1.0	+2.5 $T\mu^+$	3,414
1.0	+2.0 $T\mu^+$ + 0.5 T†	364
1.0	+2.0 $T\mu^+$ + 0.5 $T\mu^+$	3,414
1.0	+2.0 $T\mu^+$ + 0.5 $T\gamma^+$	2,167
1.0	+2.0 $T\mu^+$ + 0.5 $T\mu, \gamma^-$	283
None	+3.5 $T\mu^+$	2,333
None	+3.0 $T\mu^+$ + 0.5 T†	2,175
None	+2.5 $T\mu^+$ + 1.0 T†	2,202
None	+2.0 $T\mu^+$ + 1.5 T†	2,257

PBL, isolated according to Bøyum[13], were separated into T and non-T cells by two cycles of EAC-rosetting and subsequent density gradient centrifugation on Ficoll–Isopaque ($\rho = 1.077$)[3]. The non-T fraction was depleted of non-lymphoid cells by adherence to plastic and recovered by gently scraping with a rubber policeman as described in ref.3. *$T\mu^+$ cell isolation*: The T cell fraction was incubated with 3 mM theophylline (Sigma) for 60 min at 37 °C (ref. 14). Cells were then collected by centrifugation at 4 °C and immediately mixed with a 50-fold number of SE[15]. This mixture was layered on Ficoll–Isopaque ($\rho = 1.077$) and centrifuged for 30 min at 1,000 g. After the separation procedure the theophylline-resistant cells in the pellet were freed of contaminating SE by lysis with NH_4Cl and tested for purity. It was found that the above mentioned procedure resulted in a population of 95–99% $T\mu^+$ cells. *$T\gamma^+$ and $T\mu, \gamma^-$ isolation*: The interphase fraction of the above mentioned gradient, consisting of the theophylline-sensitive cells, was rosetted with ox erythrocytes coated with rabbit anti-ox IgG[16]. The cell mixture was pelleted and incubated on ice for 20 min. Then the pellet was gently resuspended and layered on Ficoll–Isopaque ($\rho = 1.077$). After centrifugation (30 min, 1,000g) the pellet was freed of contaminating ox erythrocytes by NH_4Cl-induced lysis. The interphase (mainly consisting of $T\mu, \gamma^-$ cells) and the pellet fractions were tested for purity by determination of the number of $T\mu^+$ and $T\gamma^+$ cells present in the various fractions. Immediately after separation the pellet fraction contained 99% $T\gamma^+$ cells. After culturing overnight the FcγR could no longer be detected (ref. 3). Primed $T\mu^+$ cells were cultured with B cells, SE and when mentioned, with the various T-cell fractions. One representative experiment out of five is given.

* Assay system: 1.5 B cells + 5 SE (cell numbers × 10^6). † Unprimed, unselected, autologous T cells.

To characterise further the cell type receiving the signal from the primed $T\mu^+$ cells, we separated the unprimed, unselected T-cell suspension into $T\mu^+$, $T\gamma^+$, and T cells without detectable Fc receptors for IgM and IgG (therefore referred to as $T\mu, \gamma^-$ cells), by means of a number of isolation procedures (see Table 2 legend). We tested the cells for their potential inhibitory capacity by adding the different subsets separately to cultures of primed $T\mu^+$ cells and B cells, supplemented with a low dose of antigen. It is clear from the results listed in Table 2 that: (1) unprimed $T\mu^+$ cells apparently do not receive the signal provided by the primed $T\mu^+$ cells; (2) unprimed $T\gamma^+$ cells which can suppress the response when they are cultured with a high dose of antigen, do not suppress the response in this system when they are co-cultured with primed $T\mu^+$ cells and a low dose of antigen; (3) unprimed $T\mu, \gamma^-$ cells are capable of interacting with the primed $T\mu^+$ cells, resulting in an inhibition of the PFC response (results of five independent experiments). It is postulated that unprimed $T\mu, \gamma^-$ cells are capable of recognising the stage and intensity of the immune response by detecting or receiving signal(s) from primed T-helper cells, which can result in a feedback mechanism and thereby regulate the response.

The activity of the supernatants of the primed $T\mu^+$ cell cultures was examined for their capacity to induce feedback inhibition of the PFC response *in vitro* because of an increasing awareness of the fact that many T–T interactions do not seem to require contact of the relevant cells, but are mediated by soluble factors released from the participating cells[8,9]. As can be seen in Table 3, no inhibition of the PFC response could be obtained by the use of supernatants of primed $T\mu^+$ cells. On the contrary,

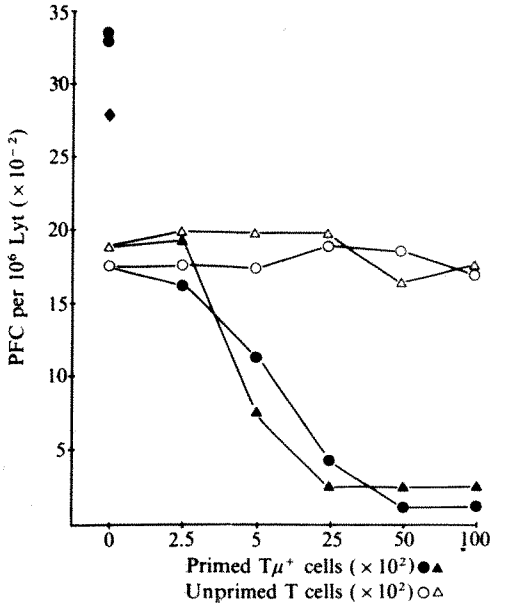


Fig. 1 Different numbers of primed $T\mu^+$ cells were added to a fixed number (3.5×10^6) of unprimed, unselected T cells (▲, ●; two donors). As a control the same numbers of unprimed, unselected T cells, derived from the two donors, were added to the same fixed number of unprimed, unselected T cells (△, ○;). ◆, ● represent the PFC responses when only primed $T\mu^+$ cells from the two donors were cultured with purified B cells. Every culture contained a low dose (5×10^6) of SE.

only a potentiating (helper) effect of the T-cell supernatant was observed.

We do not yet know whether this type of T–T interaction plays any physiological role in the regulation of the *in vivo* immune response in man. Cantor *et al.*[10] reported the absence of feedback suppression and a concomitant defect in the differentiation and/or function of T cells expressing the Lyt 123^+ phenotype in NZB mice. It was suggested[10] that the evidence provided by *in vitro* and *in vivo* experiments indicates that the absence of functional Lyt 123^+ cells and consequent lack of feedback suppression are somehow related to the development of autoimmune reactivity in NZB mice.

For the present we accept as a working hypothesis that a subset of T lymphocytes characterised as $T\mu, \gamma^-$ can be regarded as the human analogue of the murine Lyt 123^+ cell in the loop of a feedback inhibition mechanism of T-helper function. Therefore, we are investigating T-cell-mediated feedback suppression of the antigen-induced antibody response of peripheral leukocytes of patients suffering from systemic lupus erythematosus and myasthenia gravis. This investigation may yield information concerning the biological and clinical relevance of feedback inhibition of T-helper function in man. Moreover, it may to

Table 3 The activity of supernatants of primed $T\mu^+$ cell cultures

Primed $T\mu^+$ cells or supernatant	Assay system*	PFC per 10^6 Lyt
25% Supernatant $T\mu^+$	+None	4,935 (PFC per B Lyt)
3.5 $T\mu^+$	+None	3,601
25% Supernatant $T\mu^+$	+2.5 $T\mu^+$ + 1 T†	2,670
1 $T\mu^+$	+1.5 $T\mu^+$ + 1 T†	232
None	+2.5 $T\mu^+$ + 1 T†	2,275

The supernatant of the primed $T\mu^+$ cell culture in the inner tube was collected after 6 d and centrifuged for 10 min at 2,300g. After Millipore filtration (0.22 μm) the supernatant was immediately added to the cultures as listed in Table 3.

* Assay system: 1.5 B cells plus 5.0 SE (cell numbers × 10^6) plus indicated T-cell fractions. † Unselected, unprimed T cells.

some extent explain the dysregulation of T-cell-dependent antibody responses which may have a role in the aetiopathogenesis of these autoimmune diseases.

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Does lectin–receptor complex formation produce zones of restricted mobility within the membrane?

IT is well established that component lipids and proteins are mobile in the plane of the membrane[1,2]. This lateral mobility could have an important role in the transmission of signals through the membrane. For example, there is much evidence that aggregation of receptors induced by divalent antibodies or by chemical cross-linking can reproduce the biological action of the corresponding ligands[3–5]. It was shown recently that receptors for monovalent ligands like insulin and epidermal growth factor aggregate into patches on the cell surface after interaction with the corresponding hormone. Clustered receptors accumulate at coated regions of the membrane and become internalised in endocytic vesicles[6,7]. Aggregation is dependent on receptor occupancy by the active ligand[7]. Here we show that lateral mobility is also dependent on receptor occupancy. Our approach has been to compare the lateral mobilities of free and ligand-bound receptors using cultured cells placed in a steady electric field. This permits the measurement of lateral long-range (over the whole cell) mobility of the receptors with and

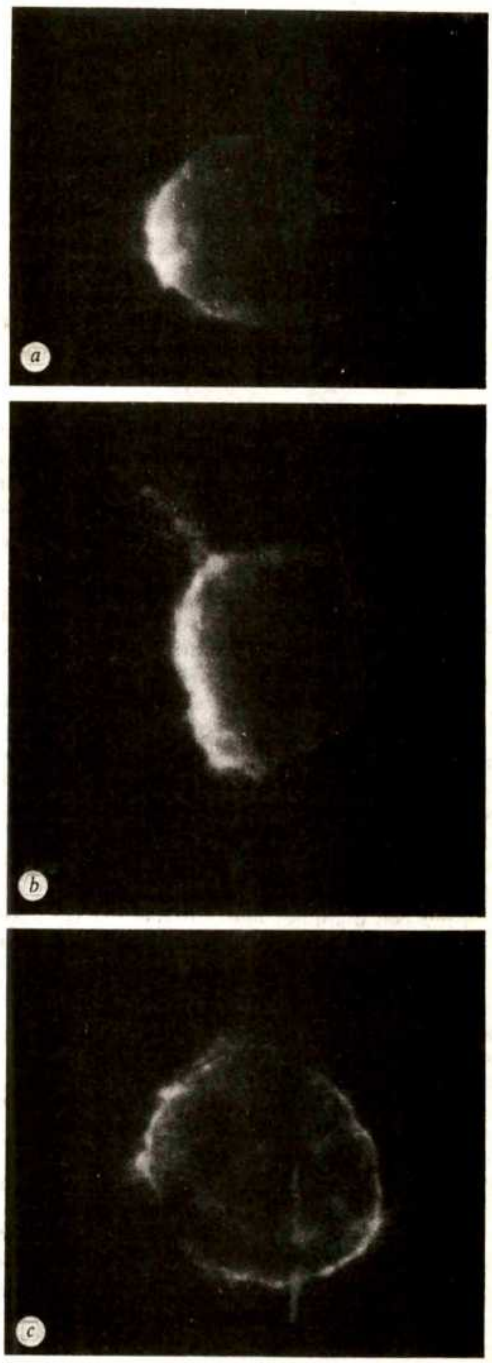


Fig. 1 TRITC-Con A-labelled NS-20 cells in a steady electric field observed by fluorescence microscopy. Conditions for a steady electric field were similar to those described by Poo *et al.*[8], Ag–AgCl electrodes, agar bridges and culture chamber made from microscope slide and pieces of no. 1 coverslides. After finding the 'stationary conditions' we used a uniform electric field of 12 V cm^{-1} for 15 min at room temperature (19 ± 2°C) in PBS. The cells (~100,000 cells for the chamber) were grown for 1 day in the chamber with F10 medium for the C_6 and with minimum essential medium for the NS-20 and 3T3 lines. All media were supplemented with 10% fetal serum and antibiotics. The conjugates TRITC (Mérieux) with Con A (Pharmacia) and ricin (molecular weight 120,000) were attached to Sephadex G-200 or Sepharose 4B, respectively, with subsequent elution of the sugars and passage through biobel P30. The cells were labelled with TRITC-labelled lectins at a concentration of 150 μg ml^{-1} for 10 min at 0–4°C before or after electrophoresis, and fixed with 2% paraformaldehyde (0–4°C for 10 min). Cells rapidly fixed with cold acetone (0–4°C, 30 s) had a similar distribution of receptors. *a*, *b*, The cells were exposed to an electric field before Con A–TRITC labelling and either fixed immediately (*a*) or were allowed to remain in MEM medium at room temperature for 35 min before fixation (*b*). *c*, The cells were exposed to a field after Con A–TRITC labelling. The photographs were taken using a Zeiss microscope equipped with Opak-Fluor veritcal illumination. Control cells not exposed to the electric field showed uniform ring staining (×2,124).

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Table 1 Values of the diffusion constant (D) obtained by the electrophoretic (without ligand) and FPR methods

Cells	Electrophoresis ('free receptor') Con A D	Ricin D	FPR (ligand–receptor complex) Con A* D	$\%R$	Ricin D	$\%R$
NS-20	$2\text{–}3\times10^{-10}$	$2\text{–}3\times10^{-10}$	$6.0\pm0.5\times10^{-11}$	30% (20–50%)	$5.5\pm0.5\times10^{-11}$	~30%
C_6	$2\text{–}3\times10^{-10}$	2×10^{-10}			$6.0\pm0.5\times10^{-11}$	~40%
3T3	$1\text{–}2\times10^{-10}$					

In the photobleaching experiments carried out on dispersed Con A–receptor complexes, a Spectra-physic argon ion laser operating at <10 mW at 514 nm was used to bleach the spot and the same attenuated beam to excite fluorescence in the spot laser beam was passed into a Leitz microscope[9,10]. The laser beam diameter (3.4 μm) and the D values were determined with the calibration scan described by Axelrod *et al.*[12]. Coefficients expressed in $cm^2\ s^{-1}$; temperature of measurements, 19 ± 2°C; $\%R$, the per cent of the fractional fluorescence recovery.

*Also published values of Y.A.Z. *et al.*[10] obtained by the 'initial slope' calculations.

without the corresponding ligands[8]. We have compared this method with the dynamic method of fluorescence photobleaching recovery (FPR) which only permits measurement of the mobility of fluorescent ligand–receptor complexes in the range of the size of the laser beam diameter (~3 μm)[9–12]. We have studied the lateral mobility of concanavalin A (Con A) and ricin receptors from non-malignant fibroblasts 3T3 and malignant neuroblastoma NS-20 and C_6 glial cells with both techniques. Our results suggest that lectin–receptor formation results in the production of zones of restricted mobility in the membrane.

Results obtained by the 'electrophoretic' technique are shown in Figs 1 and 2. Application of an electric field to the extracellular compartment produced a migration of free Con A receptors towards the cellular membrane facing the positive pole (Fig. 1*a*). Removal of the electric field resulted in a redistribution of receptors over the whole cell surface. Determination of the time course of the redistribution (Fig. 2) enabled the lateral diffusion constant, D, to be calculated, using the equation $D=r^2/2\tau$, where r is the cell diameter and τ is the $1/e$ relaxation time. Values of D obtained in the various experimental situations tested are listed in Table 1. For the free receptors, all values were in the range $1\text{–}3\times10^{-10}\ cm^2\ s^{-1}$. These values are close to that obtained using the FPR technique on the pool of surface membrane proteins accessible to nonspecific labelling by fluorescein isothiocyanate[9,13]; they are also similar to values obtained for free Con A and surface antigen receptors[8,14]. In agreement with the conclusions of Edidin and Wei[14], the cytoskeletal elements do not seem to have any effect on the mobility of 'free' lectin receptors, as shown by experiments with drugs like cytochalasin B, colchicine and vinblastine. Finally, we note that the asymmetry observed after application of an electric field was less than 100% (Fig. 2). This reflects the fact that the cell population was heterogeneous with respect to the mobility of lectin receptors. For any given cell exhibiting receptor mobility the per cent asymmetry between the cellular borders facing the negative and positive poles was close to 100% (Fig. 1).

To measure the lateral mobility of ligand-bound receptors, free receptors were made to accumulate at one cell pole. The fluorescent lectins were then added immediately after removal of the electric field and the ligand-bound receptors were allowed to diffuse for various time intervals before the cells were fixed for microscopic examination. In all cases studied (Con A receptors from all cell lines used and ricin receptors from C_6 cells), no noticeable redistribution occurred within 35 min (Fig. 1*b*). We considered that this could mean the ligand–receptor complexes on the cells had become immobilised. To confirm this suggestion, the cells were treated with the ligand before electrophoresis, but there was no asymmetric redistribution of the receptors (Fig. 1*c*). This last result agrees with the observation of Poo *et al.*[8] for Con A receptors of frog muscle cells. In contrast, the use of the FPR technique to measure the lateral mobility of the lectin–lectin receptor complexes gave strikingly different results. The apparent diffusion constant ($5\text{–}6\times10^{-11}\ cm^2\ s^{-1}$, Table 1) was only 5 times less than the diffusion constant of the free receptors as determined by the electrophoretic technique. Values of D did not change during at least 2 h of measurement at room temperature in phosphate-buffered saline (PBS).

We can resolve this apparent discrepancy by suggesting that after interaction with the ligands the mobility of the receptors becomes restricted in specific regions. To investigate this suggestion, we carried out successive bleachings of tetramethylrhodamine isothiocyanate (TRITC)–Con A complexes of the same region of the membrane of NS-20 cells (Fig. 3). If the receptors have complete mobility, we would expect (after the third bleaching) ~90–100% recovery of fluorescence ($\%R$) (almost all the immobile molecules in the spot should be bleached with a bleaching parameter $K\simeq5$), but if receptor mobility is restricted fluoresence recovery should be substantially less than this. The results obtained (mean $\%R$ value after third bleaching $\simeq50\%$, nine experiments) support the suggestion that mobility of the receptors occurs in limited regions. Although the topology of the fluid stationary 'fields' in which these complexes move is unknown, the area of the fields seems to be of the order of the area of the laser beam ($\sim10\ \mu m^2$).

Cross-linking of receptors by multivalent lectins does not seem to be a major factor in the process of ligand-induced receptor immobilisation, because (1) receptor immobilisation could be induced using ricin R 60 (60,000 molecular weight form) which has only one binding site for sugar, and (2) the polyene antibiotic amphotericin B suppressed ligand-induced immobilisation of Con A and ricin receptors from C_6 and NS-20 cells[15]; amphotericin B did not affect lectin binding to receptors when added either before or after Con A or ricin and therefore cannot interfere with eventual receptor cross-linking.

We suggest that the appearance of these fields reflects a phase separation of membrane lipids which is triggered by ligand–receptor interaction[16–18]. There is evidence that a specifically

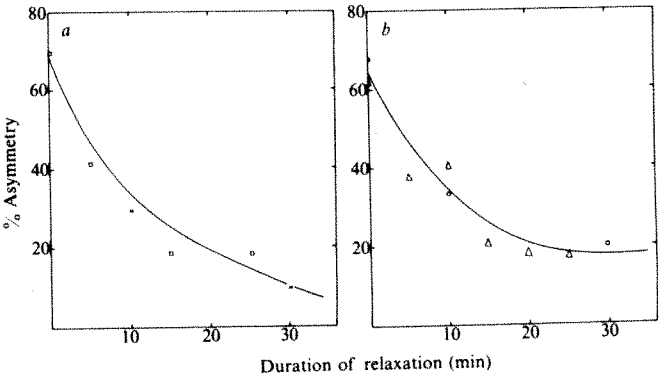


Fig. 2 Recovery of asymmetric distribution by relaxation. After removal of the field, the cells remained in the medium at room temperature for various periods of time before TRITC–lectin labelling and paraformaldehyde fixation. Per cent asymmetry $=(A-B/T)100\%$, where A and B are the number of cells showing asymmetric distribution of fluorescence towards the positive and negative poles, respectively, and T is the total number of cells examined. (Total number examined for each point was not less than 80.) In the case of long cells, we examined only cells which had their long axis aligned within 30° with respect to the direction perpendicular to the direction of the field. *a*, The relaxation of Con A receptors of NS-20 cells; the mean diameter of NS-20 cells was 14 ± 0.5 μm. *b*, The relaxation of Con A receptors of C_6 cells after treatment with colchicine, 5 $\mu g\ ml^{-1}(0)$ and 1 $\mu g\ ml^{-1}(x)$; the mean diameter of the C_6 cells was 16 ± 0.5 μm.

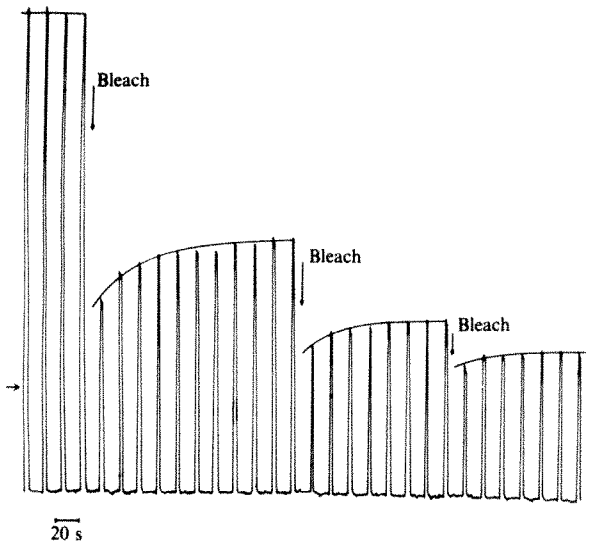


Fig. 3 Recorder trace of the fluorescence intensity of a succesively bleached spot on TRITC–Con A-labelled NS-20 cells. Values for the recovery of fluorescence after the 1st, 2nd and 3rd bleachings were 21%, 40% and 43%, respectively. The modulation was made with an optic-acoustic modulator (SORO). The intensity of fluoresence below the horizontal arrow corresponds to light scattering.

immobilised boundary layer of lipid surrounds the integral membrane proteins. Interaction of a membrane protein with a specific ligand might modify its interaction with surrounding lipids through conformational changes[18–20], or, as recently suggested by Hirata *et al.*[21] in the case of β-adrenergic receptors, through enzymatic conversion and catalysed 'flip flop' of membrane phospholipids in the vicinity of the receptor. In addition, changes in lipid fluidity after interaction of lectins with the membrane have been demonstrated[22]. A change in the lipid environment of lectin–lectin receptor complexes might facilitate their aggregation, leading to the occurrence of clusters surrounded by a more fluid zone of the membrane co-existing with the remaining pool of the membrane lipids and proteins. Our experimental results obtained with lectins at 20°C indicate that this state could correspond to a steady-state dynamic equilibrium.

We believe that the simplest way to explain this 'stationary phase' separation is to suggest a key role for cholesterol in the process. Cholesterol plays a critical part in creating a generally fluid phase, which would separate into liquid and gel phases if cholesterol were not present[23,24]. Cholesterol has a preference for some lipids (for example, lecithin) and a tendency for self-aggregation, but has no affinity for proteins[24]. Therefore, during microaggregation of receptors, cholesterol, which could have a preference for the new 'coat' lipids (like lecithin) of the receptors, could move from the 'common' membrane pool into the cavity created by the aggregated proteins, and the region external to the restriction zone could be the phase of the lipids with which the cholesterol has weak interactions (like phosphatidylethanolamine)[25] and which could be stabilised by Ca^{2+} ions[24]. This could enable restricted zones of the membrane to be in equilibrium with the remaining membrane pool of lipids and proteins. Suppression of ligand-induced receptor immobilisation by amphotericin B, which specifically interacts with cholesterol (refs 15, 26) supports the proposed interpretation.

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(DTrp^6-Pro^9-NEt)-luteinising hormone-releasing factor inhibits follicular development in hypophysectomised rats

THE super-luteinising hormone-releasing factors (super-LRFs) are a group of synthetic analogues at least 100 times more potent than the native peptide *in vitro* and *in vivo*[1]. Several laboratories have demonstrated inhibitory effects of super-LRFs on ovulation, egg implantation, pregnancy, testicular weight and spermatogenesis[2–6]. Their proposed mechanisms of action involve some type of desensitisation of the pituitary to the highly potent LRF analogue and of the gonads to the gonadotropins released in large quantities by the super-LRF, in both target tissues through receptor down-regulation. Another, seemingly unlikely, explanation for the gonadal inhibitory effects of super-LRFs is that the synthetic peptide acts directly at the level of follicular ovarian tissues. Such a direct effect of super-LRF on the gonads has been suggested by the observation that large doses of LRF prevent cultured granulosa cells from aromatising androgens to oestrogens[7]. We now report that a super-LRF inhibits follicular development in hypophysectomised rats pretreated with pregnant mare serum gonadotropin.

Pregnant mare serum gonadotropin (PMSG) has predominantly FSH-like (follicular-stimulating hormone) activity and some LH-like (luteinising hormone) activity, and induces ovarian follicular development in intact as well as hypophysectomised immature rats[8]. Various doses of PMSG dissolved in 0.2 ml of 16% gelatin were given subcutaneously (s.c.) to 26-d-old female rats (from Hormone Assay, Chicago) approximately 24 h after hypophysectomy by the parapharyngeal route. Various amounts of the super-LRF, (DTrp^6-Pro^9-NEt)-luteinising hormone-releasing factor (pGlu-His-Trp-Ser-Tyr-DTrp-Leu-Arg-Pro-N.ethylamide) dissolved in 0.1 ml physiological saline were given s.c. at 8 a.m. and 5 p.m. daily for 3 d. The ovarian weights were recorded on day 31 as the endpoint. In some groups 20 IU of HCG (human chorionic gonadotropin) in 0.25 ml of physiological saline was given

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intraperitoneally at 1.30 p.m. on day 30 and the incidence of ovulation was determined as reported previously[9]. The sella turcica of each rat was examined under magnification to determine whether the entire pituitary had been removed. The few rats in which pituitary fragments were found were not included in the results. The multiple comparison tests of Dunnett and Duncan for significance of differences were used for statistical evaluation of the experiments after an analysis of variance (computer program EXBIOL).

Administration of various doses of PMSG in 16% gelatin, a vehicle which delays absorption, produced a dose-dependent increase in ovarian weight (Table 1). Injection of the super-LRF inhibited the ovarian follicular development induced by PMSG. In rats treated with 50 IU PMSG, the minimum effective dose of super-LRF to prevent follicular induction was 0.08μg twice daily. A similar amount of native LRF or another analogue of LRF with D-amino acid substitutions [(DPhe^2-DPhe^6)-LRF] given in the same manner did not inhibit follicular development in animals pretreated with 50 IU PMSG, suggesting that the inhibition was not due to chronic injection of any decapeptide with or without a D-amino acid substitution. Experiments were also carried out to determine the responsiveness of these PMSG-primed follicles to HCG in animals concomitantly injected with the super-LRF nonapeptide: none of the animals treated with super-LRF ovulated, whereas treatment with HCG-PMSG without super-LRF resulted in the well known super-ovulation (Table 2).

These results clearly show that an analogue of the hypothalamic LRF, the super-agonist (DTrp^6-Pro^9-NEt)-LRF, has a direct effect on the ovary *in vivo*, inhibiting the follicular maturation normally induced by large doses of PMSG. Hseuh and Erickson have reported *in vitro* inhibitory effects of large doses of LRF on the aromatisation of androgens to oestrogens[7] by granulosa cells in monolayer cultures. Oestrogens are known to stimulate follicular maturation[10]; granulosa cells from medium-sized and small follicles do not grow or luteinise unless they are first primed with both FSH and LH[11], but oestrogens seem to facilitate stimulation of the mitosis of granulosa cells which is primarily responsible for follicle growth[10–12]. In the experiments reported here, no physiological responses to oestrogen secretion, such as extended uterine horns, increase in ovarian weight, and presence of proestrous vaginal smears and vaginal opening, were observed in super-LRF-treated rats whereas all these criteria were observed in the PMSG control animals. Thus, super-LRF itself seems to have adverse effects on steroidogenesis *in vivo* and consequently interferes with follicular development.

Table 1 Inhibition by super-LRF of follicular development in hypophysectomised rats

Treatment				*n*	Average ovarian weight ± s.e. (mg)
PMSG (IU)	Super-LRF (μg)	LRF (20 μg)	(DPhe^2-DPhe^6) LRF (20 μg)		
—	—	—	—	6	15.8 ± 0.9
12	—	—	—	6	16.8 ± 2.3‡
12	20	—	—	4	6.8 ± 0.6‡
12	1.2	—	—	3	6.2 ± 0.4*
12	0.3	—	—	4	8.0 ± 0.6*
12	0.08	—	—	3	8.2 ± 1.1†
12	0.02	—	—	5	16.5 ± 2.0‡
25	—	—	—	8	42.5 ± 2.4
25	20	—	—	7	7.1 ± 0.7*
25	5	—	—	4	8.5 ± 1.0*
25	1.2	—	—	4	10.3 ± 0.8*
25	0.3	—	—	4	17.4 ± 2.5*
25	0.08	—	—	4	36.4 ± 3.7‡
50	—	—	—	14	69.4 ± 5.7
50	—	—	+	5	83.1 ± 5.2‡
50	—	+	—	5	79.2 ± 7.9‡
50	20	—	—	12	13.6 ± 0.8*
50	5	—	—	5	7.0 ± 0.3*
50	1.2	—	—	5	10.4 ± 1.4*
50	0.3	—	—	7	24.4 ± 2.5*
50	0.08	—	—	8	49.7 ± 7.4*
100	—	—	—	4	93.5 ± 7.5
100	20	—	—	4	8.5 ± 1.6*
100	5	—	—	3	29.3 ± 2.0*
100	1.2	—	—	3	44.7 ± 2.7*
100	0.3	—	—	4	85.2 ± 11.8‡

Super-LRF [(DTrp^6-Phe^8)-LRF] at various doses, 20 μg LRF, or (DPhe^2-Phe^6)-LRF in 0.1 ml physiological saline were given s.c. at 8 a.m. and 5 p.m. for 3 d. Significance from PMSG control group: *$P < 0.01$; †$P < 0.05$; ‡ not significantly different (multiple comparison test of Dunnett). Applies also to Table 2.

Table 2 Inhibition of ovulation by super-LRF in hypophysectomised rats

Treatment			No. of rats ovulated total no. of rats	Average ovarian weight ± s.e. (mg)	Average no. of ova/per ovulating rat ± s.e.
PMSG (IU)	Super/LRF (μg)	HCG (μg)			
25	—	—	0/4	42.5 ± 2.4	—
25	20	—	0/3	5.7 ± 0.3*	—
25	—	20	4/4	56.1 ± 3.3‡	15.6 ± 0.8
25	20	20	0/6	6.7 ± 0.8*	—
50	—	—	0/4	75.7 ± 2.4	—
50	20	—	0/5	19.9 ± 0.9*	—
50	—	20	5/5	112.5 ± 6.0†	29.0 ± 0.4
50	20	20	0/5	21.1 ± 1.2*	—

Super-LRF (20 μg per 0.1 ml per injection) was given s.c. at 8 a.m. and 5 p.m. for 3 d.

The inhibitory effect of super-LRFs has been suggested to be due to down-regulation of gonadotropin receptors in the gonads resulting from the extremely high level of gonadotropins induced by the super-LRFs[6,14]. Regulation of gonadotropin receptors in the gonad is highly sensitive to the circulating gonadotropin concentration, a marked loss of receptor sites occurring after treatment with exogenous gonadotropins. Through such a negative regulation of gonadotropin receptors and desensitisation of gonadal responses, super-LRFs would adversely affect ovarian steroidogenic function[6], ovulation[4] and growth of endocrine-dependent mammary tumours[15]. Indeed, Catt *et al.* have reported that the gonadotropin receptor content of the gonad exhibited delayed and protracted changes after single doses of super-LRF[14], and binding sites were totally abolished after treatment with 10 μg LRF. When the pituitary gland is removed as we report here, super-LRF must inhibit gonadal response to exogenous gonadotropin by acting directly at the gonadal level; this suggests that down-regulation of gonadotropin receptors may not be the only interpretation.

Judging from our observations that super-LRF directly inhibits follicular development, presumably due to interference with steroidogenesis, it is obvious that super-LRF may either bind to granulosa cells to alter the gonadotropin receptors or antagonise the subsequent secretory step of gonadotropin (PMSG) molecules by binding to the ovarian receptor site. Such a direct action of super-LRF on gonads and possibly on other reproductive organs, including the uterus, to explain its effects on egg implantation[6,16], would lead to the possibility of the local administration of super-LRF as a totally novel approach to contraceptive medication. Furthermore, we should not neglect the possibility that the inhibitory effects of super-LRFs on oestrogen-dependent mammary tumours could similarly be explained by or related to such a direct effect of the super-LRFs on the mammary tissues. Moreover, these observations and data reported by others[11–13] suggest that the gonad may contain and possibly secrete substances that might be included in a multi-step feedback system involving hypothalamic–pituitary peptides. Indeed, recent data from this laboratory have shown the presence in aqueous extracts from PMSG-stimulated ovaries, of both LRF-like and LRF-antagonistic activities, presumably of peptidic nature[17].

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Endogenous opiates block dopamine inhibition of prolactin secretion *in vitro*

OPIATES stimulate prolactin secretion *in vivo*; plasma prolactin levels are increased by morphine administration in normal[1] and steroid-primed male rats[2], as well as in immature[3] and oestrogen-treated female rats[4]. Met-enkephalin and β-endorphin were also stimulatory[1–6] as were enkephalin analogues[3,7,8]. Naloxone or naltrexone, specific opiate antagonists, reduce basal prolactin levels when given alone[1,3,9], and block, at least partially, the effect of opiate agonists[1–4,7,8]. None of these drugs is active *in vitro* when tested alone on anterior pituitaries[3] or dispersed pituitary cells[2,4]. However, as we have previously shown in a preliminary experiment, the inhibitory effect of dopamine on prolactin secretion *in vitro* was antagonised by the addition of morphine to the incubation medium[10]. In the present work, we have attempted to clarify further the mode of interaction of opiates with prolactin secretion. We confirmed that morphine, Met-enkephalin and β-endorphin do not affect the spontaneous release of prolactin *in vitro*, but found that they suppress the inhibitory effect of dopamine on prolactin release. We have also characterised the specificity and the kinetics of this interaction.

To demonstrate the effect of these substances, paired control and experimental hemi-pituitaries of male rats (Wistar, Evic Seba) were incubated, under constant gassing (95% O_2, 5% CO_2), in medium 199 HEPES (Gypco) containing bacitracin (2×10^{-5} M) to prevent enzymatic degradation of peptides[11] and ascorbic acid (10^{-4} M) to protect dopamine against oxidation. Incubation time was 1 h following two 45-min preincubation periods. Both the incubation medium and extracts of the pituitary tissue were assayed for prolactin content against NIAMDD rat RP1 standard. Results are expressed as prolactin released into the medium as a percentage of prolactin content of the pituitary, as a good statistical correlation has been found between these two prolactin values[12].

Table 1 shows that morphine (10^{-6} M), Met-enkephalin (10^{-6} M), β-endorphin (5×10^{-7} M) or naloxone (10^{-6} M) alone did not affect basal prolactin secretion *in vitro*. As expected, dopamine (10^{-7} M) strongly inhibited the secretion of prolactin from hemipituitaries. The three opiates tested significantly

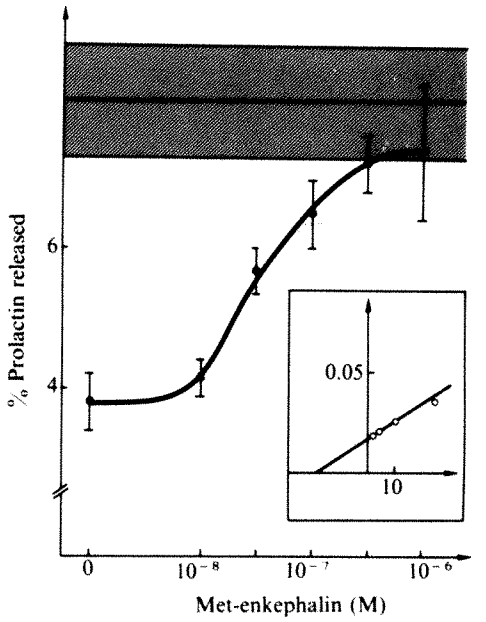


Fig. 1 Dose–response curve for the effect of Met-enkephalin on dopamine inhibition of prolactin release. The shaded area corresponds to the mean prolactin release of control pituitary ± s.e.m. Data are expressed as the percentage of the hormone content of the tissue released into the medium. Inset, Lineweaver–Burk plot of the data; ordinate: reciprocal of prolactin released; abscissa: reciprocal of Met-enkephalin concentration (μM^{-1}). Each value is the mean ± s.e.m. of 16 determinations. Average prolactin release of corresponding controls was 414 ± 36 ng ml^{-1}. In the presence of dopamine (10^{-7} M) increasing concentrations of Met-enkephalin progressively restored prolactin release to control levels.

blocked the dopamine inhibition. Their action could be antagonised by naloxone, which had no direct effect on the inhibition of prolactin secretion by the amine.

The effect of Met-enkephalin and β-endorphin on dopamine inhibition of prolactin release is dose dependent (Figs 1, 2). In the case of Met-enkephalin we were able to calculate kinetic constants for this interaction according to the method of Parker and Wand[13]. The apparent affinity was $4.1\pm0.8\times10^{-8}$ M. Complete reversal of dopamine inhibition occurred when 10^{-6}M Met-enkephalin was added to the incubation medium. The Hill constant calculated from the Met-enkaphalin inhibition curve ($n = 1.2\pm0.3$) was indicative of simple Michaelis–Menten kinetics. A similar result was obtained using the Lineweaver–Burk[14] plot of the data (Fig. 1), giving an apparent affinity of 4.5×10^{-8} M. Naloxone antagonised the effect of Met-enkephalin on dopamine inhibition in a dose-related manner (Table 2). The dopamine effect antagonised by 10^{-7} M Met-enkephalin was completely restored in the presence of 10^{-7} M naloxone. Another opiate antagonist, Mr 2266 (ref. 15), had a similar effect when added to the medium at a concentration of 10^{-7} M. In contrast, the optical isomer of this antagonist, Mr 2267 (ref. 15), was ineffective in the same conditions (Table 2).

We have thus confirmed previous data showing that incubation of pituitaries with opiates does not affect spontaneous prolactin release. This has led several authors to postulate that the morphinomimetic peptides may interact with prolactin regulating mechanisms at a higher level than the pituitary, possibly by modulating the turnover rate of tubero-infundibular dopaminergic neurones[16]. Although the present data do not exclude this hypothesis, they demonstrate that the prolactin response to opiates can be accounted for by a direct interaction of these substances with the effect of dopamine at the level of the pituitary prolactin cell.

Opiates do not affect dopamine receptors, as shown by ^{3}H-dihydroergocryptine binding to pituitary membrane preparations[17]; naloxone, morphine, Met-enkephalin and β-endorphin

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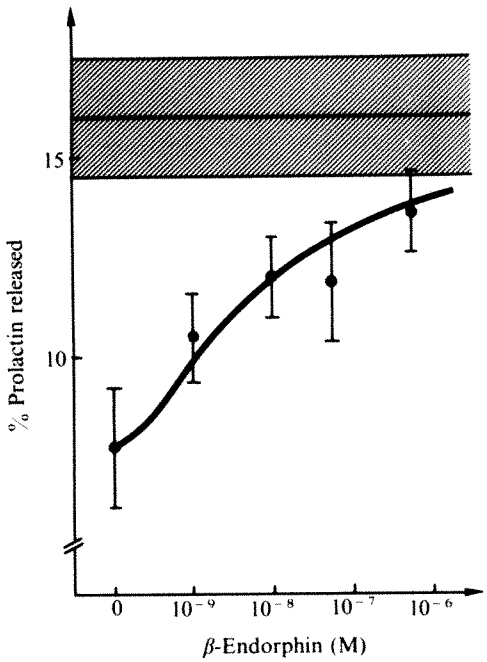


Fig. 2 Dose–response curve for the effect of β-endorphin on dopamine inhibition of prolactin release. Details as in Fig. 1 legend. Each value is the mean ±s.e.m. of eight determinations. Average prolactin release of corresponding controls was 709 ± 59 ng ml^{-1}.

do not affect ^{3}H-spiroperidol binding to striatal membranes[18]. Furthermore, in our experimental conditions, naloxone had no effect by itself on the dopamine-induced inhibition of prolactin release. We conclude from these results that two independent receptors mediate the action of dopamine and opiates on the pituitary.

Interactions between opiates and dopamine have already been described in other structures of the central nervous system[19,20]. In the case of the pituitary, reversal of the opiate effect by Mr 2266 or naloxone suggests that this interaction with dopamine is mediated by specific opiate receptors. In addition, as already reported for studies using different conditions[15,21], ineffectiveness of the optical isomer Mr 2267 to antagonise the action of Met-enkephalin demonstrates that the interaction of Mr 2266 with opiate receptors is stereospecific. Suppression of the opiate effect with low doses of antagonists also suggests that a specific opiate receptor is involved; interaction with other receptors only occurs with much higher concentrations of antagonists. For example, naloxone displaces ^{3}H-GABA binding in the cerebellum with an IC_{50} of 3×10^{-4}M (ref. 22). *In vivo* experiments using several enkephalin analogues also suggested that the effect of prolactin release is mediated by a specific opiate receptor[7,8].

Binding sites for morphinomimetic peptides have been identified in both the central and the peripheral nervous systems[23,24]. The affinity of various opiate antagonists depends on the structure on which the receptor is located. Opiate receptors have been identified in the pituitary[25], where the receptor density is much lower in the anterior lobe than in the neurohypophysis. The small proportion of prolactin cells in normal male pituitaries where, according to the present data, these receptors might be located, may account for the small number of receptors. The affinity of opiates for this receptor was lower than that calculated for central receptors which is in the range of 10^{-9} M. Our results agree with these data, for the apparent affinity for Met-enkephalin in our system is approximately 4×10^{-8} M.

Endogenous opiates, released either by the median eminence into the hypothalamo-hypophyseal portal system or by the pituitary into the general circulation, may be involved in the physiological regulation of prolactin secretion, as stress-induced prolactin release is blocked by naloxone[26,27] and naltrexone[9]. The opiate–dopamine interaction at the hypophyseal level which we have demonstrated may account for this physiological regulation.

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Table 1 Interaction of morphine, Met-enkephalin, β-endorphin and/or naloxone with basal and dopamine-inhibited release of prolactin from incubated hemi-pituitaries

	Naloxone	
	0	10^{-6} M
Control	15.1 ± 0.8	14.5 ± 0.7
Dopamine 10^{-7}M	8.1 ± 1.5*	9.0 ± 1.0*
Morphine 10^{-6}M	14.6 ± 0.9	
Dopamine + M	14.1 ± 1.4†	10.6 ± 1.0§
Met-enkephalin 10^{-6}M	14.4 ± 1.0	
Dopamine + Met-enkephalin	13.9 ± 1.6†	9.4 ± 1.1§
β-endorphin 5 + 10^{-7}M	14.8 ± 1.1	
Dopamine + β-endorphin	14.0 ± 1.0‡	9.2 ± 1.4§

Prolactin release is expressed as per cent of the hormone content of the tissue. Significance of the differences was assessed by variance analysis: *, highly significant inhibition as compared with controls; †, significant, or ‡, highly significant interaction of opiate agonists with dopamine inhibition; §, significant effect of naloxone as compared with corresponding dopamine + opiate agonist-treated hemi-pituitaries. Each value is the mean ± s.e.m. of eight determinations. Average prolactin release of corresponding controls was 722 ± 50 ng ml^{-1}.

Table 2 Dose dependence and stereospecificity of the effect of opiate antagonists on Met-enkephalin interaction with dopamine-inhibited prolactin release

Dopamine	Opiate antagonists	Met-enkephalin 0	Met-enkephalin 10^{-7} M
0	0	11.2 ± 1.1	—
10^{-7} M	0	6.2 ± 0.5*	9.3 ± 0.2‡
0	Naloxone 10^{-8} M	10.2 ± 0.9	—
10^{-7} M	Naloxone 10^{-8} M	—	9.5 ± 1.2
0	Naloxone 10^{-7} M	10.8 ± 0.7	—
10^{-7} M	Naloxone 10^{-7} M	—	6.7 ± 0.9§
0	Naloxone 10^{-6} M	10.8 ± 0.8	—
10^{-7} M	Naloxone 10^{-6} M	6.7 ± 0.5*	7.3 ± 0.5§
0	0	14.0 ± 0.7	—
10^{-7} M	0	8.1 ± 0.8*	11.0 ± 0.6†
10^{-7} M	Mr 2266 10^{-7} M	—	8.2 ± 0.9§
10^{-7} M	Mr 2267 10^{-7} M	—	11.6 ± 0.3

Increasing doses of naloxone restored dopamine (10^{-7} M) inhibition in the presence of Met-enkephalin (10^{-7} M). Another opiate antagonist, Mr 2266, was only effective at a concentration of 10^{-7} M, whereas its optical isomer Mr 2267 was inactive. Statistical data are indicated with the same symbols as in Table 1. Each value is the mean ±s.e.m. of eight determinations. Average prolactin release of corresponding controls was 393 ± 39 ng ml^{-1}.

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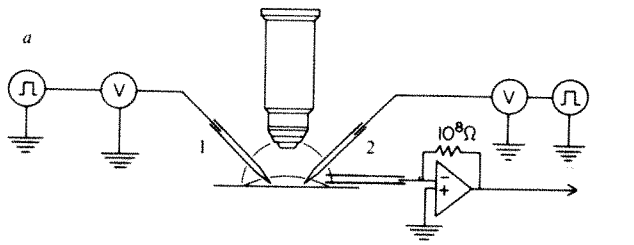
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Electrical properties of giant mitochondria studied with a double impalement technique

THE electrical properties of the membrane of giant mitochondria (4–10 μm in diameter) can be studied directly with microelectrodes. Tupper and Tedeschi[1–4] have studied mitochondria isolated from *Drosophila* (approximately 4 μm in diameter), and found a positive potential in the range of 5–15 mV and a specific resistance in the range of 1–4 Ω cm^2 (assuming no invaginations of the mitochondrial membrane). The potentials were not found to depend on metabolic state[2]. The microelectrodes were judged to be in the internal mitochondrial space from the fact that the potentials were osmotically sensitive (decreasing with osmotic swelling in a predictable manner). The positive potentials correlated with the distribution of carboxylic anions, which partition with a higher concentration inside the mitochondria. Furthermore, the potentials were collapsed by the addition of acetate[3,4], but not chloride[4] to the external medium. Presumably acetate enters and depolarises the potential. These observations further support the idea that the electrodes are in the inner mitochondrial space.

More recently, we have carried out studies with giant mitochondria from mice maintained on a diet containing cuprizone[5–8]. The isolated giant mitochondria seem to be in good physiological condition, as shown by the respiratory control of single isolated mitochondria[9], the P:O ratios of the mitochondrial suspensions[7,10], the direct bioassay of the ATP produced by single mitochondria or the ability to accumulate calcium phosphate[7,11]. The potentials were found to be in the range of 10–20 mV (positive inside) over a wide range of ionic composition of the medium. The specific resistances were found to be in the range of 1–5 Ω cm^2 (neglecting the membrane invaginations). The microelectrodes were in the inner mitrochondrial space as shown by the following criteria[6]. (1) In the presence of valinomycin, the potentials reversed in polarity and became sensitive to the K^+ concentration of the medium. Over a range of external K^+ concentrations, the measured potentials followed the Nernst equation approximately. (2) Almost identical results were obtained with mitochondria from which the external membrane was removed. (3) In a variety of conditions, advancing the electrode through the mitochondrion revealed a single potential, as long as the microelectrode had not emerged to the other side.



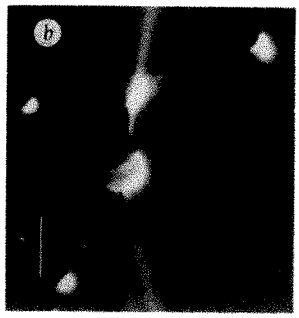


Fig. 1 *a*, Diagrammatic representation of the experimental design. 1 and 2 correspond to the electrodes used. The resistor shown is 10^7 or 10^8 Ω. The capacitor corresponds to 0–20 pF. *b*, A doubly impaled mitochondrion seen with Nomarski optics, water immersion objective (see ref. 6). The bar indicates 10 μm.

The measured positive potentials cannot be ascribed to artefacts involving the electrode tip, as the results were the same, regardless of whether the microelectrodes were loaded with KCl, NaCl (ref. 8) or potassium acetate (unpublished results).

Individual, intact or impaled mitochondria were found to be metabolically viable by two kinds of assays[7]. In one assay, the individual mitochondria were shown to exhibit granules in conditions in which mitochondria were known to accumulate massive amounts of calcium phosphate. The granulations were shown to correlate with increases in dry mass. They were interpreted to correspond to sites of accumulation of calcium phosphate. In another assay, individual mitochondria were shown to be capable of inducing the contraction of glycerinated myofibrils in close proximity to the mitochondrion (in some cases in the absence of other mitochondria[11]). Both the apparent

Table 1 Resistances after using the techniques represented in the figures

Experiments	Experimental design	Resistances (MΩ)
Series 1		
1	Single impalement (*b*)	3.7 ± 1.1 ($n = 12$)
2	Double impalement; measurement with the electrode delivering the current (*c*)	1.9 ± 0.8 ($n = 12$)
3	Double impalement; measurement with the electrode not delivering the current (*c*)	2.0 ± 1.1 ($n = 12$)
Series 2		
1	Single impalement (*b*)	3.8 ± 2.6 ($n = 6$)
2	Double impalement; measurement with electrode* delivering the current (*c*)	2.9 ± 2.5 ($n = 6$) 3.2 ± 2.0 ($n = 6$)
3	Double impalement; measurement with electrode* not delivering the current (*c*)	4.6 ± 2.4 ($n = 6$) 4.5 ± 2.2 ($n = 6$)

Values listed represent means ± s.d. The letters correspond to the diagram of Fig. 2. For series 1 the mitochondria were in 0.30 molal sucrose, 40 mM KCl, 1 mM $MgCl_2$, 3 mM sodium succinate, 2 mM potassium phosphate and 5 mM HEPES, *p*H 7.3. Excluding one value (−13 mV) for series 1, the membrane potentials were 1.2 ± 1.8 mV. For series 2, the mitochondria were in 0.30 molal sucrose, 10 mM KCl and 5 mM HEPES, *p*H 7.3. Only one membrane potential (+5 mV) was recorded in series 2.

* The induced potential was recorded from both electrodes.

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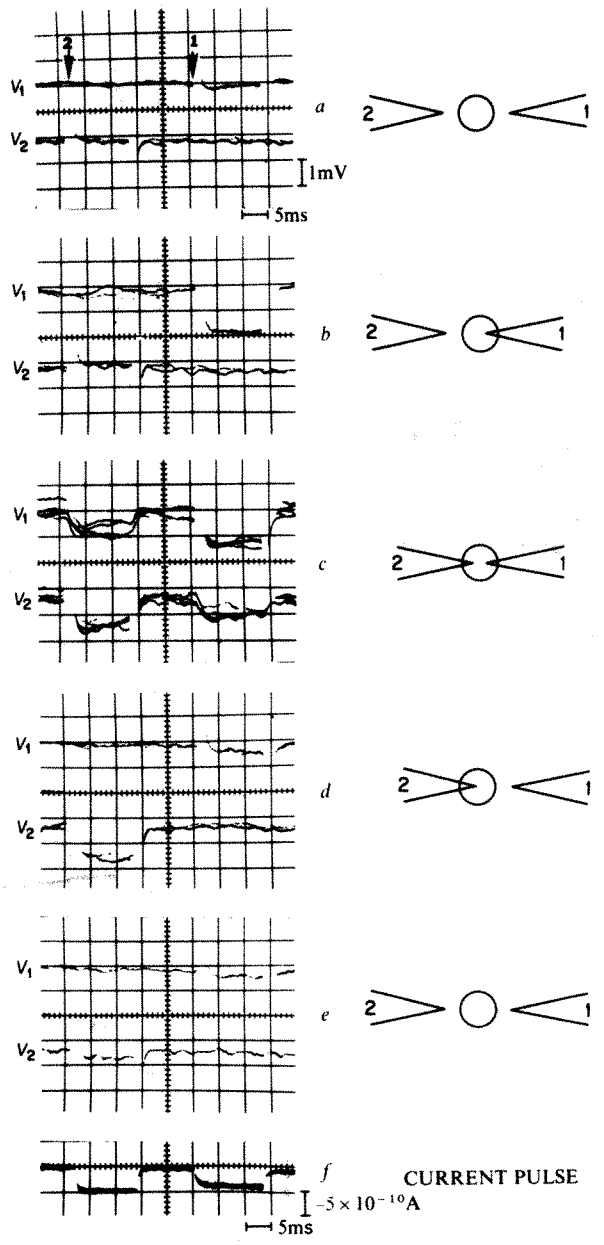


Fig. 2 The position of the electrodes and the electrical recording from a mitochondrion. The oscilloscope tracings are indicated on the left. The arrows indicate the start of the current pulses. The contrast has been reversed for ease in viewing. The corresponding location of the microelectrodes are indicated by the diagrams on the right. The microelectrodes are conventional micropipettes filled with 2 M KCl. The ground electrode consisted of a glass pipette containing 2 M KCl in 2% agar with a Ag–AgCl wire inside and connected to the virtual ground amplifier. Two electrometers (W-P Instruments, model M4A) with a minimum input resistance of 10^{10} Ω were used to pass current and simultaneously record potentials. The bath was held at virtual ground with the reference electrode by means of a current to voltage amplifier (Burr-Brown, operational amplifier, model 3308/12C) using a feedback resistor of either 10^7 or 10^8 Ω (wire wound, Victoreen). The output of this amplifier was monitored by a Tektonrix oscilloscope model 5110, using a 5A20N differential amplifier and a 5B10N time base (Tektronix). The output of electrometers was recorded with a Tektronix storage oscilloscope type R 564 B using a type 3A3 dual-trace differential amplifier and a type 2B67 time base. Current was injected using two type 161 Tektronix pulse generators controlled by a type 162 waveform generator. In this experiment negative current pulses of -4×10^{-10} A were delivered from electrode 1 and -3.5×10^{-10} A from electrode 2. The mitochondrion (8 μm in diameter) was in 0.3 molal sucrose, 10 mM KCl and 5 mM HEPES, *p*H 7.3 (≈25 °C).

accumulation of calcium phosphate and the contraction of the myofibrils were sensitive to the appropriate inhibitors or uncouplers of oxidative phosphorylation.

Despite the evidence summarised above, some investigators seem to harbour considerable reservation about the location of the microelectrodes. We have carried out a new series of experiments that bear on this question. Electrical tests of the location of microelectrodes in doubly impaled cells[12,13] or nuclei[14] have been in use for some time in electrophysiology. Characteristic voltage changes can be obtained with the delivery of current, depending on the location of the microelectrodes. In these experiments one microelectrode is used to deliver current and another to record potential. The experimental design allows one to distinguish the different possible locations of the microelectrodes from a record of the potential changes. In the case of cells, for example, one can distinguish whether both electrodes are outside the cell, both electrodes are pressed against the cells, (in the cases studied, the procedure actually produces deep invaginations where the electrodes are pressed against the cell surface (see Fig. 1 or ref. 12)), or both electrodes are inside the cell (see, for example, Fig. 4 of ref. 12). Only the latter experimental situation demonstrates a significant voltage response.

The microelectrodes and the pertinent circuits of our experiments are represented in Fig. 1*a*. The design allows recording and/or current delivery from either electrode. A doubly impaled mitochondrion is shown in Fig. 1*b*. The tracings correspond to oscilloscope records. Generally we used microelectrodes approximately 70 MΩ in tip resistance. In previous studies we have found that the results were independent of the tip resistance in the range of 10 to approximately 80 MΩ (refs 1 and 5). The records indicated in Fig. 2 were obtained after an initial balancing of the tip resistance. The impalements and withdrawals were sequential from *a* to *e*. *f* represents the current pulses, the first delivered from electrode 2 (-4×10^{-10} A), the second from electrode 1 (-3.5×10^{-10} A). In the oscilloscope record corresponding to the double impalement (*c*), five separate pulses have been superimposed. When both electrodes are in the external medium (*a* or *e*) there is no significant response from either electrode. We have found an absence of an induced potential at these current levels even when the electrodes are held at a distance well below 1 μm. With a single impalement (*b* and *d*), a response is obtained only from the impaling electrode. When both electrodes are in the mitochondrion (*c*) either electrode records the potential regardless of which delivers the current. Table 1 summarises two series of comparable experiments. The potentials across the mitochondrial membrane are in the legend to Table 1.

These experiments demonstrate that the two microelectrodes impaling a mitochondrion are in the same membrane-bounded space. Previous studies[6] have shown the same resistances in the presence and in the absence of the mitochondrial outer membrane, suggesting that in the present experiments the two electrodes are in the inner mitochondrial space.

These experiments and those previously presented[1-8] constitute evidence that the electrodes are in the mitochondrial matrix space and indicate the absence of a significant role of the membrane potential in the coupling of oxidation to phosphorylation. The details of a study on the membrane resistance of mitochondria using the double impalement technique will be presented elsewhere.

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Use of Panamanian sea urchins to test the molecular clock

THE 'molecular clock' hypothesis of protein evolution holds that each protein changes at a constant rate, so that the degree of molecular divergence between two species is linearly related to the time for which their lineages have remained separate[1]. This assertion, however, has been challenged repeatedly by authors who discovered taxa and peptides in which the proposed uniformity of molecular evolution did not hold[2,3], who noted that biochemically and palaeontologically determined dates of separation between lineages conflicted[4,5], introduced tests that pointed to significant variation in the rates of evolution of the same proteins[6,7], or dismissed the hypothesis as a confusion of averages with constants[8]. Others have postulated that, although the same proteins evolve at different rates in different lineages, the average amount of molecular change over many proteins is sufficiently uniform to provide approximate dates for the splitting of two lines of descent[9,10]. Here I present evidence from sea urchins separated by the Isthmus of Panama which indicates that even this compromise position is not tenable.

It is important to resolve this issue for two reasons: first, the assumption of a linear relationship between time since separation and degree of molecular divergence has been used extensively to assign dates to the splitting of two lineages on the basis of biochemical evidence[11–15] and second, the proposed constancy of rate of amino acid substitution has frequently been cited as the strongest evidence for the neutral mutation theory, which maintains that the major cause of molecular evolution is random fixation of selectively neutral mutations[16–19]. In spite of one model that tries to account for the phenomenon in terms of natural selection[20], the most parsimonious theoretical basis for such a uniformity of rates remains that proposed by Kimura[17]. If most amino acid substitutions were neutral, their rate of fixation would equal the mutation rate, and it would be constant over long periods of time, provided that mutations are random events.

Because of the uncertainties involved in assigning fossils to either branch of a phylogenetic tree if they lie close to the node, rate determinations that use dates based on palaeontological evidence have been challenged by both proponents[1,14,15,21] and opponents[22] of the molecular clock. The best way to test the clock hypothesis is to assess the relative amounts of divergence within two or more groups, all of which split into new lines of descent at the same time[1]. Such a situation holds for the species pairs of Panama[11], where the emergence of the Isthmus in the Pliocene[23–25] fragmented the ranges of many marine species simultaneously. Populations thus separated have become known as geminate species.

There are seven genera of shallow water regular echinoids in the Caribbean, each of which is also represented in the eastern Pacific by populations that have been assigned a different specific name. On morphological grounds the Atlantic and eastern Pacific members of each genus are presumed to comprise geminate pairs[26]. I compared three pairs: *Diadema antillarum* (Caribbean (C)) and *Diadema mexicanum* (Pacific (P)); *Eucidaris tribuloides* (C) and *Eucidaris thouarsi* (P); *Echinometra lucunter* (C) and *Echinometra vanbrunti* (P). *Echinometra viridis*, a species sympatric with *E. lucunter*, was also included, because of the uncertainty as to which of the two is the true geminate of their Pacific counterpart. Enzymatic proteins were compared by electrophoresis; 18 presumptive loci (15 for *Eucidaris*) were sampled for two populations per species. Trans-isthmian divergence is measured relative to differentiation between conspecific population on the same coast. Such a calibration corrects for the possibility that electrophoretically undetected heterogeneity[27] may be unequally distributed among the genera or that the enzymes assayed are a nonrandom sample of the existing rates of molecular evolution. Differences are quantified by Nei's[28] standard genetic distance.

The clock hypothesis predicts that the extent of molecular differentiation in all pairs should be equivalent. Clearly, this is not the case (Table 1). Pacific populations of *Diadema* have diverged from their Atlantic counterparts no more than they have from populations on the same coast. *Eucidaris* and *Echinometra*, on the other hand, exhibit trans-isthmian distances 16 and 37 times greater than intraspecific ones. Even if we take the conservative stand that intraspecific distances are roughly the same for all genera, we find that the values of Nei's index for inter-oceanic comparisons of *Echinometra* are more than 20 times larger than those for *Diadema*. This conclusion holds whether *E. vanbrunti* is compared with either *E. lucunter* or *E. viridis*.

It is unlikely that the determined dissimilarities in divergence between geminates in the three genera are artefacts of the limited resolving power of electrophoresis. If 'hidden variation' in *Diadema* were 20 times lower than in *Echinometra*, we would expect the former also to exhibit lower values of intraspecific differentiation and heterozygosity. No such trend is apparent (Tables 1, 2).

The 20-fold difference between *Echinometra* and *Diadema* is also too great to be the result of different times of isolation during the gradual closing of the portals connecting the two oceans. All three genera produce planktonic larvae[29], and cessation of gene flow between their members must have been almost simultaneous. Furthermore, although the duration of the actual geological event is unknown, there is a range of estimates for the date of final completion of the land bridge between North and South America; the highest currently accepted estimate is 3.5–5.7 Myr ago[23,30], and the lowest about 2 Myr ago[24,25]. If we place the separation of *E. vanbrunti* from *E. lucunter* (or *E. viridis*) at this date and accept that divergence is linearly related to time, the analogous event in *Diadema* would have to be dated at 100,000 to 285,000 yr ago, when the land bridge between North and South America was continuous[25]. Similarly, the postulate that *Diadema* split at the time the Isthmus rose would require the speciation event in *Echinometra* to have occurred between the Eocene and the Cretaceous. Such an early origin of the extant species, whether due to the emergence of the Isthmus or some other geological occurrence, is unlikely as, despite a good echinoid fossil record, the genus is unknown before the Pliocene in the eastern Pacific[31] and the Oligocene in the Caribbean[32].

The disparity of the genetic distance values is also too great to be due to the stochastic variation allowed by proponents of the clock[1,19]. Nor can it be the result of generation time[33], as *Diadema* reaches sexual maturity sooner than *Eucidaris* or *Echinometra*[34].

The possibility that the extant species of *Echinometra* and *Eucidaris* may have speciated after the rise of the Isthmus from true geminates which subsequently became extinct would not redeem the clock hypothesis as molecular clocks are based on the assumption that divergence is dependent on the time two lineages have remained separate and not on the number of speciation events in each.

Different amounts of divergence between the species of *Diadema*, *Eucidaris* and *Echinometra* must, therefore, reflect

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Table 1 Nei's standard genetic distance (D) between populations in each genus ± s.e.

		Atlantic				Pacific
		E. lucunter		*E. viridis*		*E. vanbrunti*
Species	Locality	Maria Chiquita	B. del Toro	San Blas Is	B. del Toro	P. Paitilla
E. lucunter	B. del Toro	0.009±0.004 (0.012±0.006)				
E. viridis	San Blas Is	0.117±0.075 (0.180±0.121)	0.109±0.073 (0.169±0.119)			
	B. del Toro	0.117±0.073 (0.177±0.120)	0.111±0.072 (0.172±0.119)	0.007±0.003 (0.008±0.005)		
E. vanbrunti	P. Paitilla	0.557±0.209 (0.655±0.288)	0.531±0.203 (0.649±0.290)	0.620±0.218 (0.771±0.303)	0.612±0.214 (0.790±0.304)	
	Isla Uva	0.561±0.213 (0.658±0.295)	0.547±0.209 (0.672±0.299)	0.666±0.233 (0.847±0.333)	0.653±0.225 (0.854±0.328)	0.021±0.011 (0.032±0.018)

E. lucunter–E. vanbrunti
Mean intraspecific genetic distance: 0.015 (0.022)
Mean transisthmian genetic distance: 0.549 (0.659)
E. viridis–E. vanbrunti
Mean intraspecific genetic distance: 0.014 (0.010)
Mean transisthmian genetic distance: 0.638 (0.816)

		E. tribuloides		*E. thouarsi*
		San Blas Is	B. del Toro	P. Paitilla
E. tribuloides	B. del Toro	0.016±0.014 (0.021±0.018)		
E. thouarsi	P. Paitila	0.292±0.143 (0.400±0.193)	0.307±0.150 (0.419±0.202)	
	Isla Uva	0.357±0.166 (0.480±0.221)	0.360±0.166 (0.482±0.221)	0.024±0.012 (0.028±0.015)

Mean intraspecific genetic distance: 0.020 (0.025)
Mean transisthmian genetic distance: 0.329 (0.445)

		D. antillarum		*D. mexicanum*
		Ft Randolph	B. del Toro	Isla Uraba
D. antillarum	B. del Toro	0.036±0.016 (0.038±0.019)		
D. mexicanum	Is Uraba	0.040±0.026 (0.052±0.040)	0.016±0.007 (0.023±0.011)	
	Isla Uva	0.039±0.017 (0.046±0.023)	0.008±0.004 (0.012±0.007)	0.015±0.007 (0.024±0.010)

Mean intraspecific genetic distance: 0.026 (0.031)
Mean transisthmian genetic distance: 0.026 (0.033)

Values in parentheses are those obtained from an analysis restricted to the 12 loci common to all three genera. Electrophoresis was carried out on 11% starch for all enzymes except amylase; 7% acrylamide gels were used for the latter. Standard histochemical tehcniques were used for staining. Collections were made at: Fort Randolph, Canal Zone (*D. antillarum*); Bocas del Toro, Republic of Panama (*D. antillarum, E. tribuloides, E. viridis, E. lucunter*); Maria Chiquita, Republic of Panama (*E. lucunter*); San Blas Islands, Republic of Panama (*E. viridis, E. tribuloides*); Isla Uraba, Bay of Panama (*D. mexicanum*); Punta Paitilla, Bay of Panama (*E. thouarsi, E. vantrunti*); Isla Uva, Gulf of Chiriqui (*D. mexicanum, E. thouarsi, E. vanbrunti*). Loci examined for each genus were: *Diadema*: phosphoglucomutase (PGM): PGM-1, PGM-2; phosphoglucose isomerase (PGI); hexokinase (HK); peptidases (Pep): Pep-1, Pep-2, mannose-6-phosphate isomerase (M6PI); NAD-dependent malate dehydrogenase (MDH): MDH-1, MDH-2, triose phosphate isomerase (TPI); leucine amino peptidase (LAP); amylase (Am): Am-1, Am-2; tetrazolium oxidase (TO); glucose-6-phosphate dehydrogenase (G6PDH); xanthine dehydrogenase (XDH); esterases (Est): Est-1, Est-2. *Echinometra*: PGM-1, PGM-2, PGI, HK, Pep-1, Pep-2, M6PI, MDH-1, MDH-2, TPI, Am-1, Am-2, Am-3, TO, G6PDH, XDH, LAP-1, LAP-2. *Eucidaris*: PGM-1, PGI, HK, Pep-1, M6PI, MDH-1, TPI, LAP, AM-1, AM-2, TO, XDH, Est-1, Est-2, Est-3. Sample sizes per locus (number of alleles) ranged from 36 to 134 (most above 90), with one, PGM-1, *E. thouarsi*, Isla Uva, represented by only six. See ref 39 for details of the methods.

Table 2 Average heterozygosity (H) (Nei's[28] gene diversity) of populations studied ± s.e.

Atlantic			Pacific		
Species	Locality	*H*	Species	Locality	*H*
E. lucunter	M. Chiquita	0.254±0.058 (0.278±0.080)	*E. vanbrunti*	Paitilla	0.216±0.056 (0.278±0.076)
	B. del Toro	0.259±0.058 (0.291±0.081)		Isla Uva	0.182±0.052 (0.229±0.073)
E. viridis	San Blas	0.202±0.065 (0.231±0.093)			
	B. del Toro	0.183±0.061) (0.232±0.087)			
E. tribuloides	San Blas	0.192±0.045 (0.214±0.050)	*E. thouarsi*	Paitilla	0.243±0.051 (0.288±0.056)
	B. del Toro	0.202±0.048 (0.221±0.053)		Isla Uva	0.111±0.036 (0.115±0.040)
D. antillarum	Ft Randolph	0.216±0.056 (0.276±0.072)	*D. mexicanum*	Uraba	0.201±0.056 (0.249±0.072)
	B. del Toro	0.162±0.051 (0.181±0.068)		Isla Uva	0.184±0.049 (0.217±0.063)

Values in parentheses are those obtained from an analysis restricted to the 12 loci common to all three genera.

dissimilar rates of protein differentiation, and argue against the molecular clock hypothesis. West (in preparation) has reached the same conclusion on the basis of allozymic differentiation in four geminate species pairs of Panamian crabs.

The results could still be compatible with the neutral mutation theory if they reflected differences between the genera in mutation rates or in histories of population size. Both these postulates, however, would conflict with the predictions of the same theory regarding the heterozygosity values of the populations involved.

Many models[35-37] predict that variability of neutral alleles is a function of the mutation rate. If *Diadema* has a mutation rate 20 times lower than *Echinometra*, it should also display less gene diversity. This is not the case (Table 2). Average heterozygosity values are very similar in populations belonging to the two genera. A severe population restriction more recent than 10^7 generations ago in *Echinometra* but not *Diadema* could also result in dissimilar rates of divergence compatible with the neutral mutation theory[38]. However, if the higher rates of divergence in *Echimometra* were the result of such a bottleneck, neutral theory would expect its populations in at least one ocean to exhibit lower levels of heterozygosity than those of *Diadema*[38]. This is not so (Table 2).

I conclude that the molecular clock hypothesis does not hold for the Panamian echinoids, and that their unequal rates of protein divergence with similar levels of heterozygosity are in direct opposition to the predictions of at least one model based on the neutral mutation theory. The most plausible explanation for the observed discrepancies is that molecules have been evolving under the influence of natural selection.

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Placental alkaline phosphatase in Hominidae

HUMAN placental alkaline phosphatase has several unique properties. It is stable to heating at 65 °C (ref. 1), is immunochemically distinct from the isoenzymes in other adult organs[2,3], and has a large number of allelic variants[4–6], with a 'degree of heterozygosity' much larger than that of any other human enzyme studied to date[7]. If placental phosphatase is specific to fetal development, it would be logical to expect that a similar isoenzyme would be present during fetal development in other mammalian species. This is not the case, however, as several species, including rhesus monkey, African green monkey and baboon, do not have in their placentae an isoenzyme which resembles human placental phosphatase[8]. The presence of the placental isoenzyme in humans therefore seems to be a late evolutionary event. To determine the species specificity of this enzyme, we have used the criteria which distinguish the human placental isoenzyme, including inhibition by 5 mM L-phenylalanine[1] and insensitivity to inhibition by 0.1 mM bromotetramisole[9], to compare the enzymes in species closely related to man. We report here that of the species studied, only the chimpanzee and orangutan have an isoenzyme resembling that in humans.

The enzyme assay used was as previously described[10]. Placental homogenates using full-term placentae were prepared using 3 ml of 0.01 M Tris-HCl, *p*H 7.6, per g of tissue. Antiserum used was prepared as described previously[11] and was obtained 10 weeks after primary immunisation. Enzyme staining was also as previously described[11].

Table 1 summarises the properties of the primate placental phosphatases. The enzymes from human, chimpanzee and orangutan placentae are similar in their activity, sensitivity to bromotetramisole and insensitivity to heat inactivation. The chimpanzee and human placental phosphatases are also very similar in their sensitivity to L-phenylalanine inhibition, the orangutan enzyme, less so.

The gorilla and spider monkey placental phosphatases were less active than those of the other three by two to three orders of magnitude, and were more sensitive to inhibition by bromo-

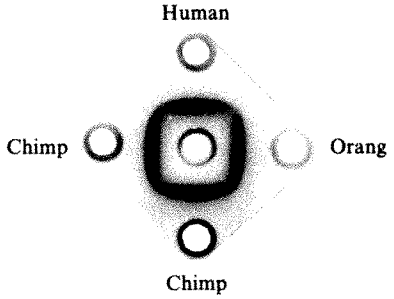


Fig. 1 Double diffusion of human, chimpanzee (chimp) and Bornean orangutan (orang) placental alkaline phosphatases against monospecific antiserum raised against human placental phosphatase. Each enzyme, 0.012 units, was placed into the appropriate wells and 10 μl of 1 : 8 diluted antiserum was placed in the centre well. Diffusion was allowed to proceed for 3 days at 37 °C, after which the gel was exhaustively washed with 0.01 M Tris-buffered saline, *p*H 7.6, and stained for enzyme.

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Table 1 Properties of alkaline phosphatases from several primates

	Activity (μmol min^{-1} ml^{-1})	Per cent inactivation by: Br-tet	L-Phe	Heat
Human	5.7	32±7	77±5	6±5
Chimpanzee	8.0	30±7	70±2	6±1
Lowland gorilla	0.01	80±2	31±7	77±4
Orangutan	2.7	35±12	45±10	15±3
Spider monkey	0.04	56±3	30±6	62±4

Br-tet, 0.1 mM bromotetramisole (Aldrich); L-Phe, 5.0 mM L-phenylalanine (Sigma); heat, heating for 10 min at 65 °C.

tetramisole, less sensitive to L-phenylalanine inhibition and markedly more readily inactivated by heat. The orangutan placental phosphatase was the least sensitive to L-phenylalanine inhibition.

Note that 20% of the lowland gorilla placental phosphatase and 38% of the spider monkey enzyme remained after heating for 10 min at 65 °C. In both cases this remaining activity was three orders of magnitude less than the amount of heat-stable enzyme found in the human, chimpanzee and orangutan placentae. Figure 1 shows the results of comparing the three related placentae by double diffusion. The absence of 'spurs' indicates that these enzymes are similar in their immunochemical structure.

The considerable difference of the human, chimpanzee and orangutan placental phosphatases from those of baboon, rhesus monkey, African green monkey and spider monkey suggests that the occurrence of the type and/or amount of alkaline phosphatase found in human placentae is a relatively late event in primate evolution. The apparent absence of a similar isoenzyme in lowland gorillas is surprising, as there is no evidence[12] for a divergence of this species from the line of man's descent after the orangutan. However, this placenta was only a single sample, and was taken from a mother with abruptio placentae and recent fetal death. The content of luteinising hormone in the placenta and its histology were normal.

The existence of a null allele for human placental phosphatase has been suggested[13]. The very low activity of the enzyme in the lowland gorilla could be due to the infant being homozygous for this null allele. Alternatively, there could be an alteration in the regulation of synthesis of the enzyme in this species, so that there is very little of this enzyme present in the species as a whole. Examination of other gorilla placentae should establish which of these possibilities is correct.

By comparing the specific activity of pure placental phosphatase with that of 150,000g max pellets of placental extracts, we have estimated that on average, 1% of the membrane protein of human placentae is alkaline phosphatase. The very low activity in rhesus, African green, baboon and spider monkeys and possibly gorillas, and the apparent biochemical distinction of the enzymes in these species is extremely interesting. No other human tissue has been found to have an enzyme which does not occur in the corresponding tissue in monkeys. The presence of this enzyme in such high concentrations in the human placenta is therefore species specific. This may account for the unusually high degree of heterozygosity at this locus[7] and the apparent role of several genetic variants in prenatal selection[14,15]. If the expression of the enzyme during human fetal development is a late evolutionary event, then selection of phenotypes could still be much more active than that of other enzymes whose organ-specific function has become specialised over a longer period of evolution.

The similarity of orangutan, chimpanzee and human placental phosphatase by double diffusion is interesting. It suggests that, despite the occurrence of this enzyme late in primate evolution, the gene coding for it is similar in all three species. It is likely that an enzyme similar to human, chimpanzee and orangutan placental alkaline phosphate is normally present in an organ(s) other than the placenta (or at much lower concentrations in the placenta), and that its presence in the placenta is the species-specific event. This could have occurred after a gene duplication or by de-repression of a regulator gene in the placenta[16].

The production of significant amounts of placental-type alkaline phosphatase, the so-called 'Regan isoenzyme'[17], and variants of this enzyme[18,19] in some cancer patients, has been interpreted as being due to de-repression of a fetus-specific gene product[20]. If the gene coding for placental phosphatase is in fact expressed in some other as yet unidentified organ, then the occurrence of the enzyme in this organ pre-dates its occurrence in the placenta. The production of placental-type alkaline phosphatase by the placenta and certain tumours may therefore be a coincidence, not the expression of fetal genes in neoplasia. This hypothesis might be tested by investigating the presence of placental-type alkaline phosphatase in non-neoplastic human tissues and studying these same tissues in non-human primates.

In view of the apparent species specificity of the placental phosphatase, it would be interesting to develop a complete catalogue of the occurrence of this enzyme type in non-human primates. It is possible that this might reveal a new biochemical aspect of primate evolution, especially if the function of this enzyme is determined.

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Human placental alkaline phosphatase differs from that of other species

AT LEAST three gene loci seem to be involved in determining the various forms of human alkaline phosphatase (ALP)—one coding for the placental form of the enzyme, at least one coding for the intestinal forms, and at least one for the liver, bone and kidney forms[1,2]. These three classes of human alkaline phosphatase can be distinguished from one another by their

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Table 1 Concentrations of various inhibitors required to produce 50% inhibition of alkaline phosphatase in placenta and liver of various mammals

		$[I]_{50}$ (mM)		
		HA	Phe	PGG
Man	Placenta	62.88	1.13	0.09
	Liver	2.83	28.62	27.44
Dog	Placenta	2.87	28.42	32.52
	Liver	2.91	22.42	27.44
Mouse	Placenta	2.73	49.08	36.30
	Liver	2.13	28.26	28.13
Rat	Placenta	2.99	42.94	29.78
	Liver	2.99	26.75	35.98
Sheep	Placenta	2.63	45.82	30.95
	Liver	2.66	30.58	32.93
Cow	Placenta	2.59	30.10	26.05
	Liver	2.83	37.61	27.40
Pig	Placenta	2.82	25.83	30.19
	Liver	2.79	39.51	38.45
Guinea pig	Placenta	2.35	33.41	26.98
	Liver	3.80	27.77	33.36
Rhesus	Placenta	2.90	40.28	38.94
	Liver	3.28	27.00	39.12
Hamster	Placenta	2.77	33.35	34.12
	Liver	2.44	38.32	34.86
Cat	Placenta	2.42	29.92	27.16
	Liver	2.41	33.73	29.95

The tissues were kept frozen at −20 °C until extracted. Aqueous solutions of the enzyme were obtained by extracting the tissue in the presence of *n*-butanol as previously described[1]. Assays were carried out using *p*-nitrophenyl phosphate (5.0 mM) as substrate in a diethanolamine buffer 1.0 M *p*H 9.85, containing 0.28 M NaCl and 0.5 mM $MgCl_2$. The inhibitors were added to the reaction mixture to produce final concentrations of 1, 2.5, 5, 10 and 20 mM. In the case of human placenta with PGG, assays were also carried out in the presence of 0.2 and 0.5 mM of this inhibitor. The reactions were run for up to 30 min at 30 °C and stopped by the addition of a volume of 1 M phosphate buffer, *p*H 9.8, equal to the volume of the reaction mixture. Absorbance was read at 405 nm. Degree of inhibition was expressed as percentage of the original activity remaining in the presence of a given concentration of inhibitor. Two or more determinations were made on each sample at each inhibitor concentration and in some cases samples from different individuals of the same species were examined. The average value was used in the subsequent calculations. Estimates of $[I]_{50}$ were obtained by calculating the least-squares regression of (100/per cent activity remaining) on concentration of inhibitor, [I], and determining from this the concentration of inhibitor required to give 50% inhibition (see Fig. 1 legend).

behaviour with certain inhibitors, their thermostabilities and their electrophoretic and immunological characteristics. Recent studies have shown that in the dog, the ALP present in placenta closely resembles that in liver, and that these enzymes are very similar to the ALP present in human liver but different from the ALP present in human placenta[3,4]. It has also been reported that human placental ALP differs from other mammalian placental ALPs in certain respects (for example, sensitivity to L-phenylalanine inhibition)[5,6]. In view of these results we have examined some of the characteristics known to distinguish human placental and human liver ALPs, in the liver and placental ALPs from a series of mammalian species. The species from which both tissues were obtained included rodents, carnivores, ungulates and one primate (rhesus). We report here that the properties of human placental ALP differ from those of human liver ALP, whereas in the other species studied, placental and liver ALPs show similar properties.

Particularly sharp discrimination between human liver and placental ALPs can be achieved using L-homoarginine (HA), L-phenylalanine (Phe) and L-phenylalanylglycylglycine (PGG) as inhibitors[1]. HA produces marked inhibition of liver ALP at concentrations which produce only slight inhibition of placental ALP[7]. In contrast, Phe and PGG produce marked inhibition of placental ALP at concentrations which produce only slight inhibition of liver ALP[1,8]. This combination of inhibitors also distinguishes intestinal ALP from both liver and placental ALP[1,9]. The percentage activity remaining in the presence of various concentrations (1–20 mM) of each of these inhibitors was determined for the different liver and placental ALPs of the various animal species. As a general rule the reciprocal of the reaction velocity of an enzyme may be expected to vary linearly with the concentration of an inhibitor[10,11]. This was found to be the case for the various ALPs with these particular inhibitors. Some typical plots are shown in Fig. 1. The greater the slope of the regression line, the greater the degree of inhibition produced by the particular inhibitor. The least-squares regression was therefore calculated for each set of data and from this the concentration of inhibitor required to produce 50% inhibition of the enzyme, $[I]_{50}$, was estimated. These estimates are given in Table 1 for the liver and placental ALPs of each species with each of the inhibitors.

It is apparent that for each of the inhibitors the estimated $[I]_{50}$ for human placenta is quite different from the estimated $[I]_{50}$ values for any of the placentas from the other species. Table 2 gives the means of the $[I]_{50}$ estimates for the series of non-human placental ALPs and for the liver ALPs. They are in close agreement. A paired *t*-test gives no evidence for significant differences between $[I]_{50}$ placental ALP and $[I]_{50}$ liver ALP in any of the species (HA, $t = 0.68$, d.f. = 9, $P > 0.5$; Phe, $t = 1.28$, d.f. = 9, $P > 0.2$; PGG, $t = 0.91$, d.f. = 9, $P > 0.2$). Note that the $[I]_{50}$ values for human liver ALP for each of the inhibitors do not deviate significantly from the mean $[I]_{50}$ values for placenta or liver ALPs of the other species. In contrast, the $[I]_{50}$ for human placental ALP with HA is about 30 times greater than the mean $[I]_{50}$ for the liver and placental ALPs of the other species; and the mean $[I]_{50}$ values for the liver and placental ALPs of the other species with Phe and PGG are about 30 times and 300 times greater, respectively, than the corresponding $[I]_{50}$ values for human placental ALP. An analysis of variance of the placental and liver ALP $[I]_{50}$ values was carried out to see whether there were significant differences between the non-human species. However, no significant heterogeneity was detected (HA, $F = 1.03$, d.f. = 9, 10, $P > 0.25$; Phe, $F = 0.41$, d.f. = 9, 10, $P > 0.75$; PGG, $F = 1.91$, d.f. = 9, 10, $P > 0.10$).

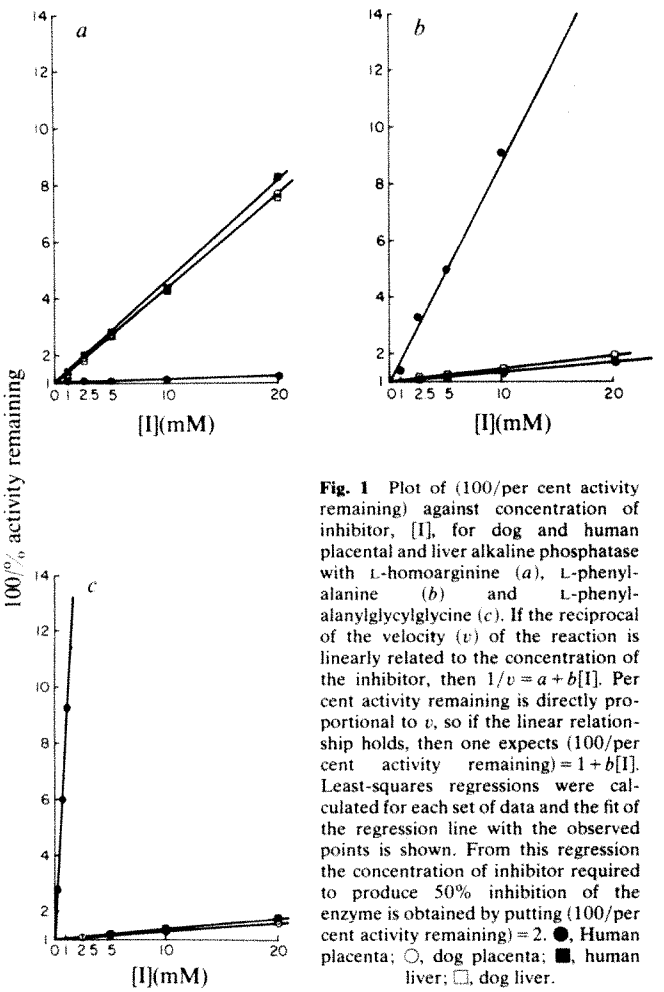


Fig. 1 Plot of (100/per cent activity remaining) against concentration of inhibitor, [I], for dog and human placental and liver alkaline phosphatase with L-homoarginine (*a*), L-phenylalanine (*b*) and L-phenylalanylglycylglycine (*c*). If the reciprocal of the velocity (v) of the reaction is linearly related to the concentration of the inhibitor, then $1/v = a + b[I]$. Per cent activity remaining is directly proportional to v, so if the linear relationship holds, then one expects (100/per cent activity remaining) $= 1 + b[I]$. Least-squares regressions were calculated for each set of data and the fit of the regression line with the observed points is shown. From this regression the concentration of inhibitor required to produce 50% inhibition of the enzyme is obtained by putting (100/per cent activity remaining) = 2. ●, Human placenta; ○, dog placenta; ■, human liver; □, dog liver.

Thus, the data suggest that the structures of the binding site(s) for HA, Phe and PGG must be identical or at least very similar in the placental and liver ALPs of these various species and also in human liver ALP. However, the structure of the binding site(s) for these inhibitors in human placental ALP is presumably quite different.

Table 2 Means and standard deviations of estimated $[I]_{50}$ values for various inhibitors for human and non-human placental and liver alkaline phosphatase

			$[I]_{50}$ (mM)					
			HA		Phe		PGG	
		No. of species	Mean	s.d.	Mean	s.d.	Mean	s.d.
Man	Placenta	1	62.88	—	1.13	—	0.09	—
	Liver	1	2.83	—	28.62	—	27.44	—
Mammals (excluding man)	Placenta	10	2.71	0.21	35.92	8.03	31.30	4.21
	Liver	10	2.82	0.47	31.20	5.80	32.76	4.40

$[I]_{50}$ values for HA, Phe and PGG are from 10 different mammalian species compared with the corresponding values for the human enzymes.

Human placental ALP is remarkably thermostable[12]. It may be heated at 65 °C for more than 1 h with little or no loss of activity. In contrast, human liver ALP is rapidly destroyed in these conditions. The thermostabilities of the various animal liver and placental ALPs were therefore determined. Each of the samples was heated at 65 °C and also at 56 °C for different times up to 30 min and the residual activities determined. In each case the regression of the (log percentage activity remaining) on time was calculated, and from this an estimate of the time required to produce 50% inactivation of the enzyme, T_{50}, was obtained. The results (Table 3) indicate that the various non-human placental and liver ALPs are relatively thermolabile and indeed similar to human liver ALP in this respect. The mean T_{50} values for placental and liver ALPs of the non-human species (Table 4) are in close agreement. A paired *t*-test showed no evidence for significant differences between the placental or liver ALP T_{50} values in any of the species. The human liver ALP T_{50} value does not deviate significantly from either the placental or liver ALP mean T_{50} values. Human placental ALP is, of course, quite stable in these conditions. An analysis of variance of the T_{50} values for the non-human placental and liver ALPs was carried out to see whether there was evidence for significant differences between the species. Significant heterogeneity was indeed detected (56 °C, $F = 3.29$, d.f. = 9, 10, $0.01 < P < 0.05$; 65 °C, $F = 7.29$, d.f. = 9, 10, $P < 0.01$). Such thermolability differences presumably reflect differences in the overall molecular structures of the ALPs in the different species.

Table 3 Time required to produce 50% inactivation of alkaline phosphatase in placenta and liver of various mammals

		56 °C T_{50} (min)	65 °C T_{50} (min)
Man	Placenta	Stable	Stable
	Liver	8.2	1.05
Dog	Placenta	23.1	1.20
	Liver	14.2	1.31
Mouse	Placenta	2.3	0.58
	Liver	5.8	0.80
Rat	Placenta	2.2	0.42
	Liver	5.0	0.84
Sheep	Placenta	10.8	0.52
	Liver	9.0	0.35
Cow	Placenta	10.9	1.04
	Liver	16.0	1.32
Pig	Placenta	9.7	0.91
	Liver	3.5	0.65
Guinea pig	Placenta	6.6	0.97
	Liver	6.6	0.92
Rhesus	Placenta	8.2	0.76
	Liver	18.7	0.45
Hamster	Placenta	4.4	0.21
	Liver	7.9	0.32
Cat	Placenta	12.3	0.67
	Liver	18.2	0.60

Thermal inactivations were carried out at 56 °C and 65 °C on tissue extracts dialysed against 10.0 mM Tris-HCl, *p*H 7.5, containing 2 mM $MgCl_2$. The samples (0.2 ml) were placed in capped plastic tubes (Falcon 2038) and heated in a waterbath for 5, 10, 15, 20 or 30 min at 56 °C and for 1, 2.5, 5, 10, 15 or 20 min at 65 °C. At the end of each period the tube was immediately placed in an ice bath where an unheated control had been kept for the same period. Thermal inactivation was expressed as percentage of original activity remaining after a given time at the particular temperature. Two or more determinations were made on each sample at each period and in some cases samples from different individuals of the same species were examined. The average values were used in subsequent calculations. Estimates of T_{50} were obtained by calculating the least-squares regression of (log per cent activity remaining) on time and determining from this the time required for 50% inactivation.

Table 4 Means and standard deviations of estimated T_{50} values for human and non-human placental and liver alkaline phosphatase

		56 °C T_{50} (min)		65 °C T_{50} (min)	
Man	Placenta	Stable	—	Stable	—
	Liver	8.2	—	1.05	—
Mammals (excluding man)	Placenta	9.1	6.1	0.73	0.31
	Liver	10.5	5.7	0.76	0.36

The T_{50} values at 56 °C and 65 °C for placental and liver alkaline phosphatases were from 10 different mammalian species compared with the corresponding values for the human enzymes.

The results show that in terms of the inhibition and thermostability characteristics studied here, placental and liver ALPs are indistinguishable in each of these mammalian species other than man. The data suggest that the same gene locus is being expressed in liver and placenta of the various species and that this locus corresponds to the ALP locus being expressed in human liver. It is, however, presumably different from the ALP locus expressed in human placenta.

These findings are of evolutionary interest. It has been suggested that the liver/bone/kidney, intestinal, and in man, placental, ALPs represent a so-called multilocus enzyme system, the loci coding for these enzymes having been derived in the course of evolution from a common ancestral gene as a result of successive gene duplications[4,13]. Mutations occurring in the genes subsequent to the duplications would have led to some divergence in their base sequences, and thus to characteristic differences in the properties of the ALPs they now determine. If this is correct, a plausible interpretation of the data is that the gene duplication giving rise to the human placental ALP gene locus was a relatively late evolutionary event which occurred subsequent to the divergence of the evolutionary lineage leading to man from the various lineages leading to most other mammalian species, including rhesus, an Old World monkey. An alternative hypothesis would be that a gene locus corresponding to the human placental ALP locus does exist in these other mammalian species but is not expressed in their placentas, so that the late evolutionary event would have involved its derepression or activation accompanied by repression of the locus coding for liver ALP. This seems a less economical hypothesis. However, eventually it should be possible to distinguish critically between the two hypotheses, by direct studies of the genes at the DNA level using the newly emerging methods of DNA analysis, such as restriction endonuclease mapping. The apparently late evolutionary appearance of the human placental ALP locus is also of interest in connection with the fact that human placental ALP, unlike the intestinal and liver ALPs, is genetically highly polymorphic.

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α-Globin gene organisation in blacks precludes the severe form of α-thalassaemia

IN most human populations, the α-globin structural gene loci are duplicated so that each diploid cell contains four copies of α-globin genes[1–3]. In α-thalassaemia, a hereditary disorder of α-globin chain synthesis, the most common molecular lesion is due to the deletion of the α-globin genes[4–7]. In the Asian population, four main α-thalassaemia syndromes of increasing clinical severity are recognised: (1) the silent carrier state (α-thalassaemia-2) with no clinical manifestation; (2) α-thalassaemia trait (α-thalassaemia-1), characterised by microcytic red blood cells but little or no anaemia; (3) haemoglobin-H disease, which manifests as haemolytic anaemia; and (4) homozygous α-thalassaemia, in which the afflicted fetus dies at or around term from hydrops fetalis. These four syndromes are due to the deletion of from one to all four copies of the α-globin genes. In this study, we have characterised α-thalassaemia in people of African origin. Haemoglobin screening programmes have shown that α-thalassaemia occurs in the black population[8–10]. Recently we have demonstrated by complementary DNA–DNA hybridisation that in black individuals with clinically well defined α-thalassaemia trait, two of the four normal α-globin genes were deleted[11]. However, in this population, haemoglobin-H disease is rare and homozygous α-thalassaemia has never been found[12,13]. For this study we have used the restriction endonuclease mapping technique of Southern[14] to delineate the nature of the deletion of the α-globin genes.

The two α-globin structural gene loci are 3.7 kilobases apart[15,16]. The presence of these two loci could be deduced by digestion with the two restriction enzymes, *Eco*RI and *Hpa*I. When cellular DNA from a non-thalassaemic individual (genotype: $\alpha\alpha/\alpha\alpha$) was digested with *Eco*RI, the two α-globin loci were located in a single DNA fragment about 23 kilobases in length. In the DNA of a patient with haemoglobin-H disease who had only one α-globin gene remaining ($--/-\alpha$), the *Eco*RI fragment which contained the single α-globin locus became shortened to 19 kilobases. In the silent carrier state (α-thalassaemia-2), in which two α-globin loci resided on one chromosome and a single locus resided on the other ($-\alpha/\alpha\alpha$), both the 23-kilobase and the 19-kilobase bands were found. The phenotype of α-thalassaemia trait with two α-globin genes deleted could arise from two types of gene arrangement. The two genes could be deleted either *in cis* or *in trans*, giving rise to heterozygous state of α-thalassaemia-1 ($--/\alpha\alpha$) or the homozygous state of α-thalassaemia-2 ($-\alpha/-\alpha$), respectively. These two gene arrangements could be distinguished, as *Eco*RI digests would show a single 23-kilobase normal fragment in the former, and a single 19-kilobase fragment in the latter (Fig. 1).

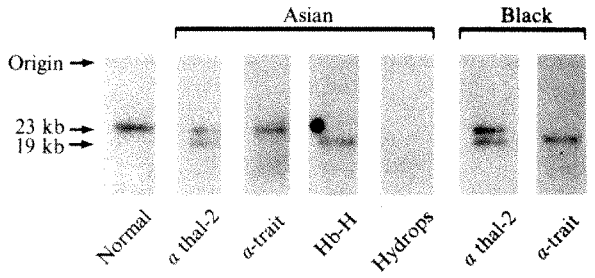


Fig. 1 *Eco*RI-digested human DNA hybridised with α-globin gene specific probe. Human DNA was extracted from white blood cells or placenta from normal individuals and patients with different α-thalassaemia syndromes using methods described previously[5]. DNA (10 μg) digested with *Eco*RI was separated by electrophoresis on 0.8% agarose gel and transferred on to nitrocellulose filters as described previously[17]. The filter was hybridised for 48 h in previously described conditions[17] with plasmid JW 101 (ref. 18) which contains the human α-globin complementary DNA insert and which has been nick translated and labelled with ^{32}P-dCTP and ^{32}P-dTTP according to the method of Maniatis *et al.*[19]. The filter was extensively washed and autoradiographed[17]. The numbers on the left indicate nucleotide pairs in kilobases (kb). The α-globin gene containing bands in Asians are shown on the left, whereas those of blacks are on the right. Note the difference in the patterns of α-thalassaemia trait (α-trait) in the two ethnic groups.

The arrangement of the α-globin genes could further be confirmed by digestion of the DNA with the enzyme *Hpa*I, which cleaves in between the two α-globin loci (Fig. 2). Normal DNA yielded two *Hpa*I fragments, 14.5 and 4.1 kilobases in length, which contained the 3′ and the 5′ α-globin loci, respectively[15,16]. In haemoglobin-H disease the single remaining locus contained in the 14.5-kilobase fragment was seen. In the silent carrier state both the 14.5-kilobase and the 4.1-kilobase fragments were present, the former containing two alleles, being

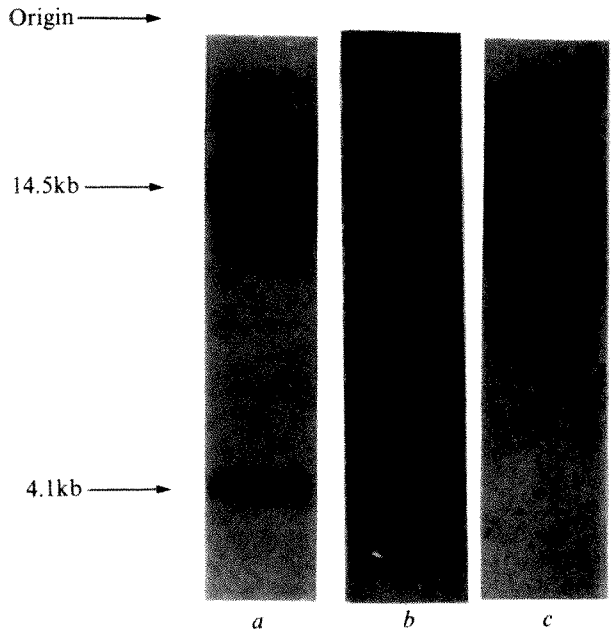


Fig. 2 *Hpa*I digested human DNA hybridised with α-globin gene-specific probe. The pattern seen in: *a*, normal ($\alpha\alpha/\alpha\alpha$) and α-thalassaemia-1 with deletion *in cis* ($--/\alpha\alpha$); *b*, α-thalassaemia-2 ($-\alpha/\alpha\alpha$); *c*, haemoglobin-H disease ($--/-\alpha$) or α-thalassaemia trait with deletion *in trans* ($-\alpha/-\alpha$).

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Table 1 Pertinent haematological data of patients with α-thalassaemia trait

	Mean corpuscular volume	Hb A_2 (%)[2]	Hb Bart's (%)	Serum iron (μg per 100 ml)	Free erythrocyte protoporphyrin (μg per 100 ml RBC)	α/β Ratio	α-Globin‡ gene no.
Normal							
Adult	80–99	1.5–3.0	—	65–165	<90	0.95–1.05	4
Cord blood	≥95	—	trace	—	—	—	4
Black patients							
Adult (9)	72 (64–75)	2.2 (1.9–2.7)	—	82 (68–130)	35 (23–70)	0.76 (0.62–0.87)	2
Cord blood (6)	92* (88–95)	—	3.1 (3.0–5.0)	70† (64–120)	—	—	2
Asian patients							
Adult (15)	70 (62–75)	2.3 (1.8–2.9)	—	98 (65–162)	46 (29–85)	0.82 (0.71–0.93)	2

* Follow-up studies showed persistent microcytosis at 6–12 months.
† Determined at 6–12 months.
‡ Determined by complementary DNA–DNA hybridisation.

more intense than the latter with one allele. Again, the two genotypes which produce the clinical state of α-thalassaemia trait could be distinguished from each other, as deletion *in cis* ($--/\alpha\alpha$) gave both the 14.5 and 4.1-kilobase bands, whereas deletion *in trans* ($-\alpha/-\alpha$) produced only the 14.5-kilobase band.

We selected 15 black individuals with clinical diagnosis of α-thalassaemia trait. Rigid criteria were used to establish the diagnosis. These were: in the adult, a mean corpuscular volume (MCV) of less than 76 fl, normal haemoglobin A_2 and F, absence of iron deficiency and decreased α/β globin synthesis ratio; in the newborn period, an MCV of less than 96 fl, haemoglobin Bart's of over 3% and persistence of microcytosis at the age of 6–12 months in the absence of iron deficiency. Table 1 summarises the pertinent haematological findings. Haemoglobin-H was not detectable in these patients and molecular hybridisation analysis showed that they had two copies of α-globin structural genes per diploid genome[11]. Restriction endonuclease mapping showed that all 15 had the 19-kilobase band on *Eco*RI digest, and only the 14.5-kilobase band on *Hpa*I digest. Thus, the two remaining α-globin genes are located on opposite chromosomes ($-\alpha/-\alpha$) (Fig. 1). We also studied 15 adult Asian patients with α-thalassaemia trait. This diagnosis was also made according to the criteria listed, and in addition, six of these patients were parents of hydropic fetuses. Molecular hybridisation confirmed

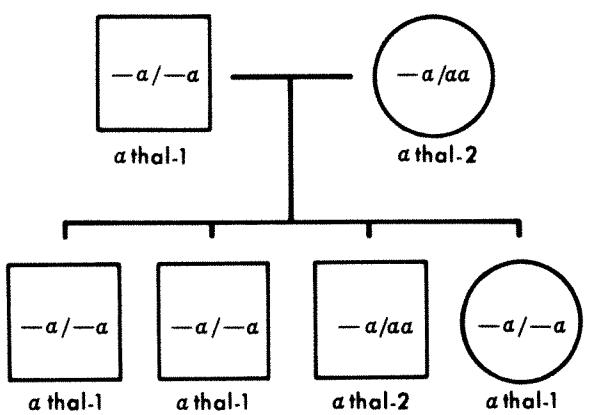


Fig. 3 Inheritance of α-thalassaemia in a black family. The mean corpuscular volume in femtolitres (normal > 80) in the family members are: I-1 : 70, I-2 : 81, II-2 : 68, II-3 : 78 (age 4 yr, normal > 75), III-4 : 62. The apparent direct transmission of α-thalassaemia trait is due to the presence of α-thalassaemia-2 in the haematologically normal mother.

that they had two α-globin genes per diploid genome[11]. Restriction analysis showed that they all had the normal 23-kilobase *Eco*RI band and both *Hpa*I bands, indicating that the deletion of the two genes had occurred on the one chromosome ($--/\alpha\alpha$).

We used these enzymes to map the α-globin genes in 211 black Americans. They were not selected for α-thalassaemia, none had haemoglobin-H disease, but some had the β-structural mutants S and C (Table 2); 70.6% had the normal α-globin genotype, 27.5% had two α-globin bands on *Eco*RI, characteristic of the silent carrier state, and 1.9% had the 19-kilobase *Eco*R1 band and only the 14.5-kilobase *Hpa*I band characteristic of the genotype of $-\alpha/-\alpha$. The frequency of the single α-globin locus ($-\alpha$) was calculated to be 0.16.

We also investigated the mechanism of transmission of α-thalassaemia. Previously, 'direct transmission' of α-thalassaemia trait from parent to offspring in the black has been taken as evidence for the presence of the α-thalassaemia-1 genotype ($--/\alpha\alpha$). When we studied with restriction enzyme analyses two families in which direct transmission seemed to have occurred, we found that the parent with α-thalassaemia trait had the genotype of homozygous α-thalassaemia-2 (that is, deletion of the two α-globin genes on opposite chromosomes), and the haematologically normal spouse had the 23- and 19-kilobase bands containing the α-globin genes characteristic of α-thalassaemia-2 carrier. The latter diagnosis was previously unrecognised without restriction endonuclease mapping. Figure 3 illustrates the findings in one such family. As the frequency of α-thalassaemia-2 is 27.5%, this type of inheritance will explain the so-called direct transmission in most cases.

These findings directly confirm the hypothesis first proposed by Lehmann on the genetic basis of α-thalassaemia and the lack of the severe form of α-thalassaemia in the black population[20]. Using the percentages of haemoglobin S in sickle-cell trait as an indicator of α-thalassaemia, Altay *et al.*[9] in the African population and Brittenham[21] in Asian Indians found a trimodal distribution of the percentage of haemoglobin S level, of about, 25, 33 and 45%. They concluded that the two lower percentages of S are due to the co-existence of α-thalassaemia trait and α-thalassaemia-2, respectively. The present study directly demonstrates the frequent occurrence of the α-thalassaemia-2 genotype. It seems that the presence of 1–2% haemoglobin Bart's in cord blood used as the criterion for diagnosis of α-thalassaemia-2 grossly underestimates the presence of the α-thalassamia-2 gene, as electrophoresis of cord blood showed that only a few per cent of the black population had increased haemoglobin Bart's, whereas restriction enzyme analysis demonstrated the prevalence of α-thalassaemia-2 carrier to be 27.5%. This finding agrees with that of Pierce *et al.*[10] who detected a high incidence of α-thalassaemia in black recruits by globin chain synthesis studies.

Table 2 α-globin genotype in black Americans

Genotype	No.	Percentage
$\alpha\alpha/\alpha\alpha$	149	70.6
$-\alpha/\alpha\alpha$	58	27.5
$-\alpha/-\alpha$	4	1.9
Total	211	100

Frequency of $(-\alpha)$ genotype = 0.16.

These results satisfactorily explain the distribution of the different clinical syndromes of α-thalassaemia in the black population. Whereas α-thalassaemia-1 with deletion of the two α-globin genes *in cis* $(--/\alpha\alpha)$ is common in the Asian, this genotype is rare in the black. The fatal form of α-thalassaemia associated with deletion of all the α-globin genes $(--/--)$ will therefore not be found. Haemoglobin H disease $(--/-\alpha)$ will also be rare as it requires the combination of an α-thalassaemia-1 gene with an α-thalassaemia-2 gene. On the other hand, in those parts of the world such as the West Indies, where there is a high incidence of mating between people of African and Asian origin, a high incidence of haemoglobin-H is found.

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Phosphorylation in a simple system of lipids and chloroplast ATP synthetase driven by pulsed ionising radiation

WE report here the observation of an ADP-dependent phosphorylation reaction in an artificial lipid vesicle system driven by pulsed ionising radiation. This reaction is completely dependent on the only enzyme incorporated into the phospholipid vesicle bilayer, the ATP synthetase (CF_0–CF_1) complex from spinach chloroplasts. Previous attempts to demonstrate directly an obligatory coupling between the proton pumping and phosphorylation activities of an ATP synthetase enzyme in resolved, reconstituted vesicle systems have required the presence of additional protein components[1–3] to generate the transmembrane *p*H gradient and/or membrane potential postulated by the chemiosmotic hypothesis[4] as the driving force for the ATP synthesis reaction:

$$ADP + P_i + nH^+_{in} \rightleftharpoons ATP + H_2O + nH^+_{out}$$

Pulsed high-energy radiolysis of water produces, in a very brief time increment, significant quantities of protons along with the highly reactive transient species e^-_{aq}, $^{\cdot}OH$, and $^{\cdot}H$ ($\Delta[H^+] \sim 10^{-5}$ M in 50 ns)[5]. Assuming a uniform production of protons throughout the aqueous media, differential buffering between the inner and outer aqueous compartments of phospholipid vesicles will result in the effectively instantaneous formation of a transmembrane proton gradient. We have applied the pulse radiolysis technique to drive the ATP synthetase activity of phospholipid vesicles reconstituted with the chloroplast CF_0–CF_1 enzyme complex.

Single-walled bilayer vesicles were prepared in dilute buffer from purified soy phospholipids (asolectin) by either a sonication[6] or a detergent–dilution[7] technique. The CF_0–CF_1 ATP synthetase complex extracted from spinach was incorporated into the vesicles as previously described[8]. Immediately before irradiation, a highly buffered solution containing ADP and $^{32}P_i$ was added; then the sample was deoxygenated with nitrogen, sealed in a small glass vial and irradiated. Details of the specific reaction mixtures for each experiment and the method of assay for esterified $^{32}P_i$ are given in the footnotes to the tables. The radiation dose given per pulse was determined by a method previously described[9].

From Table 1 it is apparent that phosphorylation only occurred in irradiated samples, and that the yield of phosphorylated product reached a limiting value at high cumulative dose levels. This is consistent with the predicted radiation inactivation of the enzyme; based on studies with rat-liver mitochondria, inactivation would be expected in the 10^2 krad range[10]. Significantly, the amount of $^{32}P_i$ esterified exceeded, on a molar basis, the amount of enzyme added by a factor of about 8 in the cholate-dilution vesicles and by a factor of about 2 in sonicated vesicles. Even assuming that all of the enzyme added was active and properly

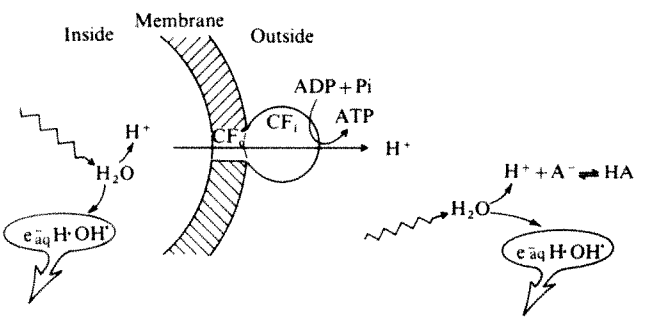


Fig. 1. Proposed mechanism for radiolytically driven ATP formation in artificial lipid vesicles reconstituted with the chloroplast CF_0–CF_1 complex.

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Table 1 Dose dependence for pulse radiolysis-driven phosphorylation in artificial lipid vesicles

No. of pulses*	Pulse frequency (Hz)	$^{32}P_i$ esterified (nmol ml^{-1})† Cholate-dilution vesicles‡	Sonicated vesicles§
0	—	0	0
100	120	0.33	
500	120		1.46
1,000	5	0.92	1.55
1;000	120	0.95	1.63
10,000	120	0.97	

*Radiation dose in each pulse, 1 krad. Pulse width, 50 ns.

†Residual radioactivity after extraction of unreacted ^{32}P-orthophosphate as phosphomolybdic acid as described[3,12] $^{32}P_i$ specific activity, 3,375 c.p.m. nmol^{-1}.

‡Cholate-dilution vesicles were prepared as follows: 20 mg purified soy phospholipid was disolved in 0.5 ml of a solution containing 0.2 M sucrose, 1.4% Na-cholate, 1 mM tricine (*p*H 8.0) and 3 mM $MgCl_2$. This was mixed with an equal volume of CF_0–CF_1 complex containing 8.0 mg protein per ml. After 20 min at 4 °C, 16-μl aliquots of the enzyme–lipid mixture were diluted into 0.8 ml volumes of a solution containing 0.8 mM tricine (*p*H 8.0), 3 mM $MgCl_2$, 12 mM glucose, 0.12 mM KNO_3. After 1–2 min, 0.1 ml 10% defatted bovine serum albumin and 0.1 ml of the external buffer solution (2 mM ADP, 20 mM $Na_2H^{32}PO_4$, 25 μg ml^{-1} hexokinase, 200 mM HEPES (*p*H 8.25)) were added, the sample (1 ml) was deoxygenated, sealed in a small glass vial (1 cm i.d.) and immediately irradiated.

§Sonicated vesicles were prepared by sonicating (in a bath type sonicator) 2.5 mg purified soy phospholipids in 1 ml of a solution containing 1 mM tricine (*p*H 8.0), 3 mM $MgCl_2$, 0.2 M sucrose. After the suspension cleared (~15 min), 0.1 ml of the CF_0–CF_1 complex (8.0 mg protein per ml) and 0.15 ml of a solution containing 200 mM glucose, 1 mM KNO_3 was added and the suspension sonicated an additional 3 min. To this reconstituted system, 0.15 ml 10% defatted bovine serum albumin and 0.15 ml of the external buffer solution were added and samples (0.5 ml) were deoxygenated, sealed and irradiated.

incorporated into the vesicles, this result indicates multiple turnover of the enzyme. Although the relative yield of phosphorylated product (per mg protein) was greater using cholate dilution vesicles, subsequent studies were performed with sonicated vesicles to permit greater variation in both phospholipid and enzyme concentrations.

The data from several preparations of sonicated vesicles are summarised in Table 2. A reasonably good correlation was observed between the yield of phosphorylated product and the absolute quantity of enzyme added. When the CF_0–CF_1 complex was irradiated in the absence of lipid vesicles, the formation of phosphorylated product was greatly diminished, although a slight incorporation of radioactive phosphate into an organic product (~0.3 nmol per mg protein) was observed. This residual phosphorylation may result from some phospholipid

Table 2 Effect of enzyme concentration on pulse radiolysis-driven phosphorylation

Enzyme (mg ml^{-1})	Lipid (mg ml^{-1})	$^{32}P_i$ esterified (nmol ml^{-1})	$^{32}P_i$ esterified per mg protein
0.47	2.6	3.20	6.80
0.53	1.7	1.46	2.75
1.24	2.6	3.51	2.80
1.60	13.3	5.25	3.28
1.71	26.7	4.48	2.62
1.87	26.6	6.61	3.53

Sonicated vesicles were prepared as described in Table 1, except the enzyme and lipid concentrations were varied to give the final concentrations indicated. Irradiation was with 500 pulses (50-ns pulsewidth) at 120 Hz. Dose per pulse = 1 krad.

Table 3 Substrate and enzyme requirements for pulse radiolysis-driven phosphorylation

Conditions*	$^{32}P_i$ esterified (nmol ml^{-1})
Control	4.48
Heat-denatured enzyme†	0.16
ADP omitted	0.36
Enzyme omitted	0.00
Sonicated in 'external buffer'‡	2.28

*Sonicated vesicles containing 26.7 mg ml^{-1} soy phospholipid and 1.71 mg ml^{-1} CF_0–CF_1 complex were prepared as described in Table 1. Each sample was irradiated with 500 pulses at 120 Hz (pulsewidth, 50 ns; dose per pulse, 1 krad).

†The enzyme was heated to 55 °C for 10 min immediately before incorporation into the vesicles.

‡Sonicated vesicles were prepared as described in Table 1, except that the external buffer was added with the enzyme before the 3 min sonication step.

contamination of the CF_0–CF_1 preparation[8], or may be related to the observation that acid denaturation of soluble CF_1, in the absence of membranes, can result in the formation of a small amount of ATP[11].

The data in Table 3 demonstrate that the radiolytically driven phosphorylation reaction required the CF_0–CF_1 ATP synthetase complex in active form. Furthermore, the esterification of $^{32}P_i$ occurred at significant levels only when ADP was added to the system. This strongly suggests that the initial product of the radiolytically driven reaction was in fact ATP. The interpretation is further strengthened by the finding that all of the $^{32}P_i$-labelled product is acid stable (100 °C, 10 min, 1 M HCl), as expected for glucose-6-phosphate. These results also eliminate the possibility of a radiolytically initiated ADP–$^{32}P_i$ exchange reaction. The trace amount of $^{32}P_i$-labelled product formed in the absence of added ADP may result from residual bound ADP added with the enzyme[13].

Considered within the context of the chemiosmotic hypothesis[4], the radiolytically driven phosphorylation reaction studied here appears to be reasonably efficient, although a definitive determination of the H^+/ATP ratio cannot be made without an accurate characterisation of the structure of the vesicles used in this system. Assuming a vesicle internal radius of 100 nm, for example, the number of radiolytically generated internal protons per ATP synthesised would be approximately 800.

Surprisingly, sonication of the vesicles in the presence of high external buffer did not completely eliminate the formation of ATP in this system (Table 3). Incomplete equilibration of the external and internal buffers could account for the residual phosphorylation activity, but this does not seem likely after sonication. Alternatively, it is probable that generation of protons in ~10^{-8} s produces a transient large *p*H drop, the relaxation of which is limited only by buffer protonation. The extent to which to the initial reaction of protons with the CF_0–CF_1 complex can compete with buffer protonation reactions in the short time scale involved here could determine, at least in part, the efficiency of the overall ATP synthetic reaction, with limited dependence on the internal buffer concentration. In any event, there is evidence to indicate that the effects of internal buffers on phosphorylation efficiency are complex[14].

Irradiation, in identical conditions (500 pulses, 120 Hz, 1 krad per pulse), of the solution which was used for the internal aqueous phase of the vesicle produced a long-term *p*H drop of ~1.2 units. Assuming a chemically inert lipid bilayer, this would allow a maximum steady state transmembrane gradient of ~1.2 *p*H units. A possible contribution by a radiolytically generated transmembrane electrical potential cannot be ruled out, although yields of ATP per mol of CF_1 comparable to those reported here have been measured in chloroplasts subjected to a similar small *p*H gradient by a dark *p*H jump (T. Graan and N. E. Good, personal communication).

The study of CF_0–CF_1-catalysed ATP formation by the pulsed irradiation technique (Fig. 1) provides an opportunity to resolve kinetically the mechanistic processes involved in the ATP synthetase and proton pumping activities of this enzyme. Further, the ability to generate a significant transmembrane pH gradient on a nanosecond time scale should contribute to the study of a wide range of membrane processes.

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In vivo reduction of cyclopropene by *Azotobacter vinelandii* nitrogenase

NITROGENASE from N_2-fixing aerobes such as *Azotobacter vinelandii* is stable to air in crude extracts but on fractionation into its two protein components, Fe–Mo protein and Fe protein it manifests the O_2 sensitivity characteristic of other nitrogenases[1]. This difference between the air-stable and air-labile forms of the enzyme has been ascribed to various causes[2–4]. More generally, the incompletely defined correlations between *in vivo* and *in vitro* nitrogenase function with respect to optimal component ratio[5], local pH[6], ATP stoichiometry[7], reductant[8], catalytic behaviour[4,9] and other important enzymatic parameters are a source of ambiguity in biochemical studies on the cell-free enzyme. In principle, effects from changes in many of these parameters can be studied by parallel *in vivo* and *in vitro* substrate reduction and inhibition studies. In practice[10], restrictive limitations are imposed by the paucity of nitrogenase substrates that are at once (1) well bound, (2) capable of diagnostically complex chemical interactions, and (3) compatible with function of the enzyme in living microorganisms. For example, among known substrates other than N_2 itself, C_2H_2 is deficient in reduction complexity[10], HCN is excluded by its cytotoxicity and acrylonitrile, which displays interesting product patterns[11], is a poor substrate[12]. Recently, cyclopropene was shown to undergo ATP-dependent nitrogenase-catalysed reduction to propene and cyclopropane *in vitro*[13]. The product ratio of ~2:1 alkene : cycloalkane was found to be insensitive to purification over a specific activity range of 40–1,200 in conventional assay conditions. We report here results of further nitrogenase–cyclopropene investigations which establish that: (1) nitrogenase in living *A. vinelandii* catalyses cyclopropene reduction to the products observed *in vitro* in the same ~2:1 ratio; the K_m for formation of either product is very similar if not identical to that obtained with cell-free enzyme; (2) with purified components (specific activity up to 2,000) at optimal stoichiometry, the K_m for formation of the reduction products (propene and cyclopropane) is 0.01 atm, corresponding to a molar K_m as low as that of N_2 (and C_2H_2). These findings strengthen the case for catalytic similarity between *in vivo* and *in vitro* nitrogenase. Conversely, they substantiate the importance of cyclopropene product ratio as a chemical probe for the enzyme and as an important new criterion for nitrogenase biomimetic chemistry.

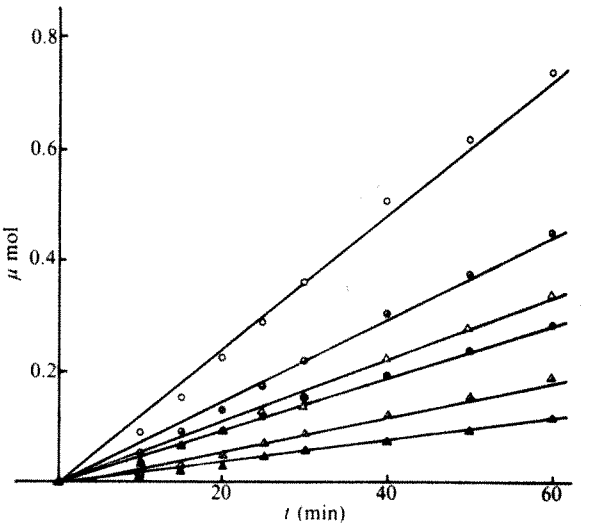


Fig. 1 CO inhibition of cyclopropene reduction by *A. vinelandii* cultured on N-free Burk's medium. The time course of formation of products was monitored by GC after addition of substrate (0.04 atm), inhibitor (as shown below), O_2 (0.1 atm) and 206 Klett-ml of log phase culture. $P_{CO} = 0.0026$ atm: propene, ●; cyclopropane, ▲. $P_{CO} = 0.0013$ atm: propene, ∅; cyclopropane, △. Control (no CO): prm, ○; cyclopropane, △.

Cyclopropene (0.05 atm in Ar or He) was incubated at 30 °C and shaken in septum-stoppered 21 ml vaccine bottles with 2 ml of log phase *A. vinelandii* OP culture grown on a modified[14] N-free or NH_3-supplemented Burk's medium. Cyclopropene was prepared purified and analysed as described elsewhere[13,15]. In aerobic assays, the gas phase also contained 1–5% O_2 (care was taken to avoid O_2 inhibition[16]). Respiration-dependent reduction to both propene (~2.1) and cyclopropane (~1.0) was observed with N_2-fixing cultures; the reduction ratio remained constant throughout the linear portion of product evolution time course experiments (Table 1). Cultures grown on the NH_3 medium were devoid of cyclopropene reduction activity (Table 1). Only respiring, N_2-fixing *Azotobacter* possess C_2H_2 reduction activity[4,9].

Table 1 Requirements for reduction of cyclopropene by *A. vinelandii*

Whole cell assay system	Medium	Propene activity (nm per Klett-ml min) × 10^3	Cyclopropane activity (nm per Klett-ml min) × 10^3	C_2H_4 activity (nm per Klett-ml min) × 10^3
Complete	N-free	61	30	152
Complete	$+NH_3$	<1	<1	<1
$-O_2$	N-free	<1	<1	<1

Values are average values for duplicate assays. See text for details of assay system.

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Table 2 Comparison of kinetic parameters and product ratios for *in vivo* and *in vitro* nitrogenase reduction of cyclopropene

Enzyme state	Propene formation		Cyclopropane formation				
	K_m(M)	V_{max}/V_{max} (C_2H_2)	K_m(M)	V_{max}/V_{max} (C_2H_2)	R*	C_2H_2 K_m(M)†	N_2 K_m(M)‡
In vivo	1.3×10^{-4}	0.38	1.4×10^{-4}	0.20	1.9	2.2×10^{-4}	—
In vitro	1.1×10^{-4}	0.29	1.0×10^{-4}	0.14	2.1	1.3×10^{-4}	1×10^{-4}

*Ratio V_{max} (propene)/V_{max} (cyclopropane). †This work. ‡Ref. 1.

Inhibition of the *in vivo* cyclopropene reduction by N_2 or CO was also consistent with identification of nitrogenase as the responsible catalyst. At a partial pressure of 0.91 atm, N_2 caused a reversible decrease in the cyclopropene reduction rate of 73% at P (cyclopropene) = 0.02 atm. CO was highly effective in inhibiting cyclopropene reduction *in vivo* (Fig. 1). CO has been previously shown to be a potent inhibitor of N_2 and C_2H_2 reduction by nitrogenase[1,4]. (The normal propene:cyclopropane ratio seemed slightly increased in the presence of CO.)

Lineweaver-Burk plots of propene and cyclopropane formation rate dependence *in vivo* on initial cyclopropene pressure were linear (Fig. 2*a*) and gave an average K_m value of 0.014 ± 0.002 atm for each product. In identical conditions, a K_m value of 0.006 atm was found for C_2H_2, in good agreement with most literature data[1,4]. Using a value for the Henry's law constant at 30 °C for cyclopropene of 4.6×10^6 mm (C.E. McKenna and M-C. McKenna, unpublished work) and a literature value of 1.1×10^6 mm for that of C_2H_2 (ref. 17), molar K_m values for the two substrates are found to be about the same (Table 2). Of other nitrogenase substrates, only N_2 has as low a K_m value[1,4]. The ratio V_{max} (cyclopropene)/V_{max} (C_2H_2) from these experiments had an average value of 0.58.

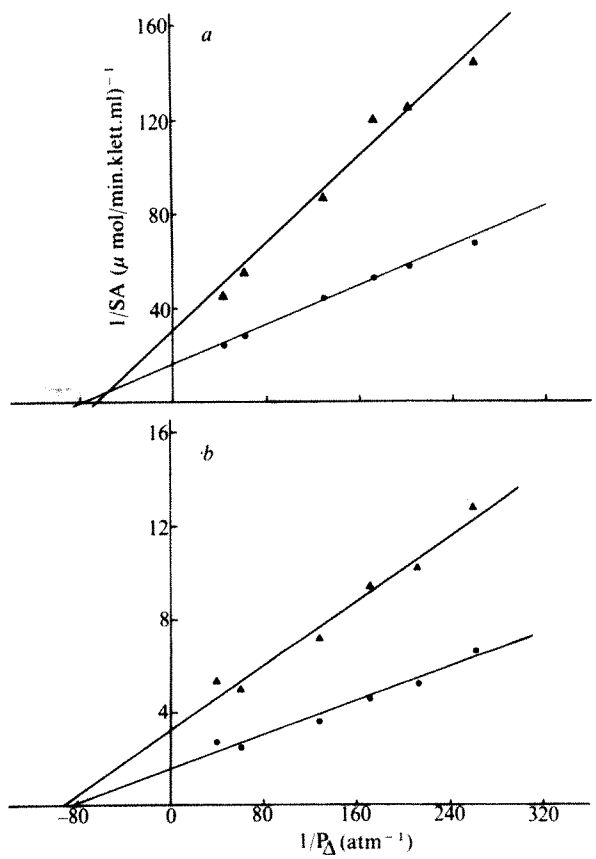


Fig. 2 *a* Double reciprocal (Lineweaver–Burk) plot of reduction rate dependence on cyclopropene pressure with *A. vinelandii* cultured on N-free Burk's medium. After addition of O_2 (0.1 atm) and 218 Klett-ml of log phase culture, time course plots for product formation (GC) were made at each substrate pressure and the initial rate (V) found from the linear portions; V values were divided by the product of the cell density (Klett units) and assay culture volume to obtain specific activities (SA values). The data were fitted to a linear regression curve as shown. Propene, ●; cyclopropane, ▲. *b*, Double reciprocal (Lineweaver–Burk) plot of reaction rate dependence on cyclopropene pressure with purified nitrogenase components from *A. vinelandii*. The component ratio was 1:2 Av1:Av2. Assay conditions were as described elsewhere[13]. Time course plots for product formation (GC) were made at each substrate pressure and the initial rates were found from the linear portion; V data were then converted to SA values (V per mg limiting protein-min). The data were fitted to a linear regression curve as shown. Propene, ●; cyclopropane, ▲.

Similar kinetic experiments were done using highly purified Av1 and Av2 recombined in a 1:2 ratio (Fig. 2*b*). Formation of both cyclopropene products was characterised by the same K_m value within the error of the measurement; the ratio V_{max} (cyclopropene)/V_{max} (C_2H_2) was about 0.43. Comparison of the *in vivo* and *in vitro* results (Fig. 2*a*,*b* and Table 2) reveals that the K_m values for both propene and cyclopropane formation are strikingly similar and that over a pressure range from 0.29 K_m to 1.8 K_m no variation in the propene:cyclopropane ratio is observed; the V_{max} (propene):V_{max} (cyclopropane) ratio is unchanged from the experimental ratio values.

Cyclopropene is thus comparable to N_2 and C_2H_2 in effectiveness as a nitrogenase substrate both *in vivo* and *in vitro*. As a catalyst for cyclopropene reduction, *Azotobacter* nitrogenase gives similar kinetics (K_m, V_{max}) whether in intact cells or assayed as an isolated enzyme in specified standard conditions. It is clear that its overall catalytic properties with the new substrate are not greatly affected by removal from the cell and purification; to the extent that a nitrogenase K_m value inversely reflects active site binding, this means that the relatively bulky cyclopropene molecule has equivalent access to the *in vivo* and *in vitro* enzymes.

The observations described here show the potential of cyclopropene product stoichiometry as a chemical probe for nitrogenase directly[10,15,18] and for chemical models of the enzyme[19,20].

The effectiveness of cyclopropene as an *in vivo* nitrogenase substrate also suggests investigation of the substitutional relationship between cyclopropene and C_2H_2 with other C_2H_2-inhibited reductases (such as hydrogenases[21]) in N_2-fixing bacteria.

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Reduction of cyclopropene as criterion of active-site homology between nitrogenase and its Fe–Mo cofactor

THE nitrogenase Fe–Mo cofactor (FeMo-co)[1] is usually characterised by its unique ability to restore N_2-fixing ability to extracts of certain nitrogenase-less mutants[1,2] and by its spectral properties, some of which can be correlated with those of the FeMo protein itself[3]. The role of FeMo-co in nitrogenase function is not yet clear. Recently, it was reported that uncomplemented FeMo-co catalysed BH_4^- reduction of C_2H_2 to C_2H_4 in a CO-inhibited, non-ATP-dependent process[4]. This was taken as evidence that FeMo-co is the active site for N_2 reduction and binding in nitrogenase, although conversion of N_2 to NH_3 by cofactor-BH_4^- mixtures was not observed[4]. C_2H_2 is readily reduced by many homogeneous and heterogeneous catalysts and can give only a single 2 e^- reduction product, urging caution in exclusive reliance upon it as a model substrate[5,6]. The observation that nitrogenase catalyses reduction of cyclopropene to both propene and cyclopropane in a ~2 : 1 ratio *in vitro*[7] and also *in vivo*[8] suggests a more demanding criterion[8] for evaluation of FeMo-co catalysis, one which still avoids the difficult energetic requirements for N_2 activation and reduction. This new criterion is strengthened by the fact that cyclopropene competitively inhibits N_2 (ref. 9). We find that incubation of FeMo-co with a suitable 'apoprotein' extract yields a reconstituted FeMo-protein activity for reduction of cyclopropene to propene and cyclopropane. FeMo-co alone, however, only catalyses cyclopropane formation in the conditions described[4] for C_2H_2 reduction. This result and other considerations suggest that active site homology between FeMo-co and nitrogenase has not yet been fully demonstrated on a function basis.

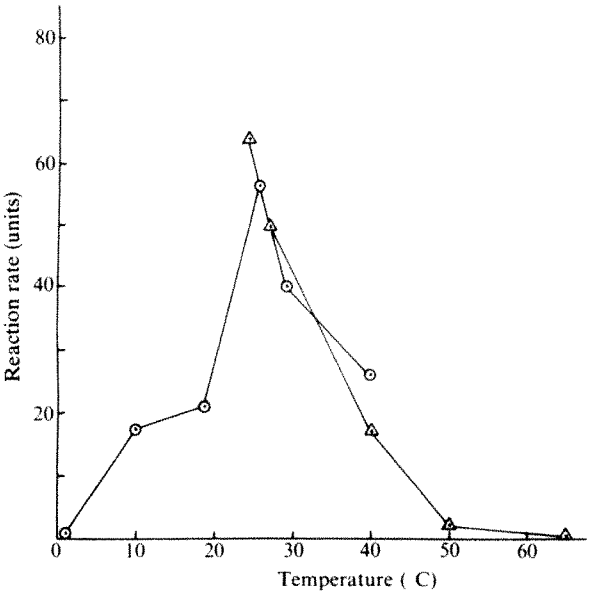


Fig. 1 Initial reaction rate–incubation temperature profile for FeMo-co-dependent BH_4^- reduction of C_2H_2 to C_2H_4. Identical 50 μl samples of FeMo-co were assayed as described in the text. ○, experiment 1; △, experiment 2.

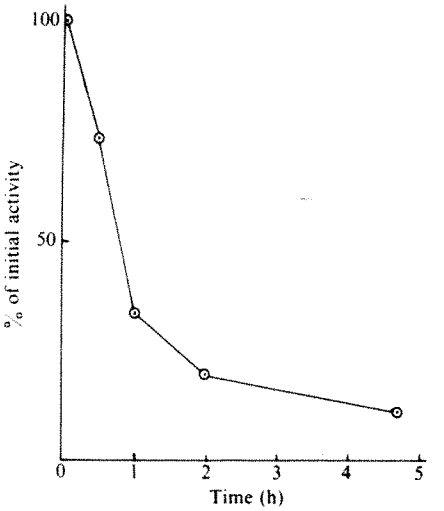


Fig. 2 Inactivation of the FeMo-co catalyst on exposure to air. 500 μl of the FeMo-co preparation was placed in an open 13 × 125 mm test tube and agitated at 150 r.p.m. in a 25 °C waterbath. At the indicated times 50 μl aliquots were removed and assayed for C_2H_2 reduction activity as described in the text.

FeMo-co was extracted from recrystallised *Azotobacter vinelandii* FeMo protein (Av1), specific activity 2,100[9], by a procedure adapted from that of Shah and Brill[1]. Typically, 140 mg Av1 in 2.8 ml of 25 mM Tris-HCl, *p*H 7.4 containing 250 mM NaCl was acidified by addition of 0.54 ml of 1 M citric acid at 4 °C. After 3 min, 2.4 ml of 0.5 M potassium phosphate, *p*H 9.0 was added dropwise with stirring and the protein precipitate was centrifuged for 7 min at 4,500 *g*. The pellet was extracted with 2.0 ml of dimethylformamide (DMF). The resulting suspension was clarified by centrifugation, and the pellet extracted with 2 ml of *N*-methylformamide (NMF). Both DMF and NMF were redistilled and contained 5 mM potassium phosphate (*p*H 8.0) and 2 mM dithionite. The NMF extract was layered on a 1 × 54 cm Sephadex G-100 column and developed in the same solvent (8 ml per h). FeMo-co elution from this column was monitored at 440 nm and was followed by 10-fold concentration under vacuum at 32 °C. All isolation and assay procedures were carried out under purified Ar. Samples were stored in liquid nitrogen and remained active for several weeks. BH_4^--dependent reductions were carried out in 22-ml serum vials containing (in 3 ml): 0.5 ml NMF; 80 mM sodium borate *p*H 9.6; 240 mM $NaBH_4$ and 4 mM sodium dithionite. Reaction was initiated with 50 or 100 μl FeMo-co concentrate and reduction of substrate (0.03–0.04 atm) was determined by GC. Activities (nmol substrate reduced per min) were corrected for background reduction, where necessary.

Shah and Brill[4] reported that C_2H_2 reduction by the BH_4^-–FeMo-co system at 25 °C was dependent on catalyst concentration but was not affected by C_2H_2 concentration (0.25–1.0 atm) nor by increasing the reaction temperature to 30 °C. No C_2H_6 was detected. Reduction activity was relatively stable to air inactivation (−16% after 18 h) at 4 °C.

Our results confirm that BH_4^--FeMo-co mixtures reduce C_2H_2 to C_2H_4 at an initial rate that depends on the FeMo-co concentration (determined over a range of 0 to 110 activity units). However, a dependence on the C_2H_2 concentration is observed at partial C_2H_2 pressures less than about 0.02 atm. Replacing the diluting gas (Ar) with He or H_2 (ref. 4) had no effect, and dithionite alone did not support reduction. Based on estimated Mo content, the specific C_2H_2 reduction activity of our FeMo-co preparations was 5–10% relative to homogeneous Av1 (Table 1), which compares well with the relative specific activity of 8% obtained by Shah and Brill[4]. The reaction rate increases above 0 °C to a maximum at 25–30 °C, then decreases (Fig. 1), presumably due to thermal lability of the catalytic species or to accelerated decomposition of the BH_4^-. The maximum in the

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rate profile occurring over 25–30 °C (Fig. 1) would explain the apparent invariability of the reaction rate to temperature reported previously[4]. The $t_{1/2}$ for loss of C_2H_2 reduction activity in air-exposed FeMo-co at 25 °C was nearly 1 h (Fig. 2), while the $t_{1/2}$ for loss of reconstitutive activity was less than 1 min[1]. The C_2H_2 reduction activity was abolished by 0.12 atm CO or 1 mM KCN in the assay system. Besides C_2H_4, the major reduction product, we detected traces (up to 2%) of C_2H_6 (Fig. 3). A low level of background C_2H_2 reduction activity present in assay blanks did not account for this minor product (Fig. 3). Subsidiary formation of C_2H_6 is not observed in ATP-dependent reductions of C_2H_2 to C_2H_4 by either intact or reconstituted nitrogenase with dithionite as electron donor, but is typical of BH_4^- reductions catalysed by simple Mo-thiol complexes[5,6,10,11].

As shown in Table 1, cyclopropene is also reduced by the BH_4^--FeMo-co system, and at a rate ratio relative to C_2H_2 reduction that approximates the corresponding ratio in reconstituted (or intact) nitrogenase assayed in standard conditions. However, formation of both propene and cyclopropane was observed uniquely with intact or reconstituted FeMo protein, the cofactor alone yielding only cyclopropane. The 'apoprotein' extract from Kp 5058 used in the reconstitution experiments had no detectable catalytic activity before incubation with FeMo-co (Table 1). CO at 0.12 atm inhibited the FeMo-co cyclopropene reduction by 89%. A comparable inhibition is observed with nitrogenase at $\sim 10^{-3}$ atm but the comparison is complicated by the presence of both low and high affinity CO-binding sites[12].

It seems clear *a priori* that care must be exercised in drawing analogies between the catalytic behaviour of BH_4^-–FeMo-co[4] and nitrogenase. ATP dependence, the reductant and the assay *p*H are quite different for the two systems, while the known instability of FeMo-co in anaerobic aqueous solution[1] places in question the actual species responsible for substrate reductions by BH_4^-–FeMo-co mixtures. The striking differences in O_2-lability between the catalytic and reconstitutive FeMo-co activities may be relevant to this ambiguity, although the possibility that catalytic and complementive functions in native cofactor involve distinct and non-interactive moieties cannot be excluded. Our experiments verify that on the basis of Mo content, FeMo-co added to aqueous BH_4^- is a highly effective, CO-sensitive catalyst for C_2H_2 reduction[4]. C_2H_2 has been recognised as a potentially misleading model substrate for nitrogenase, especially when used alone[5,6,9,13]. By the more stringent cyclopropene criterion, BH_4^-–FeMo-co is not functionally equivalent to nitrogenase. Interestingly, the inability of the cofactor to reduce cyclopropene to propene is shared by a number of transition metal complexes that also catalyse C_2H_2 reduction, including a Mo-thiol system[14]. Incubation of FeMo-co with Kp 5058 'apoprotein' extract restored propene formation. To the extent that appropriate chemical equivalence exists among (1) the FeMo-co catalyst in aqueous, alkaline BH_4^-; (2) the spectroscopically defined FeMo-co species isolated in NMF; and (3) those Mo and Fe ions in intact nitrogenase assigned to the NMF cofactor, our findings are consistent with, but do not prove, the postulate that FeMo-co may represent only a portion of the active site responsible for binding and reducing N_2.

Table 1 Comparison of nitrogenase and FeMo-co as catalysts for C_2H_2 and cyclopropene reduction

Catalyst	Relative activity (C_2H_2)	Reduction rate ratio (cyclopropene/ C_2H_2)‡	Formation rate ratio (propene/ cyclopropane)
Intact nitrogenase*	1.0†	0.58	2§
Reconstituted nitrogenase*‖	—	0.56	2.5§
FeMo-co¶	0.05–0.10 #	0.40	<0.003
'Apoprotein' extract**	<0.0001	—	—

All data represent average values from two or more experiments.
* Assayed as described in ref. 7.
† Reference activity of Av1 (text) determined with 0.04 atm C_2H_2 and optimal amount of Fe protein.
‡ Equivalent pressures (0.03–0.04 atm) of both substrates. Initial rates derived from time course data were used.
§ Compare with refs. 7, 8.
‖ FeMo reconstitution was performed according to ref. 1 using 5 or 200 μl FeMo-co (text) and crude extract from Kp 5058. Some variability in activity was encountered at the reconstitution step.
¶ Assayed (text) using 50 or 100 μl of stock preparation.
Ref. 4 gives a value of 0.08.
** Taken in an amount sufficient to give optimal C_2H_2 reduction activity with 500 μl FeMo-co preparation and tested under standard nitrogenase[7] assay conditions.

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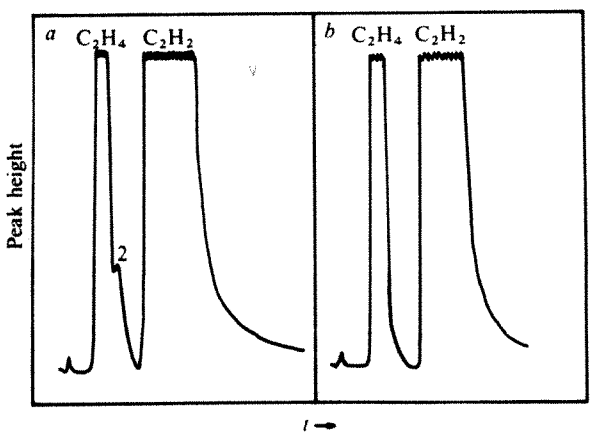


Fig. 3 Detection of C_2H_6 in FeMo-co dependent reduction of C_2H_2. The BH_4^- reduction assay conditions were as described in the text. GC analysis was carried out on a FID instrument equipped with a 2′ Porapak N column operated at 70 °C. The He carrier gas flow rate was 30 ml min^{-1}. *a*, GC trace for 0.15 ml of the gas phase from an assay vial after 4 min incubation at 25 °C in the presence of 115 units of FeMo-co. The initial blip is due to Ar in the sample. The small shoulder labelled '2' was identified as C_2H_6. *b*, GC trace for a control vial lacking FeMo-co after 45 min incubation.

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matters arising

Evolution of dominance

CHARLESWORTH[1] has noted that very harmful mutant alleles in *Drosophila* are usually almost recessive in fitness to wild type, whereas in the case of mutants which are mildly disadvantageous, dominance of the wild type is much less marked. He points out that this result agrees well with Wright's theory of dominance but argues that it does not agree with Fisher's theory, for the following reason. In the case of dominance brought about by modifiers, the rate of change in frequency of a modifier is essentially independent of the fitness of the mutant homozygote, over most of the time that dominance is evolving. Thus, given equal times for evolution of dominance, dominance should be about as marked for mildly as for seriously disadvantageous mutants, which is not in fact the case.

However, the assumption of equal times may not be justified. A mutation which is lethal or near-lethal in laboratory conditions is likely to be very harmful in almost any environment and over very long periods of time, giving ample opportunity for the evolution of dominance. A mildly disadvantageous mutation, such as a change leading to an enzyme with a slightly altered *p*H or temperature optimum, is less likely to be disadvantageous in all conditions or for very lengthy periods of time. Thus, on Fisher's theory we should expect evolution of dominance to be much more marked in the case of very harmful mutants. The same would be true for evolution of dominance as envisaged by Haldane. Thus, the observed differences in degree of dominance do not provide a critical test of the relative merits of the three theories.

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CHARLESWORTH REPLIES—The suggestion of Gale and Mackay is an interesting one, and introduces a new dimension to the theory of dominance modification. It is not obvious, however, that it invalidates my argument[1]. In the first place, there is no direct evidence for their view that mutations of large effect have lower environmental variance in their selection coefficients than mutations of small effect.

Nonetheless, even if we accept this idea, it is still not necessarily true that the conclusions of Gale and Mackay are correct. To evaluate them, we need to consider models of the modification of dominance at a locus subject to temporal fluctuations in selection coefficients. Theoretical studies[2–4] have shown that such fluctuations generate a probability distribution of gene frequency, even in an infinite population. The intensity of selection on a modifier of dominance in a given generation can be calculated by multiplying the advantage to the heterozygote by the frequency of heterozygotes[1]. The net intensity when gene frequencies and selection coefficients are fluctuating is given by the appropriate expectation over the joint distribution of gene frequency and selection coefficients. If the fluctuations in selective values are long term, the population will spend a long time in each environmental state, and so will be close to equilibrium for much of the time. When the population is in environmental state i, the intensity of selection on a modifier of effect Δh on the level of dominance of a mutant allele is thus approximated by $2u(\Delta h/h_i)$, where u is the mutation rate and h_i is the dominance coefficient in environmental state i (see ref. 1). There is thus no dependence on the strength of selection against the mutant homozygote, or on the amount of environmental change.

In the case of rapid environmental fluctuations, we need to use the gene frequency distribution at the locus subject to dominance modification[2–4]. A complete analytical solution for this is not yet available. To reconcile the experimental findings[1] with Fisher's theory we would have to find that the net intensity of selection for dominance modification is much weaker for genes of small average homozygous effect than for genes of large effect, or for genes whose selective values are constant in time. Genes of slight average homozygous disadvantage will tend to have a gene frequency distribution with a relatively high mean[3], so that the expected frequency of heterozygotes will be higher than for genes with a large effect. This will tend to counteract the smaller advantage of dominance modification in the heterozygotes, just as in the case with no fluctuations. A strong relationship between average homozygous effect, the intensity of environmental fluctuations and the advantage of dominance modification thus seems highly unlikely. A relationship of this sort may perhaps hold, but the effect might even work in the opposite direction. A definite answer to this question can only be obtained by carrying out the relevant calculations.

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Haemoglobin S and *P. falciparum* malaria

PASVOL, WEATHERALL AND WILSON[1] have proposed an hypothesis to account for the protection given by haemoglobin S (HB S) against *Plasmodium falciparum* in heterozygotes for the sickle cell gene. This hypothesis depends on the argument that the last stages of a 48-h synchronous intra-erythrocytic growth cycle do not take place in the peripheral circulation, but in the deep capillary bed of some internal organ with a low oxygen tension, and that the low oxygen tension depresses invasion and development by the parasite in red cells containing HB S.

They therefore compared the rate of invasion and subsequent growth and development *in vitro* by parasites in conditions of low and high oxygen tension in red cells containing Hbs AA, AS and SS. The data given in their Table 1 do not, in our view, support the hypothesis that low oxygen tension impedes the rate of invasion and development by merozoites of *P. falciparum* in erythrocytes containing Hb S. We feel that the use of a ratio of the number of ring-form parasites per 100 red cells under low oxygen tension to that in aerobic conditions is misleading and statistically dubious, as a normal distribution could not be expected. However, one arrives at a conclusion very similar to that of the authors if one calculates the arithmetic differences for each of the pairs of measurements.

On the other hand, examination of the mean values, rather than the differences, for invasion and development in the various conditions, leads to a rather startling conclusion (our Table 1). With the exception of SS haemoglobin in aerobic conditions, all the other means are

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virtually indistinguishable. However, Hb SS in aerobic conditions shows marked enhancement of invasion and development rather than, as suggested by the authors, suppression of growth in conditions of low oxygen tension. One accepts, of course, the statistical limitations of the relatively few experimental results for Hb SS.

Pasvol *et al.* also maintain that their hypothesis is supported by the data in their Tables 3 and 4. This interpretation ignores the significance of the large number of abnormal forms observed for red cells maintained in conditions of low oxygen tension (Table 3—74% for Hb AS; 20% for Hb SS—and Table 4). Furthermore, the use of a growth score from 1 to 6 for the different stages of parasite development, with abnormal forms being given an arbitrary value of 0, seems unjustifiable in the likely event that these abnormal forms represent the growth of the parasite to a stage at which no further development was possible, thus aborting the parasite life cycle and producing degenerate forms.

Table 1 Mean and s.e. for the number of ring-forms per 100 red cells after 12 h in aerobic or low oxygen conditions (data from Pasvol *et al.*[1])

	Aerobic	Low oxygen	
Haemoglobin			
AA	11.71±1.48	12.32±1.23	(13)
AS	11.52±1.31	10.35±1.34	(11)
SS	18.13±2.48	11.73±1.01	(4)

We have recently suggested that production of fatty acids by *Plasmodium knowlesi* and *Plasmodium berghei* may represent the molecular mechanism by which the plasmodial parasite damages red cells and produces haemolysis which may often be out of all proportion to the parasitaemia[2,3]. Similar evidence for the involvement of fatty acids in *Plasmodium lophurae* infections has been reported[4], and the whole question has been reviewed by Holz[5]. The fatty acids within the red cell are buffered by the haemoglobin that is present at high concentration, and Hbs AS, SS and SC are more effective than Hb AA at buffering fatty acids. This increase in buffering capacity has been rationalised at the molecular level in terms of the change in the amino acid residue at the 6β position[3,6], and could represent one of the mechanisms by which Hb protects against *P. falciparum* malaria.

In conclusion, we suggest that the data presented by Pasvol *et al.*[1] do not support their hypothesis concerning the mechanism for protection against *P. falciparum* malaria by sickle cell haemoglobin.

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PASVOL, WEATHERALL AND WILSON REPLY—Laser and Klein might have been less startled by the results of their analysis of our data[1] if they had considered more carefully the haematology of sickle cell anaemia; taken into account our previous work which shows that *Plasmodium falciparum* preferentially invades young, metabolically active red cells[2,3]; and realised that the data in Table 1 of our paper deal only with parasite invasion and say nothing about development. The red cells in sickle cell anaemia have a short survival time which is reflected by a marked reticulocytosis[4]. Therefore, it is only to be expected that the degree of invasion of sickle cell anaemia (SS) cells by *P. falciparum* would be considerably greater than that of sickle cell trait (AS) or normal (AA) cells. For this reason the only valid comparisons are between cells of like ages (SS with SS, AA with AA) maintained aerobically or under reduced oxygen tensions. The data in Table 1 simply show that cells containing a significant amount of Hb S, such as SS, are invaded less actively under reduced oxygen tensions. However, we are grateful to Laser and Klein for restating that young red cell populations are preferentially invaded by *P. falciparum*.

The main emphasis of our paper was on parasite development once inside the red cells (Tables 3 and 4)[1]. There is no doubt that parasites in cells containing Hb S failed to develop, but only in conditions of reduced oxygen tensions. Furthermore, sickling was not required for this to occur. Laser and Klein are quite incorrect in their assumption that the abnormal forms of the parasite maintained in low oxygen conditions represent the growth of the parasite to a stage at which no further development was possible. Rather, they represent degenerate parasites at early stages of development. This was determined both morphologically and by analysis of pigment content. Furthermore, smears prepared at an early stage in the cultures showed that these abnormal forms were already present. Even if these were entrapped mature forms, which they were not, it is difficult to understand the marked differences observed between the aerobic and low oxygen tension cultures. The growth scores used were simply a means of condensing the data for a large number of experiments. Because the abnormal forms were mainly rings which had failed to develop, it seemed logical to rate them at less than the score of 1 designated to rings. Indeed, the results of the typical experiments in Tables 3 and 4 (ref. 1) stand without the need for growth score analysis.

Laser and Klein's hypothesis that Hb S has a greater buffering capacity than Hb A, thereby reducing fatty acid-induced haemolysis and impeding merozoite release from red cells[5] is interesting. However, at the stage of development before merozoites are released, over 75% of the haemoglobin has been digested by the parasite[6]. Indeed, on this basis it could be argued that relatively increased sensitivity of Hb A-containing cells to lysis might result in premature death and hence offer relative protection to normal individuals with malaria! Furthermore, Laser and Klein's hypothesis is quite incompatible with the mass of epidemiological data suggesting that protection of sickle cell heterozygotes is primarily against *P. falciparum* and not against all species of malarial parasites[7]. Our hypothesis takes into account the behaviour of the mature forms of *P. falciparum* which lodge in deep tissues where the oxygen tissues are low[8] and similar to those used in our experiments[9]. The tendency of Hb S to sickle under reduced oxygen tensions seems more rational than a subtle buffering effect of Hb S as yet not clearly defined in *P. falciparum* infections.

Thus, we believe that our findings, taken together with the essentially similar results of Friedman, who used an entirely different culture system[10], indicate that the growth of *P. falciparum* is limited in cells containing HB S exposed to reduced oxygen tensions and hence provide a reasonable explanation for the protective effect of Hb S against *P. falciparum* malaria.

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Harvey Goldstein

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Applied Problems in Memory

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Memory plays a vital part in all human activities and a proper understanding of how it works in real life is of great importance to many practical and social problems. However, for many years there has been less emphasis on research into applied problems of memory than into theoretical aspects. The present book examines applications of memory research with contributions from eminent workers in a number of areas. The book covers an impressive range of problems in which psychologists have recently been engaged, from the more obvious improvements in techniques for better remembering and in education, to less evident areas such as reading, memory in the aged, effects of stress, memory for skills and disorders of memory. The wealth of practical examples given throughout the text contribute to make a highly readable account of the subject. Within its wide scope, the book has a great deal to offer, not only to cognitive psychologists and workers in educational, clinical and industrial psychology, and in ergonomics, but also to anyone concerned with practical problems of memory at all levels.

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T. M. Fenchel and T. H. Blackburn

August/September 1979, xii + 266pp., £14.60/$31.00 0.12.252750.X

This book describes the influences of bacterial metabolism on the chemical environment of the biosphere. Its readers will include general microbiologists who want to find out about the ecological significance of micro-organisms; ecologists whose microbiological background is limited yet who wish to understand the role of bacteria in natural ecosystems; postgraducates in microbial ecology; and, finally, applied scientists — such as soil scientists or environmental engineers — involved with microbial ecosystems. The discussion of the energetics of bacterial processes serves as a general structure for most of the topics in the book since such considerations may afford insight into which processes are important in natural ecosystems. Within this basic framework, two major aspects of bacterial metabolism are stressed. Firstly, the chemical evolution of the biosphere and secondly, the manner in which different element cycles interconnect in natural ecosystems. Appendices are included with information that will enable those with only elementary knowledge of microbiology, energetics and ecology to understand the book with no trouble.

The Mathematics of Finite Elements and Applications III, MAFELAP 1978

edited by J. R. Whiteman

July/August 1979, c 520pp., £15.00/$31.50 0.12.747253.3

This volume records the Proceedings of the Third Conference on the Mathematics of Finite Elements and Applications, which was held at Brunel University in 1978. The decision to hold the conference followed the success of the previous two. The book contains the 10 main invited papers and 30 contributed papers, which were presented as lectures at the meeting, together with the abstracts of some 30 other papers that were presented in poster sessions. As before, the aim of the conference was to bridge the gap between the mathematical theory of finite elements and their applications. In particular, the main invited lecturers and the contributed papers were chosen so as to give coverage to as many as possible of the developments that have taken place in the finite element field in the three years since the last conference. Several chapters contain suggestions by the authors as to possible future paths that finite element techniques may take.

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edited by B. Lindblom and S. Öhman

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The variety of new theories and related research is mirrored by the range of topics covered in this book: the quantitive modelling of the human vocal tract and its acoustical properties; acoustic phonetic description of various languages; the perception of speech and the development of objective measures of speech quality; applications of speech research; and the development of distinctive feature frameworks for linguistic theory. These original research reports, together with a number of review articles, provide a broad, up-to-date survey that makes the book a valuable reference work. The scope of the contributions will attract students and professionals engaged in research into speech communication, and those involved in the related fields of speech pathology, psychology, physiology and acoustics, and relevant areas of computer science and engineering.

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Volume 1, Number 1 scheduled for March 1980

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The Cretaceous system is of interest in many different disciplines, including palaeontology, stratigraphy, geophysics, oceanography, petroleum geology, geochemistry, sedimentology and tectonics. This new and wide-ranging journal aims to cover all these fields in their applications to Cretaceous research. It will also consider methodological papers if they can be applied to problems of interest to workers on Cretaceous rocks, and will give an important place to regional geology. Articles both long and short will be accepted but priority of publication will be given to short reports of research papers, in order to reduce publication delays to a minimum. All papers will be refereed, and may be submitted in English or French — papers in French must have a detailed summary in English. There will be scope for book reviews and the proceedings of symposia on subjects of relevance to the Journal's readers. *Cretaceous Research* will aim at high-quality contributions from the outset, and will be of great interest and value to all petroleum companies, libraries and individual workers on the Cretaceous or in foraminifera, plate tectonics, deep-sea research, regional geology or stratigraphy.

APPETITE

The Journal for Research on Intake, its Control and its Consequences

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Publication: Quarterly
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This new interdisciplinary journal concerns appetite — the tendency to seek and consume substances, be they food, water or any other ingestable materials — in all its aspects. It will cover the basic appetitive and ingestatory mechanisms of interest, including neural, systematic physiological, biochemical, pharmacological, sensory, psychological, social, cultural and economic processes. The applied study of appetite may be *clinical* (weight control, obesity, dietary health risks, anorexia nervosa and malnutrition), *industrial* (sensory evaluation, consumer research on foods and drinks and feed regulation for animal production) or *zoological* (conservation and pest control). Papers are invited from any of these areas, although material submitted should have substantial relevance to intake or attitudes to ingestable substances. An important aim of the journal will be to publish innovatory papers crossing the traditional boundaries between disciplines: reviews, comments and mini reviews with an integrative bias are welcomed as well as reports of new investigations. There will be a place for book reviews, abstracts or reports, summaries of papers published elsewhere, bibliographies and announcements of relevant meetings — indeed anything appropriate to the general area of appetite. This major new publication, while not affiliated to any society, is intended for researchers in a wide range of disciplines including psychology, anthropology, zoology, pharmacology, psychiatry, medical research, nutrition, food science and applied social science.

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reviews

Prediction of a global cooling

William W. Kellogg

Ice or Fire? Surviving Climatic Change. By D. S. Halacy, Jr. Pp. 212. (Harper and Row: New York and London, 1979.) $9.95; £5.95.

THE fascinating subject of climate, and the likelihood that the world may soon witness another climatic change, has attracted the attention of several semi-popular book writers in the past few years (*Hothouse Earth,* by Howard Wilcox; *The Weather Machine,* by Nigel Calder; *The Genesis Strategy,* by Stephen H. Schneider; and *Climates of Hunger,* by Reid A. Bryson and T.S. Murray, to mention but four of the more celebrated ones). As one contemplates these very different approaches to climatic change and its many implications for mankind one is struck by the difference in tone and approach taken by the scientists actually doing climate research (Schneider and Bryson) and the science-writers who are not actively working in the field (Wilcox and Calder — and now Halacy).

It is not surprising that this difference should exist. Research scientists are trained to be critical and questioning, and are usually very cautious about jumping to conclusions too quickly (though some do, of course). This trait tends to make their scientific writings a bit dull for the average intelligent layman, although it should be quickly added that Schneider and Bryson are among the liveliest of scientist-writers.

On the other hand, a science-writer approaches his subject with the eye of the journalist, and may be on the lookout for a good story rather than a presentation of a balanced account. Halacy, in *Ice and Fire?* like Calder, has chosen to write a book whose central theme is the prediction of a global cooling as the beginning of a new ice age — perhaps occurring very quickly. Although he mentions the fact that Calder's book was criticised by many climatologists and other scientists as "irresponsible scaremongering", he has followed the same chilling path: The coming ice age, the slow growth of ice sheets over North America and Europe, the lowering of sea level as the water is trapped as ice on land, the changing patterns of precipitation, and the global scramble for new sources of food as the old ones become unfavourable are all described — almost, it would seem, with relish.

There is a strong counterpoint to this prediction of an imminent ice age, and that is the thought that we might be able to modify or avert it if we tried hard enough. This theme is developed in Part I, which occupies more than half of the book. Halacy wrote a book called *The Weather Changers*, in 1968, and the part on weather (and climate) modification has the same title and seems to be an update on the earlier work, with an emphasis on the subject of *weather* modification — notably rainmaking. There is relatively little said about *climate* modification that would be relevant to the prospect of a coming ice age.

The book is written with an engaging style, but it gives the overall impression of a clever collage of anecdotes, theories, and facts, rather than a development of a main thesis. Furthermore, even a non-expert will notice that he has blurred his timescales cleverly (as did Nigel Calder, whom he quotes extensively), giving the impression that the advent of an ice age could occur in a matter of a decade or so — perhaps it will take a century if we are lucky, he says. This will be recognised as Calder's "snowblitz". Halacy does mention that the periodic fluctuations that have accounted for major natural climate changes are 2,500 to 100,000 yr long (although there seem to be some weaker climate changes associated with shorter periods), but this matter of time-scale is never made clear in talking about the impending ice age. Few climatologists would question the likelihood of a new ice age coming *eventually* — but how many thousands of years from now?

Similarly, he does mention the idea that the burning of fossil fuels and the addition of carbon dioxide to the atmosphere is likely to cause a global warming in the next few decades, and that a "worst case" estimate calls for a warming of over 3°C by the year 2050. This, he concedes, "would play havoc with weather and climate". However, he dismisses the prospect of a warming due to human activities by saying, "There are those who reason that other pollutants stop more incoming radiation and will outbalance the CO_2 to create a cooling rather than a warming trend." This is an idea proposed some time ago by Bryson and others, but there are few left who now subscribe to it.

The expectation of a global warming in the next few decades and centuries, rather than the onset of an ice age, was expressed most authoritatively by the more than 100 experts assembled by the World Meteorological Organization (WMO) for the World Climate Conference in Geneva in February 1979. Said the Declaration of that Conference: "We can say with some confidence that the burning of fossil fuels, deforestation, and changes of land use have increased the amount of carbon dioxide in the atmosphere . . . and it appears plausible that [this] can contribute to a gradual warming of the lower atmosphere, especially at high latitudes . . . It is possible that some effects on a regional and global scale may . . . become significant before the middle of the next century."

Thus, Halacy seems to have a rather poor batting average in his predictions, if we can judge them against current evidence; and one of his statements, presumably written a year or more ago, has already turned out to be quite wrong! "There is no evidence of a global or national [US] climate programme of significance" (p 205). Early in 1979 the US Congress passed the National Climate Program and it is now part of the law of the land; and in May 1979 the Congress of the WMO adopted the World Climate Programme. These are both official programmes being actively pursued on many levels, and the WMO's Programme in particular demonstrates the serious concern of all nations with the problems of climatic variability and climatic change.

Although *Ice or Fire?* deals with a vital set of questions, and although it contains a great deal of information recounted in a lively manner, it is seriously flawed by a combination of emphasis on subjects only remotely relevant to the main theme, and by a main theme that is itself based on a faulty premise. Perhaps if Halacy had written his book a year or two later he would have followed the lead of Wilcox and played on the prediction of a warmer Earth instead of an ice age. That too would have been a good story. □

William W. Kellogg has just completed a 14-month appointment with the WMO in Geneva, where he served as Adviser to the Secretary-General on the World Climate Programme. He has now resumed his post as Senior Scientist at the National Center for Atmospheric Research, Boulder, Colorado.

Geometrical aspects of general relativity

Gravitational Curvature: An Introduction to Einstein's Theory. By Theodore Frankel. Pp. 172. (Freeman: San Francisco, 1979.) £5.20.

TEXTBOOKS on general relativity fall roughly into two classes — the geometrical and the physical. As an example of the former class we have J. L. Synge's fine book *Relativity: The General Theory* (North-Holland: Amsterdam); and in the latter class there is S. Weinberg's excellent *Gravitation and Cosmology* (Wiley: New York). Theodore Frankel's book falls squarely into the geometrical division, as suggested by his title.

Gravitational Curvature starts with a short chapter on special relativity which does no more than set the scene for what is to follow. In chapters 2 and 3 Einstein's equations are approached through the principle of equivalence, the gravitational red shift and Poisson's equation. It is in the essence of the author's treatment that Poisson's rather than Laplace's equation should appear at this stage because throughout he is as much interested in the right-hand side of Einstein's equations

$$R_{ik} - \tfrac{1}{2} g_{ik} R = 8\pi\kappa T_{ik} \qquad (1)$$

as he is in the left, an attitude which is still fairly uncommon.

It is in chapter 4 that the author declares his hand, in a section headed "A Geometric Form of Einstein's Equations", where he rewrites (1) as

$$8\pi\kappa T(\zeta, \zeta) = -\tfrac{1}{2}\varepsilon_\zeta R_{V^3} + \mathrm{tr}(\mathbf{b} \wedge \mathbf{b}). \quad (2)$$

Here ζ is a unit vector at a point p in space-time V^4, and V^3 is any hypersurface normal to ζ; R_{V^3} is the scalar curvature of V^3, and $\mathrm{tr}(\mathbf{b} \wedge \mathbf{b})$ depends on the extrinsic curvature of V^3 embedded in V^4 (ε_ζ is ± 1). What (2) says is that however V^3 may curve at p, the left hand side (which in an older notation is $8\pi\kappa T^{ik}\zeta_i\zeta_k$) depends only on the normal ζ. As an application consider a perfect fluid and let V^3 be a totally geodesic space-like hypersurface (that is, one whose second fundamental form vanishes). The second term on the right of (2) disappears and we have

$$R_{V^3} = 16\pi\kappa\varrho.$$

ϱ being the proper density, which, as the author remarks, is a particularly beautiful consequence of Einstein's equations.

The author has not derived (2) just for fun. On the contrary, he uses this form of the equations repeatedly — to derive the Schwarzschild exterior solution in chapter 5, for the general static spherical mass distribution in chapter 11, for cosmology in chapter 12, and for other applications. In the remaining chapters are treated the perfect fluid, equations of motion, light rays, and, in some detail, electromagnetism in special and general relativity.

The treatment throughout is quite advanced. No introduction to tensor calculus or differential geometry is given, and there are no examples or exercises to help the student. Not merely is a knowledge of tensors assumed, but the reader is expected to be acquainted with differential forms, and the index-free notation as in (2) above. *Gravitational Curvature* is therefore not really a textbook. The author writes in the Preface that "ideally, the material here would be given as the third quarter of a year course devoted to manifolds and Riemannian geometry", but it seems to me that the first two quarters of such a course would be pretty indigestible.

Who then will benefit from the book? I believe that to trained relativists it should cast a refreshing new light on the geometry of their subject. It would be nice to think that there will be some enlightenment passing in the other direction, by which I mean that differential geometers looking at the book may see how close relativity is to their subject, and even turn their attention to it for a while.

W. B. Bonnor

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Fludd debates

Robert Fludd: Hermetic Philosopher and Surveyor of Two Worlds. By J. Goodwin. Pp. 96. (Thames and Hudson: London, 1979.) Paperback £2.95.

CHIEFLY remembered today as the target of polemical attacks by seventeenth-century men of science like Kepler, Mersenne and Gassendi, Robert Fludd (1594—1637) was among the most famous physicians and occultists of his time. In an interesting paper, published in English translation in 1955 ('The Influence of Archetypal Ideas on the Scientific Theories of Johannes Kepler'), the physicist Wolfgang Pauli showed that the Fludd—Kepler debate could not simply be regarded as one between an obscurantist occultist and a modern 'scientist'. The polemic of Mersenne and Gassendi more directly rejected Fludd's ideas in the light of the corpuscularian and mechanistic philosophies then being developed. However obscure and repellent they may now seem, some grasp of Fludd's doctrines is essential for a genuine historical understanding of an influential current which was to be vanquished and eclipsed by the science of Galileo and Descartes.

Fludd's works are rare and have become expensive collectors' items chiefly for the beautiful engravings with which they were adorned by de Bry and Matthieu Merian in their sumptuous Frankfurt editions. A study of the engravings is essential for making it possible to penetrate Fludd's strange and fantastic cosmology. That has been made much easier by Dr Joscelyn Goodwin, a musicologist, who has brought together 120 of the plates in a well-printed and reasonably priced book.

According to Dr Goodwin in his biographical introduction, the chief value of his collection of engravings lies in reminding us "of the possibility, indeed the imminence, of a cosmic view free alike of the myopia of materialism and the absurdities of naive spiritualism'. But his full bibliography will usefully guide the reader to other recent sources in which an attempt has been made to place the Fludd debates within the scientific and philosophical *milieu* of the seventeenth century.

P. M. Rattansi

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Biological foundations of sensory processing

Sensory Processing, Perception, and Behaviour. By R. B. Livingston. Pp. 106. (Raven: New York, 1978.) Paperback $11.05.

IN his preface to this mercifully slim volume, which was aimed originally at psychiatrists, the author says his intention is "to present the main biological foundations for perception, judgement, and behavior, in an evolutionary and developmental context.". The ground is covered in 83 pages, but the ride is uncomfortably rough.

The book is extraordinarily hard to read, and I had great difficulty in making sense of parts of it. The principal problem is the pretentious prose, which actively hinders comprehension. Here are two representative samples: "Humankind is presently in need of experiencing unprecedented perceptual, judgemental, and behavioral discontinuity, from prior to new forms of imaging"; and "similarity of views are attractive to one another, leading to greater introversion of perspectives and greater societal self-righteousness".

The other difficulty is the organisation; although the book is divided into four chapters, Behavior and Evolution, Perception, Sensory Processing, and Sensation, the order of topics in the last three is arbitrary, and seems quite confused. I often failed to grasp the thread of an argument simply because the discussion was so disconnected.

Underneath these deficiencies, I found the work interesting, principally because its broad coverage introduced me to some unfamiliar observations and ideas. However, I valued the book less for the author's synthesis of general principles than for the provision of references and pointers to other reading.

The chapter on Behavior and Evolution sets the tone for the rest of the monograph. Here Livingstone makes the point (not new to psychologists) that the idea of tight connections between sensory stimulus and behavioural response is an impoverished view of what controls behaviour. He then goes on to develop the argument that man's flexible and complex behaviour depends upon his development of a large brain, which is only possible if infants are immature at birth, which in turn means that most of man's skills have to be learned. So man becomes the flexible animal. These arguments are plausible but, in common with many made in the book, are not developed thoroughly enough to permit careful examination.

The chapter on Perception introduces the idea that our capacity to structure our sensory input depends on our having, in our brains, adequate models of the world. Here Livingston touches upon ideas that have been influential in perceptual psychology for nearly forty years, but he does not acknowledge this work. His discussion also neglects many worthwhile and exciting developments in 'Cognitive Psychology', and the insights into perceptual processing that have been obtained from work on the recognition of objects by computer programs. The result is a naively optimistic view of the ease with which psychological phenomena can be related to physiological events in the brain.

The chapter on Sensory Processing contains an odd mixture of topics that are not well integrated. Lateral Inhibition is included, as might be expected, but so is a summary of the psychology of perpetual development, which I found surprising. I had expected to see some discussion of broad principles that are common to the operation of the different sensory systems —for example, Müller's doctrine of specific nerve energies and its modern counterpart, the single unit doctrine—but nothing of the sort is mentioned. Moreover, there is no discussion of the sensory codes used for the transmission of information. There are interesting sections on feedback and feedforward control mechanisms, and on the centrifugal control of afferents, but again the author fails to place these in any larger context.

The final chapter, on Sensation, is a good deal more specific than the others, and the major part of it is devoted to a straightforward review of the physiology and anatomy of the different receptor systems and their associated pathways. I was again disappointed that the author did not bring out common principles in their operation.

Livingston's aim was to provide a coherent view of how modern biology can help us better understand some old problems in philosophy and psychology. He falls short of his goal.

Peter Lennie

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Victorian medicine

The Scientific Revolution in Victorian Medicine. By A. J. Youngson. Pp. 237. (Croom Helm: London, 1979.) £9.95.

THE bookshop browser whose eye is caught by this title might well turn to the preface to learn about the aims of the book and to the concluding chapter to find a summary of the content of the book. Impressed, he might take it home, where a more leisurely inspection would reveal a strangely unrelated text sandwiched between the preface and conclusion.

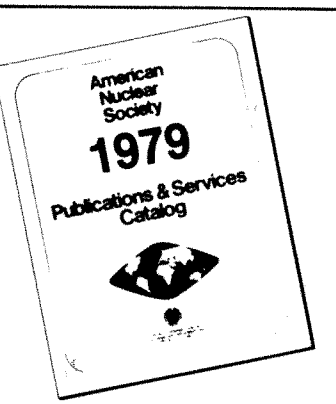
We are wisely advised in the preface to avoid the trap of thinking that all who resisted new ideas were merely dull or obscurantist, but the sandwich filling falls into this very trap: "pre-scientific" medical men suffered from "slovenly thinking"; they "did not know enough medicine" (the author means *our* medicine); Sir Henry Holland was "not altogether foolish" in following Celsus' advice about the need for cheerfulness in the sickroom — that of six prime ministers and two queens, in Sir Henry's case — but in doing so he "seriously underestimates the importance, or at least the potential importance, of scientific and technical [*viz*, twentieth century] medicine".

The modern observer of the early nineteenth-century hospital might well be "struck with the utmost astonishment" if he was a modern doctor, but, I suggest, if he were a modern historian he would understand the absence of precautions when the threat was not known; he would have doubted whether some speculations of the time were "sensible" simply because they were later proved to be right; and he would certainly have disagreed with the notion that the renaissance idea of the generation of the barnacle goose was a *denial* of *modern* biology.

The concluding chapter attempts a formal analysis of the reasons for resistance against new ideas, and a number of interesting points are raised. These should have been tied in more closely with the previous chapters, where the examples that illustrate the general statements are comparatively standard accounts of the introduction of anaesthesia, antisepsis and related topics. These accounts differ from others largely in the author's dependence on the *Lancet*. To have these accounts together in one volume is convenient, at least, and the historical style might well prove attractive to those with an interest in the history of their profession.

R. K. French

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Plant protection

The Plant Protection Discipline: Problems and Possible Developmental Strategies. By W. H. Sil, Jr. Pp. 190. (Wiley: Chichester, UK, and New York, 1979.) £17.75.

THE title of this book is misleading, although the subtitle does imply that it is concerned not with a factual account of the subject, but with a discussion of certain aspects. It is essentially a series of essays which consider the problems caused by pests, diseases and weeds in the major agricultural crops of the world, the ways in which research, development and advice are used to bring them under control, and the conflicting interests involved.

The problems illustrated are almost exclusively American, although there is some reference to the work in developing countries of international agencies such as FAO and the Ford and Rockefeller Foundations. There is an excess of quotations which are so numerous and conflicting that it is difficult to discern the author's own views. Indeed he apologises for them in his introduction: "This is not a scientific treatise. Rather it is a compendium and summary of the thoughts of the leading proponents or opponents of this new discipline. Consequently, there are many quotations, far more than most scientists, including the author, might prefer."

The book is intended for "government officials, administrators and leaders in the various sub-disciplines." It argues the case for an interdisciplinary approach to the increasingly serious problem of crop losses from pests and diseases. This subject is certainly one of urgency and the reader is given a comprehensive account of a situation which has arisen chiefly from increasing concern about the pollution of the environment by pesticides and an increase in the incidence of resistance and tolerance of pests and diseases to chemical control. (A recent report from the UN Environment Programme shows that the number of insect and mite species which have developed tolerant strains increased from 182 in 1965 to 364 in 1977.) The crop losses caused by pests, diseases and weeds are serious and worldwide, and the sophisticated systems of monoculture in the developed countries are as dependent on effective pests and disease control as are the more primitive systems of the developing countries which are seeking to exploit the improved varieties bred specifically for the Green Revolution.

To meet this challenge Dr Sil maintains that there is a need for a new profession to educate 'general practitioners' of plant protection. He complains with some reason and factual support that agriculture is a low prestige subject, especially in developing countries and that applied research in agriculture is less prestigious than pure research. He distinguishes differences in attitudes between entomologists and plant pathologists, a distinction which is not nearly so significant in Europe as it seems to be in America. There is brief reference to legislation governing the use of pesticides and schemes for certification of pesticides and those who apply them. There is a lengthier consideration of the need for cooperative efforts between the various disciplines and agencies involved and at times, the author becomes obsessed with the technical jargon of 'crop protection', 'plant protection', 'pest management', 'Biological control', 'integrated pest control' and so on, so that he loses sight of his main theme in the minutiae.

The remaining chapters of the book are devoted to a consideration of possible organisational strategies as they relate to collaboration between official research and advisory organisations and the chemical industry. The emphasis throughout is on the integration of education, research and advice.

There is little new in this treatment but the book serves a useful purpose in bringing together many topics that are of current worldwide concern. Interesting though the discussion may be, however, the readership of the book is likely to be very limited. It is hardly a book of reference because both the situation and attitudes are changing all the time; and it is expensive for what it is.

D. Rudd-Jones

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● Paul Colinvaux's *Why Big Fierce Animals are Rare* (Princeton University Press: New Jersey; for review, see *Nature* **273**, 787; 1978) will be published in the UK in November by George Allen and Unwin (London) at £6.50.

HLA and H-2

HLA and H-2: Basic Immunogenetics, Biology and Clinical Relevance. By H. Festenstein and P. Démant. Pp. 215. (Edward Arnold: London, 1978.) Paperback £9.

TRANSPLANTATION immunology has evolved in an attempt to understand, and prevent, rejection of foreign grafts. Although we may have been getting closer to this goal, the impact of this research has not been restricted to that old dream of restorative medicine but has contributed fundamentally to a better understanding of vertebrate genetics and immunological phenomena in general, ranging from tumour immunology and serology to such complex phenomena as immune response regulation and the fascinating linkage between susceptibility to disease and major transplantation antigens.

This booklet on HLA and H-2 covers this wide area in a short and accessible way. It is intended for the many clinicians and immunologists who do not have the time, or courage, to study the encyclopaedic treatises on H-2 by J. Klein or by Snell, Dausset and Nathenson. Taking transplantation immunology as "art pour art" the book is teaching a great deal: How to perform HLA typing economically and reliably, how to inbreed mice, the grand rules of serology and genetic analysis, how to cope with statistics of gene linkage, that complement factors and other MHC products may have interesting things in common, some ideas about the mysterious MIs locus or the L antigen in mice, a simple method to use sperm as Lad typing cells, and a great number of most helpful tables on various antigen systems, frequencies of antigenic specificities, cross-reactivities, the newest rules in terminology and designation of specificities. Although the booklet has a small format, it is really an extended review, and it has been difficult to treat the immense abundance of facts and speculations on HLA and H-2 even in 200 pages. That the intended brevity has, except for MHC-coded *Ir* genes, left out some of the recent insights into the biological role of major histocompatibility gene products in defining T cell effector function, restriction specificity and responsiveness is (and my view is obviously biased) a pity. Some explanation of the probably real biological functions of major transplantation antigens might make it easier for the reader and student to get a feeling for the linkage between cell-mediated immunity against intracellular parasites (for example, viruses) and graft rejection (with all consequences for immunosuppressive therapy), for polymorphism (really the basis for transplantation reactions), and associations of disease susceptibilities and major histocompatibility antigens. However, people concerned with the practical consequences of major transplantation antigens in graft rejection are interested in the hard facts, methods of analysis and their correct evaluation of everyday experiments in the clinic or the laboratory, rather than in philosophical discussions on evolution and function of major transplantation antigens. For these practical aspects this booklet is of great value to students and practitioners in transplantation immunology and will serve as excellent and handy *vademecum*.

Rolf M. Zinkernagel

Rolf M. Zinkernagel is in the Department of Immunopathology at the Scripps Clinic Research Foundation, La Jolla, California.

Recent scientific and technical books

Technology

ENGINEERING EQUIPMENT FOR FOUNDRIES. (Proceedings of the Seminar on Engineering Equipment for Foundries and Advanced Methods of Producing such Equipment, organized by the United Nations Economic Commission for Europe, Geneva, 28 November — 2 December 1977. Pp.xviii + 499. ISBN-0-08-022421-9. (Oxford and New York: Pergamon Press, 1979.) Published for the United Nations.) £27.

FYNN, G. W., and POWELL, W.J.A. The Cutting and Polishing of Electro-optic Materials. Pp.xv + 216. ISBN-0-85274-302-5. (Bristol: Adam Hilger, Ltd., 1979.) £25.

HAIST, Grant. Modern Photographic Processing. Vol. 1: Pp.xii + 701. ISBN-0-471-02228-4. (v.1.) £29.95. Vol. 2: Pp.ix + 635. ISBN-0-471-04285-4 (v.2.) J25.95. (Wiley Series on Photographic Science and Technology and the Graphic Arts.) (New York and Chichester: Wiley-Interscience, John Wiley and Sons, 1979.)

HAPPEY, F. (ed.) Applied Fibre Science, Vol. 2. Pp.xvi + 511. ISBN-0-12-323702-5. (London and New York: Academic Press, 1979.) £35.

HARDMUTH, Henning F. Acoustic Imaging with Electronic Circuits. (Advances in Electronics and Electron Physics, Vol. II.) Pp.xiv + 231. ISBN-0-12-014571-5. (New York and London: Academic Press, 1979.) £25.

HOBBS, F.D. Traffic Planning and Engineering. Second edition. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.xv + 543. ISBN-0-08-022697-3. (Oxford and New York: Pergamon Press, 1979.) Hard J27.50; Flexi £10.

HUNT, V. Daniel. Energy Dictionary. Pp.ix + 518. ISBN-0-442-27395-9. (New York and London: Van Nostrand Reinhold Company, 1979.) £16.90.

JAEGER, Charles. Rock Mechanics and Engineering. Second edition. Pp.xiv + 523. ISBN-0-521-21898-5. (Cambridge, London and New York: Cambridge University Press, 1979.) £32.50.

KNOLL, Glenn F. Radiation Detection and Measurement. Pp.xv + 816. ISBN-0-471-49545-X. (New York and Chichester: John Wiley and Sons, 1979.) £17.25.

McCARTHY, Gregory J., BARRETT, Charles S., LEYDEN, Donald, NEWKIRK, John B., and RUUD, Clayton (ed.) Advances in X-ray Analysis, Vol. 22. Pp.xvii + 492. ISBN-0-306-40263-0. (New York and London: Plenum Press, 1979.) $42.50.

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NOBLE, Dave. The War and Peace Book. Pp.96. ISBN-0-904613-57-7. (London: Writers and Readers Publishing Cooperative, 9-19 Rupert Street, W1, 1977.) £3.50.

PEDLER, Kit. The Quest for Gaia: A Book of Changes. Pp.231. ISBN-0-285-62398-2. (London: Souvenir, 1979.) £5.50.

PEETERS, Peter. Can We Avoid a Third World War Around 2010?—The Political, Social and Economic Past and Future of Humanity. Pp.xii+266. ISBN-0-333-25928-9. (London and Basingstoke: The Macmillan Press, 1979.) £6.95 net.

PROCEEDINGS OF THE ATOMS FOR PEACE AWARDS, 1957–1969. (A Memorial to Henry Ford and Edsel Ford.) Pp.xxii+327. ISBN-0-262-11068-7. (Cambridge, Mass. and London: The MIT Press, 1978.) £10.50.

RUSSETT, Bruce M., and BLAIR, Brice G. (with introductions by). Progress in Arms Control? Pp.238. ISBN-0-7167-1061-7. (San Francisco and Reading: W. H. Freeman and Co., 1979.) Hard £10; Soft £4.60.

SCHAEFER, Barbara Kirsch. Using the Mathematical Literature: a Practical Guide. (Books in Library and Information Science.) Pp.ix+141. ISBN-0-8247-6675-X. (New York and Basel: Marcel Dekker, Inc., 1979.) $19.75.

SIMMS, Eric. The Public Life of the Street Pigeon. Pp.144. ISBN-0-09-133150-1. (London: Hutchinson and Co. (Publishers), Ltd., 1979.) £6.95 net.

STEVENS, L. Robert. Charles Darwin. (Twayne's English Authors Series.) Pp.159. ISBN-0-8057-6718-5. (Boston, Mass.: Twayne Publishers, a Division of G. K. Hall and Co., 1978.) $8.95.

TEWARI, Paramhamsa. Space Vortices of Energy and Matter. Pp.xii+142. (Aligarh, UP, India: M/S Bharat Prakashan Mandir, 1978.) np.

The Insight Team of *The Sunday Times*. Suffer the Children: The Story of Thalidomide. Pp.vi+309. ISBN-0-233-96873-3. (London: Andre Deutsch, 1979.) £5.95 net.

TISDELL, C. A., and McDONALD, P. W. Economics of Fibre Markets: a Global View of the Interdependence Between Man-made Fibres, Wool and Cotton. Pp.xiv+261. ISBN-0-08-022468-7. (Oxford and New York: Pergamon, 1979.) £15.

TWOMBLY, Robert C. Frank Lloyd Wright: His Life and His Architecture. Pp.x+444. ISBN-0-471-03400-2. (New York and Chichester: Wiley, 1979.) £13.50.

VOIGT, Melvin J., and HANNEMAN, Gerhard J. (ed.). Progress in Communication Sciences, Vol. 1. Pp.ix+198. ISBN-0-89391-010-4. (Norwood, NJ.: ABLEX Publishing Corporation, 1979.) np.

WALFORD, George. Ideologies and Their Functions: a Study in Systematic Ideology. Pp.163. ISBN-0-9505445-1-5. (London: George Walford, 186 Upper Street, N1, 1979.) Clothbound £3.95; Paperback £1.95.

WEINBERG, Alvin M. (general editor). Economic and Environmental Impacts of a U.S. Nuclear Moratorium, 1985–2010. (The Institute for Energy Analysis and The MIT Press Perspectives in Energy Series.) Pp.xi+381. ISBN-0-262-23093-3. (Cambridge, Mass. and London: The MIT Press, 1979.) $17.50.

WINIKOFF, Beverly (ed.). Nutrition and National Policy. Pp.xi+580. ISBN-0-262-23087-9. (Cambridge, Mass. and London: MIT, 1978.) £15.75.

WAYRE, Philip. Lutra: The Story of an Otter. (Collins Animal Lives.) Pp.96. ISBN-0-00-195611-9. (London and Glasgow: William Collins Sons and Co., Ltd., 1979.) £3.95 net.

WEST, Anthony. John Piper. Pp.224 (238 illustrations, 33 colour plates. ISBN-436-56590-0. (London: Martin Secker and Warburg, Ltd., 1979.) £14.95 net.

WOOD, Eric S. Collins Field Guide to Archaeology in Britain. New and revised edition. Pp.383+59 photographs. ISBN-0-00-219235-7. (London: Collins, 1979.) £6.50.

YULE, John-David (edited by). Phaidon Concise Encyclopedia of Science and Technology. Pp.590. ISBN-7148-1891-7. (Oxford: Phaidon Press, Ltd., 1978.) £9.95.

A Selection of Physics Texts

Modern Atomic Physics
Fundamental Principles
P. Cagnac and J-C. Pebay-Peyroula
Translated by J. S. Deech

This introductory volume discusses the crucial experiments that demonstrate the limitations of the classical theory, sets down the fundamental laws governing the internal structure of atomic systems, and surveys current experimental work. Special attention is also given to orders of magnitude calculations.
1975, £5.95

Galaxy Formation: A Personal View
John Gribbin

The debate about the structure and origin of the Universe has attracted a diversity of plausible but conflicting explanations. In this personal view of galaxy formation, Dr Gribbin criticises theories and examines current research and attitudes.
1976, £5.95/£2.95

Atomic Dynamics in Liquids
H. N. March and M. P. Tosi

A difficult and challenging area of physics has here been lucidly and authoritatively explained. This text deals with the dynamics of electrons in liquids at a level suitable for research workers and advanced students.
1976, £25.00

Thermal Physics
P. C. Riedi

An introductory text which places roughly equal emphasis on classical macroscopic thermodynamics and statistical mechanics, with a pronounced bias towards discussing applications.
1976, £10.00/£4.95

Forthcoming:

Thermodynamics of Irreversible Processes
B. H. Lavenda

A clear exposition of the basic formalism applicable to non-linear thermodynamic processes, written at a mathematical level accessible to physicists, theoretical chemists and biologists. Examples are given showing the application of the formalism in various physical and biological situations.
December 1977, £12.50.

Inspection copies of titles costing less than £6 are available from Miss Kim Mitchell, Dept P3(N), The Macmillan Press, Little Essex Street, London WC2R 3LF

To order a copy of any of these books, please send the correct amount, plus 50p per volume carriage, to Technical Book Services, P.O. Box 79, Maidenhead, Berkshire SL6 2EG

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APPOINTMENTS VACANT

UNIVERSITE DE LAUSANNE
Faculté de Médecine

Un poste de 4ème professeur de physiologie est à pourvoir. Entrée en fonction à convenir. Les candidats doivent envoyer leur curriculum vitae, liste de publications et 3 références au prof. H. Van der Loos, Institute d'Anatomie, Bugnon 9, Ch-1011 Lausanne-CHUV, Suisse, auprès duquel ils peuvent se renseigner.

W296(A)

UNIVERSITY OF WARWICK

POSTDOCTORAL RESEARCH FELLOW IN BIOLOGICAL SCIENCES

To join a group led by Dr A Colman of the Department of Biological Sciences, on a project involving the secretion of mouse immunoglobulins by oocytes and embryos of *Xenopus laevis*. Previous experience in messenger RNA isolation and purification would be an advantage, but not a necessity. The successful candidate will be required to use various techniques involved in the *in vitro* and *in vivo* translation of messenger RNAs and the immunoprecipitation of the products already in use in the laboratory. The post is supported by the Cancer Research Campaign from 1st October, 1979, renewable on an annual basis, but a suitable applicant may start at a later date.

Starting salary on the IA scale: £4,333 (£5,777) — 7,521 pa plus USS.

Further particulars can be obtained from Dr A Colman in the Department of Biological Sciences. Application forms from the Academic Registrar, University of Warwick, Coventry CV4 7AL quoting ref no 1/A/79. 2900(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL
(University of London)
Denmark Hill
London, SE5 8RX
DEPARTMENT OF MEDICAL MICROBIOLOGY

A Postgraduate Research worker is required from 1st October 1979 or as soon as possible thereafter, to participate in an MRC funded project investigating the interactions between interferon and natural killer (NK) cells. Candidates should possess a good honours degree and the successful applicant will be expected to register for a higher degree.

Salary will be on the University scales for Research and Analogous staff, according to age, qualifications and experience. For further information please contact Dr Mary Anderson on 01 274 6222 Ext 2480.

Applications, giving full curriculum vitae and the names and addresses of 2 referees should be sent to the Secretary of the Medical School. Closing date 14th September, 1979. 2929(A)

UNIVERSITY OF LONDON GOLDSMITHS' COLLEGE
New Cross, London SE14 6N
School of Science & Mathematics
BIOLOGICAL SCIENCES DEPARTMENT
TEMPORARY LECTURESHIP IN ZOOLOGY

Applications are invited for a tempor lectureship in Zoology, in the Departm of Biological Sciences. The Departmen looking for candidates with good qua ications in cell biology and special inter in cytology/histology but is also prepa to consider applications with interests other fields of Zoology.

The appointment will be from September, 1979 or as soon after possible until 30th August, 1980 with possibility of an extension for a seco year.

Salary on scale £4,470 × 12 increments £7,701 per annum plus £474 Lond Allowance.

Write for further information to Personnel Officer, to whom applicatic should be sent by 14th September, 1979 2948(A

Coal Scientists—

Carboniferous Palynology

The National Coal Board is seeking an Honours graduate with research experience in carboniferous palynology, to based at the Regional Laboratory at Wath Upon Dearne, South Yorkshire.

The successful candidate will assist with the solution of stratigraphical problems connected with exploratory boreholes and the identification and correlation of coal seams at colleries using palynological methods. It will also be necessary to undertake research towards refining the existing palynological schemes for zoning the Coal Measures. A scanning electron microscope is available within the Department.

The appointment will be made within an inclusive salary range of £6,000 – £7,500. Details of other benefits, including superannuation, annual leave, etc., will be explained at interview.

Please apply, with full career details and qualifications quoting reference AA265, to:

NCB **Headquarters Staff Manager (Doncaster), National Coal Board, Coal House. Doncaster DN1 3HG.**

2916(A)

HERIOT-WATT UNIVERSITY
Riccarton, Edinburgh
DEPARTMENT OF CHEMISTRY
The Synthesis of New Antibiotics

(a) POSTDOCTORAL RESEARCH ASSOCIATE
(2 years)

Candidates must be highly qualified, a have a thorough background in orga synthesis. The programme will involve synthesis of a new group of potential a biotics; and will encompass the chemis of β-lactams and peptides. Salary £4,91 £5,199, plus related superannuati benefits.

(b) RESEARCH TECHNICIAN
(2 years)

The successful applicant will h experience of organic synthesis and work on the above programme collaboration with the postdocto worker, but pursuing independ research. An honours degree is prefer and registration for a higher degree ma possible. Salary £3,513 to £3,609, related pension benefits.

Applicants should send a curricul vitae as soon as possible to Dr. M. Campbell, Department of Chemist Heriot-Watt University, Riccart Currie, Midlothian EH14 4AS and sho arrange for referees to send references. 2893(A

THE UNIVERSITY OF LANCASTER

DEPARTMENT OF BIOLOGICAL SCIENCES

RESEARCH ASSISTANT

Applications are invited for a post to work in the Electron Microscope Unit (Mr K Oates) on the low temperature microscopy of articular tissues in health and disease. The appointment, supported by the Medicl Research Council, is tenable for two years in the first instance. The research programme has been developed in close association with the Department of Histopathlogy (Professor D L Gardner) University of Manchester, co-holder of the relevant MRC grant.

Applicants should have a good honours degree in an aspect of the biological sciences, and they should have a sympathetic interest in the manipulative skills of instrument technology. The possibility exists to study for a higher degree. Salary on the scale £3,775 to £5,488.

Further particulars may be obtained quoting reference L156/B) from the Establishment Officer, University House, Bailrigg, Lancaster, LA1 4YW to whom applications (6 copies) naming three referees, should be sent not later than 21 September, 1979. 2943(A)

QUEEN ELIZABETH COLLEGE
Kensington
(University of London)

HEAD TECHNICIAN GRADE 7

CHEMISTRY DEPARTMENT

Applications are invited for Head Technician (Grade 7) in the Chemistry Department. Applicants should have appropriate qualifications and experience, including the supervision of technical staff, wide laboratory experience and the maintenance of instruments.

Salary within range £5,162 to £5,737 pa inclusive of London weighting.

Application forms from Mrs G Howard, Administrative Assistant, Queen Elizabeth College, Campden Hill Road, London W8 7AH. Tel: 01 937 5411 ext 499. 2940(A)

MACDONALD CAMPUS OF McGILL UNIVERSITY

DEPARTMENT OF PLANT SCIENCE

ASSISTANT PROFESSOR IN PLANT SYSTEMATICS

Applicants invited for a teaching and research tenure-stream position in *Plant Systematics*. Teaching duties will include undergraduate courses in Plant Systematics, Morphology and General Biology of Organisms (plant oriented), and graduate course in Advanced Systematic Botany or other as developed by the appointee.

The appointee will also hold the position of Curator of the McGill Herbarium with approximately 100,000 specimens. Appointment will be made at the Assistant Professor level. Applicants must have a PhD or expect to receive it shortly in plant science with a major in plant systematics/botany.

A complete curriculum vitae, a short statement of research interest and names of three referees should be sent to Prof W F Grant, Chairman, Selection Committee, Department of Plant Science, Macdonald Campus, McGill University, Ste. Anne de Bellevue, Quebec, H9X 1CO, Canada. Closing date for application, 1 November 1979 or later if position not filled.

W304(A)

CAREERS IN SCIENTIFIC COMPUTING AND ELECTRONIC ENGINEERING

Challenging opportunities to work on:

MICROPROCESSORS NETWORKS
REAL TIME PROGRAMMING LOGIC DESIGN

Daresbury Laboratory is developing major national facilities in the fields of computer networking, atomic, molecular and biological sciences and nuclear physics. The exploitation of these facilities requires first-class computing support. Computers at the laboratory include an IBM 370/165, GEC 4070 PDP-11s and Interdatas.

COMPUTER NETWORKS

Development and support of communications software for interactive terminal, graphics and remote job entry access to the SRC's computing centres using microprocessor based communications hardware and mini-computer pocket switches and front end processors.

DATA ACQUISITION

Programmes are required for the development of data acquisition systems using LSI and PDP-11 computers connected to an IBM 370/165. There are opportunities to work with large and small systems in a stimulating scientific environment. Relevant programming experience would be an advantage but is not essential.

LOGIC DESIGN

Interface design for mini-computers using the latest microprocessor techniques, also communications interfacing to remote data processing. Specialised design of data acquisition systems.

Applicants, of either sex, should have a good honours degree in a relevant scientific discipline. Appointments will be made depending on qualifications and experience at SO level.
Scientific Officer £3,289 - £5,085 (salaries are currently under review)
Closing date: 7 September 1979
Please write or telephone: Warrington 65000 Ext. 467, for an application form quoting reference DL/694/

The Personnel Officer
DARESBURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD

2926(A)

TECHNICIAN/ RESEARCH OFFICER

to head a small fermentation unit aimed at the production of enzymes. Preference given to someone interested and experienced in isolation of enzymes. Degree/HNC. Salary range £3,600 to £5,250.

For further information and application form, write or telephone to Miss S M Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, WC2 on 242 0200 ext 305 quoting ref BG1. 2932(A)

ANTHONY NOLAN Bone Marrow Research Laboratories, St. Mary Abbots Hospital, Marloes Road, London W8. Senior grade person required to take technical and partial administrative charge of these laboratories mainly responsible for tissue typing bone marrow volunteer donors. Applicant should be a senior graduate or senior technician with considerable experience of tissue typing. Salary on higher University Biochemist or Whitley council MLSO scales as appropriate. Apply in writing to Dr D C O James, Blood Transfusion Unit, Westminster Hospital, Dean Ryle Street, London SW1. 2915(A)

UNIVERSITY OF READING

DEPARTMENT OF SOIL SCIENCE

Applications are invited for a Lectureship in Soil Science with responsibility in soil physics or mineralogy or soil biochemistry.

The appointment will be made within the first five points of the Lecturer salary scale (£4,333 to £5,488 per annum).

The person appointed should take up duties on 1 January 1980.

Further information may be obtained from the Registrar (Room 214, Whiteknights House), The University, Whiteknights, Reading RG6 2AH, by whom applications should be received not later than 30 September, 1979.

2895(A)

Synthetic Organic Chemists

Salary Range £4,500-£6,500 p.a.

The Pharmaceuticals Division of Imperial Chemical Industries is seeking a small number of recent chemistry graduates who would enjoy the challenge of working in organic synthesis directed toward medicinal targets. A good honours degree is essential and previous experience of organic synthesis desirable. Successful candidates will operate with a fair amount of independence whilst remaining under the overall guidance of a Ph.D chemist and have the opportunity of tackling a variety of chemical problems.

The Division's research laboratories are situated in pleasant surroundings but within easy reach of main road and rail routes. Conditions of service, salary and assistance given to married staff in moving house are designed to attract and retain staff of high calibre.

Applications in writing should quote reference D.B.6 and should be addressed to:

ICI

Mrs D. Bass,
Assistant Personnel Officer,
I.C.I. Pharmaceuticals Division,
Mereside Research Laboratories,
Alderley Park, Macclesfield, Cheshire.

2949(A)

PRESIDENT

DESERT RESEARCH INSTITUTE

Applications and nominations are invited by the Board of Regents, University of Nevada System, for the position of President of the Desert Research Institute.

This autonomous, statewide division of the University System conducts research programs in Atmospheric Sciences, Water Resources, Energy Systems, Social Sciences and Bioresources, funded by both governmental and private sources. Established in 1959, the Institute has a current annual budget of about six million dollars and a faculty of 90 professionals. There is a close association between institute research and University graduate programs. The President reports directly to the Board of Regents, with a position equivalent to the Presidents of the two University campuses. The present incumbent is retiring.

Qualifications include demonstrated leadership abilities, a doctoral degree, a background in scientific research, a distinguished record of intellectual achievement, a broad background in the funding, conduct and management of research, and proven effectiveness in the areas of governmental, university and community relations.

Applications will be accepted until October 1, 1979. The position will be filled at the convenience of the candidate, but no later than July 1, 1980.

To apply, or for further information, contact:

Presidential Search Committee
Desert Research Institute
University of Nevada System
PO Box 60220
Reno, Nevada 89506

AN EQUAL OPPORTUNITY/
AFFIRMATIVE ACTION EMPLOYER

W300A

POSTDOCTORAL
RESEARCH FELLOW

PROTEIN METABOLISM

Applications are invited for an appointment of up to two years, to work with Professor J C Waterlow at the Clinical Nutrition & Metabolism Unit, St Pancras Hospital, on studies of the regulation of protein synthesis and breakdown in animal tissues. The salary will be in the 1a range for research staff, £4,260 to £7,145 (£4,333 to £7,521 from 1 October 1979) plus £502 London Weighting.

Applications, consisting of full curriculum vitae and naming two referees, should be sent to Secretary (A1), London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT.

2913(A)

UNIVERSITY
OF BRISTOL

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a post-doctoral appointment, supported for three years by the Medical Research Council, to work on the glycoproteins of human mucous secretions, particularly the bronchial secretions of chronic bronchitics and asthmatics.

The work will involve preparation of the glycoproteins, physico-chemical characterization, and a study of the products of limited degradation. Candidates should have experience in protein chemistry; an interest in physical biochemistry would be advantageous. Initial salary £4,232 (Range 1A), to commence 1 October 1979 or as soon thereafter as possible.

Enquiries should be addressed to Dr J M Creeth, Department of Biochemistry, University of Bristol Medical School, Bristol BS8 1TD. 2933(A)

UNIVERSITY
OF ST ANDREWS

DEPARTMENT OF ZOOLOGY

Applications are invited for the post o

POSTDOCTORAL RESEARCH ASSISTANT
(Molecular Biology)

to work in collaboration wit Dr J Sommerville on an investigation of th packaging and processing of RNA trar scripts in eukaryote cells. Applicants shoul have a training in biochemistry or micr biology. Experience in nucleic acid biochen istry or the electron microscopy of nucle acids would be an advantage. The appoin ment is for up to 3 years, starting date b arrangements, and is supported by the SR(Starting salary within range 1A £4,333 t £4,910 (under review), plus FSSU/US: Informal enquiries may be made t Dr J Sommerville (Tel: St Andrews 72823)

Applications (two copies preferably i typescript), with the names of two referee should be lodged by **12th September 19** with the Establishments Officer, Th University, College Gate, St Andrews, Fif KY16 9AJ. 2832(A)

UNIVERSITY OF
LONDON KING'S COLLEC

DEPARTMENT OF
ANATOMY

Applications are invited for an

MRC POSTDOCTORAL
RESEARCH ASSISTANT

in

DEVELOPMENTAL
NEUROBIOLOGY

to work on the development of li innervation in the chick embr Experience in electrophysiology and developmental biology would be advantage.

The post will be available from October 1979 for up to 3 years, salary in range £4,333 to £4,910 p.a. plus £502 p London Allowance.

Applications, together with c.v. a names of two referees, to: Dr J.H. Lev Department of Anatomy, King's Colle Strand, London WC2R 2LS as soon possible, quoting 191689/ 2858(A

UNIVERSITY
OF STRATHCLYDE

Applications are invited for the post of

POSTDOCTORAL
RESEARCH FELLOW

in the

DEPARTMENT OF PURE
AND APPLIED SCIENCE

to work on the study of polymer subst interactions. The research will involve characterization of metal substrates us ESCA and XPS and the characterisatio the effects of various surface treatment the adhesion of a polymer film t pretreated substrate. This project associated with studies of polyme materials as electron beam developm agents.

The post, which is supported by SR(for two years.

Commencing salary on Range 1A of national salary structure for research analogous staff up to £4,910 per ann with placing according to a qualifications and experien Superannuation benefit.

Applications naming two referees including a curriculum vitae should be s (quoting R34/79) to Dr R A Pethr Department of Pure and Appl Chemistry, Thomas Graham Building, Cathedral Street, Glasgow G1 1XL. 2930(A

UNIVERSITY OF THE WITWATERSRAND
Johannesburg

DEPARTMENT OF PHYSIOLOGY

Applications are invited from suitably qualified persons for appointment to the posts of:

1. SENIOR LECTURER/ LECTURER (two posts)

2. RESEARCH FELLOW

Applicants for these posts should have a medical or veterinary degree or a PhD in physiology, Biochemistry, Pharmacology, zoology or any other biological field.

The Lectureships and Senior Lectureships involve teaching undergraduate and graduate medical and science students, research, and participation in the administrative work of the department. There is no restriction on the field of interest of applicants.

The Research Fellow will be involved in an on-going programme of research, at a biochemical, pharmacological and physiological level, in cardiovascular physiology and health and disease. Applicants with a background in protein biochemistry, radio-ligand binding studies, or adrenergic pharmacology are particularly invited to apply.

The salary range for Lecturer is R8,100 to R16,800; for Senior Lecturer is R15,000 to R17,400 and for Research Fellow is R8,100 to R13,200 per annum (£1 = R1.89 approx).

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion or colour.

Further particulars relating to this practice and to the above posts are included in an information sheet obtainable from the London Representative, University of the Witwatersrand, Chichester House, 278 High Holborn, London WC1V 7HE, or from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, with whom applications should be lodged by 15 September, 1979. 2928(A)

UNIVERSITY COLLEGE LONDON
DEPARTMENT OF PHARMACOLOGY

POST DOCTORAL RESEARCH ASSISTANTS

Two Post-doctoral research assistants required for work on cholinergic transmission supported by 5-year MRC Programme Grant under the supervision of Professor H. P. Rang and Dr. D. Colquhoun. One post is for an electrophysiologist (with experience of microelectrode recording) to work initially on voltage clamp studies on neuromuscular and ganglionic synapses, and eventually to develop new recording techniques. The laboratory is well equipped for electrophysiological recording and on-line computer analysis.

The other post is for a physiologist or biochemist to undertake mainly biochemical work on transmitter synthesis and release, receptor labelling, and membrane permeability changes evoked by neurotransmitters.

Those appointed will form part of a group of about seven scientific staff, which is to be set up at University College in newly built and newly equipped laboratories. Starting salaries will be about £5,307 (inc. London Allowance). Applicants should send full curriculum vitae and names of two referees before 31 August 1979 to Professor P. Rang, Dept. of Pharmacology, University College London, Gower St, London WC1E 6BT. 2911(A)

PRINCIPAL SCIENTIFIC OFFICER
(Tidal Waters)
Grade 12

(£9,333 to £10,059 plus £312 supplement)
(Salary Award Pending)

An energetic and enthusiastic Scientist (male or female) is required to lead a small team of Tidal Water specialists based at the Headquarters of the Welsh Water Authority at Brecon and also to be responsible for advising on and co-ordinating the work programmes of Divisional Tidal Waters Staff. The duties will include the setting of quality objectives. Advice on the formulation of consents to discharge. Advice and co-ordination of monitoring programmes to acquire routine physical, chemical and biological data from estuaries and coastal waters. Planning, directing and evaluating the hydrographic surveys and environmental appraisals necessary for the effective management of disposals of wastes to tidal waters. Liaison with staff of the Directorates of Operations and Resource Planning concerning remedial works. Involvement with existing and potential industrial dischargers with regard to their effluents.

The successful applicant will report to the Assistant Director of Scientific Services (Water Quality) and will represent the Authority on a number of national working parties.

Applicants should have a degree or equivalent in a Marine Science, proven management ability and considerable experience of pollution control in tidal waters.

This post carries a Regular User Car Allowance and Disturbance Allowances will be paid in appropriate cases.

Application forms are available from:

The Assistant Personnel Officer (Ref. 320)
Welsh Water Authority, Cambrian Way
Brecon, Powys LD3 7HP
Telephone: Brecon (0874) 3181 Extn 373
Closing Date: Wednesday 29th August, 1979

2934(A)

WELSH WATER AUTHORITY
AWDURDOD DWR CYMRU

UNIVERSITY OF WARWICK

POSTDOCTORAL RESEARCH FELLOW in Biological Sciences

To join a group led by Dr J Bennett in the Department of Biological Sciences, on photocontrol of thylakoid structure and function in higher plants. The successful candidate will explore the role of thylakoid protein phosphorylation in photosynthesis. Previous experience in physical or enzymic aspects of the light reactions of photosynthesis would be an advantage. The post is supported by the Science Research Council from 1st October, 1979 for two years, but a suitable applicant may start at a later date. Salary on the 1A scale: £4,333 (£5,777) to £7,521 pa plus USS.

Further particulars from Dr J Bennett, Department of Biological Sciences. Application forms from the Academic Registrar, University of Warwick, Coventry CV4 7AL quoting Ref No 1/3A/79. 2902(A)

KENNEDY INSTITUTE OF RHEUMATOLOGY

POST DOCTORIAL RESEARCH ASSISTANT IN DIVISION OF BIOCHEMISTRY

Applications are invited for the post of Research Assistant for the further development of a project investigating the molecular basis of the pathological changes in connective tissues associated with articular joint degeneration (osteoarthritis).

The work is fundamental and forms part of an integrated programme of connective tissue research within the division of biochemistry (Head, Dr Helen Muir, FRS).

Previous experience of connective tissue or protein biochemistry, proteolytic enzymes or tissue culture technique would be an advantage. The position is funded in the first instance for three years; salary in the range of £4,835 to £5,701.

Applications enclosing a curriculum and vitae and the names of two referees should be sent to Dr Helen Muir, The Director, Kennedy Institute of Rheumatology, 6 Bute Gardens, Hammersmith W6 7DW. 2945(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL
DEPARTMENT OF IMMUNOLOGY

RESEARCH/SENIOR RESEARCH OFFICER

required for the remaining 2 years of an MRC funded project concerned with a clinical and experimental study of immunological function and graft versus host disease following bone marrow transplations, undertaken jointly between the Immunology and Haematology Departments at the Royal Postgraduate Medical School, Hammersmith Hospital. The post becomes vacant October 1st 1979, but an overlap of 2-4 weeks with the present holder is desirable and could be arranged.

Starting salary up to £5,777 a year. Applicants should have experience and preferably a higher degree in cellular immunology,and be familiar with tissue culture techniques.

Application forms and further particulars may be obtained from the Royal Postgradualte Medical School, Personnel Office, 150 Du Cane Road, London W12 0HS quoting ref no 21/200/N.

Closing date: 10th September 1979. 2873(A)

BIOGEN S.A.

a young company engaged in basic and applied research in the field of molecular biology and microbiology, and with the objective of utilizing the potential of genetic engineering for the production of substances of commercial and medical interest in such areas as health care, agriculture and energy, is inviting applications from

SENIOR AND JUNIOR SCIENTISTS

with experience in molecular biology, nucleic acid biochemistry and microbiology,

for its **Research Laboratory in Geneva,**

Switzerland.

Excellent working conditions, in close contact with leading scientists from Europe and the US.

Salary scale: Group Leaders = SFr 60-85,000, Research Associates = SFr 35-55,000.

Applicants with real interest in both basic research and its applications should send their curriculum vitae (with a description of previous experience and a list of publications) to BIOGEN Laboratory, 3 Route de Troinex, 1227 Geneva, Switzerland. W248(A)

THE BRITISH FOUNDATION FOR AGE RESEARCH

has funds available and invites applications for support of proposed research projects in ageing or relevant to the elderly.

Please write for application form to:

The Director,
The British Foundation for Age Research,
49, Queen Victoria Street,
London, EC4N 4FA

Closing date for applications: Monday, 18 October, 1979.
2903(A)1

THE MULTIPLE SCLEROSIS SOCIETY

Invites applications for three

Career Development Posts at senior lectureship level in multiple sclerosis research.

The positions will be offered in established M.S. Research Centres.

Applicants for these posts should be proposed by another research worker or person with an association with M.S. research.

A summary proposal of the project should be submitted in the first instance.

The application to be based on an MRC programme grant application.

Laboratory site at which proposed project will be carried out to be indicated in the application.

All applications will be considered by the Society's Medical Research Advisory Committee.

Applications to be sent to: The General Secretary, Multiple Sclerosis Society, 4 Tachbrook Street, London, SW1J 1SJ. 2894(A)

UNIVERSITY OF OTTAWA

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANTS

Two recent PhD graduates required to join research group working on plant protein utilization. An enzymologist with experience preferably in cereal chemistry or plant physiology is needed for characterization of lipase in high-protein oats during maturation and storage. A microchemist experienced in stain development is needed to study localization of phytate, glucosinolates, etc in rapeseed.

Research involves collaboration with the food industry and the Food Research Institute of Agriculture, Canada. Salary minimum $12,200.

Please apply to Dr I Altosaar, Biochemistry Dept, Univ of Ottawa, 40 Somerset St E, Ottawa, Ontario K1N 6N5, Canada. W298(A)

IMPERIAL COLLEGE of Science and Technology, South Kensington, SW7. Technical Staff Grade 3 vacancy in a group interested in the molecular biology of transformation by DNA tumour viruses. The work depends heavily on the techniques of genetic manipulation. Previous experience in animal cell culture and/or microbiology would be a considerable advantage. The appointment, which is supported by the cancer research campaign will be for one year in the first instance with the possibility of renewal for two further years. Salary will be within the range £3,455 to £3,860 pa (under review). Superannuation. Five weeks leave plus additional days at Christmas and Easter. Application forms can be obtained from Mr I P Blench, Department of Biochemistry, Imperial College, London SW7 2AZ or phone 589 5111 ext 1130. 2941(A)

UNIVERSITY OF HONG KONG

LECTURESHIP/ ASSISTANT LECTURESH IN PHYSIOLOGY

Applications are invited for a post Lecturer/Assistant Lecturer in Physio which has become available as a resu the institution of dental studies in University. The successful applicant wi required to participate in the teachin physiology to dental students and me students. Applicants with higher degre Physiology or medical degrees, or de degrees will be given preference.

Applicants should have some rese and teaching experience and a sp interest in one of the following ar 1. Gastro-enterological Physiolo 2. Neurophysiology, 3. Cardiovasc Physiology, 4. Reproductive Physiolo

Annual salaries (superannuable) Lecturer HK$63,540 × 4,260 — 72,060 76,320 × 4,260 — 106,140; Assis Lecturer HK$46,260 × 4,320 — 59, (£1 = HK$11.60 approx).

Starting salary will depend qualifications and experience. A medic qualified appointee who has complete pre-registration year will be appointed Lecturer at the minimum point of the s

At current rates, salaries tax will exceed 15% of gross income. Housing rental of 7½% of salary, educa allowances, long leave and medical ben are provided.

Further particulars and applica forms may be obtained from Association of Commonwe Universities (Appts), 36 Gordon Sq London WC1H 0PF, or the Assi Secretary (Recruitment), University Hong Kong, Hong Kong.

Closing date for applications i September, 1979. 2907(

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF VETERINARY PARASITOLOG

Applications are invited for the po Lecturer in the Department of Veter Parasitology. The Department is hous the School of Tropical Medicin Liverpool and is responsible for tea all aspects of Parasitology to veter undergraduates and for the organisati the post-graduate MVSc course in Ap Parasitology. Applicants must ha veterinary qualification which is regist in the United Kingdom and experien laboratory animal welfare as the succ candidate will also be Curator o Animal Unit in the Tropical School.

The initial salary will be on the £4,232 to £8,452 per annum.

Applications, together with the nan three referees, should be received not than 28th September, 1979, by Registrar, The University, PO Box Liverpool L69 3BX, from whom fu particulars may be obtained. Quot RV/742/N. 2921

UNIVERSITY OF BRISTOL

POSTDOCTORAL RESEARCH ASSISTAN

An NIH funded post for work o isolation, affinity labelling and cat mechanism of mammalian glycosida available immediately.

Applications are invited from pe holding, or expecting to hold, degrees in Biochemistry, Organ Physical Chemistry, or Biol Applicants should send their curric vitae and the names of two refere Dr M L Sinnott, School of Chem University of Bristol, Cantock's C Bristol BS8 1TS. 2888

UNIVERSITY
OF BRISTOL

SCHOOL OF CHEMISTRY

POSTDOCTORAL
RESEARCH ASSISTANT

ıplications are invited for this post in the partment of Organic Chemistry, to ry out work on the structures, origins d significance of alkyl porphyrin ments in sedimentary organic matter d petroleums. This work is part of a der study directed towards derstanding the sedimentary fate or orophylls. Instrumentation includes LC, mass spectrometry (EI and CI), urier Transform 1H and 13C NMR, illary GLC and computerised GC-MS. vious experience of the chemistry of rphyrins, chlorophylls or related npounds, or of natural product mistry would be advantageous, though essential.

The appointment, funded by the SRC is one year in the first instance, at a ting salary within the range £4,232 to 776 pa (scale subject to revision with ect from 1st October, 1979).

Please send applications, with details the names of two referees, to Professor Eglinton, FRS, Organic Geochemistry it, School of Chemistry, University of stol, Cantock's Close, Bristol BS8 1TS. 2897(A)

UNIVERSITY
COLLEGE, CARDIFF

plications are invited for the post of

DEPARTMENTAL
SUPERINTENDENT

in the

DEPARTMENT OF PHYSIOLOGY

e Technical establishment of the partment includes 5 T6 members in ırge of the general laboratories, animal use, mechanical and electrical rkshops plus 12 lower grade staff. There 15 academic staff and research fellows h several research students. The partment extends over 10 floors. Salary ge: Tech 8c £5,172 to £5,625 pa (under iew). Duties to commence as soon as ssible.

Applicants, who should hold qualifi-ions of HNC level or equivalent and e extensive experience of working in ical or pre-clinical laboratories, are uested to submit a curriculum vitae ing full details and the names and dresses of two referees to the Vice-ncipal (Administration) and Registrar, iversity College, PO Box 78, Cardiff 1 1XL. Particulars of duties and terms employment will be sent to short-listed ndidates. Closing date 31st August, 9. Reference 1884. 2924(A)

UNIVERSITY
OF OXFORD

DYSON PERRINS
(ORGANIC CHEMISTRY)
LABORATORY

POSTDOCTORAL
RESEARCH ASSISTANT

postdoctoral Assistant is required to join group engaged in the synthesis of logically active peptides. Experience is sirable but not essential. The pointment, which is for one year, ewable for a second year, will start as n as possible. Salary (Grade 1A) in the ge £4,776 to £5,850 (under review) ording to age and experience, plus erannuation.

Applications, with curriculum vitae and names of two referees, should be sent as n as possible to Dr G T Young, The son Perrins Laboratory, South Parks ad, Oxford OX1 3QY. 2944(A)

ASSISTANT MICROBIOLOGIST OR CHEMIST
(Post 6S016)

Due to the current reorganisation of the Authority, a vacancy exists for an Assistant Microbiologist or Chemist in the new Gwynedd Division.

The post is to undertake analytical work on raw water, treated water, sewage and effluents in a Process Control Laboratory at Caernarfon. Though experience would be an advantage, applications from recent graduates in microbiology or chemistry interested in a career in the Water Industry will be considered.

The current salary is Grade 4 £4,179 to £4,476 *or* Grade 5 £4,635 to £4,962 with a pay award pending.

The Terms and Conditions of employment are as laid down by the National Joint Council for Water Service Staffs.

Assistance with relocation will be given where appropriate.

Closing date: 3rd September, 1979.

Application forms available from:

Personnel Officer, Gwynedd Division,
Welsh Water Authority, Bron Castell,
High Street, BANGOR, Gwynedd.
Telephone: Bangor (0248) 52881.

2942(A)

WELSH WATER AUTHORITY
AWDURDOD DWR CYMRU

UNIVERSITY
OF EDINBURGH

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL
RESEARCH FELLOW

This SRC financed post is available in the molecular beam group investigating inelastic scattering at super-thermal energies. The work involves on-line data processing and machine control, potential surface modelling and trajectory calculations. A background in molecular scattering, computing or spectroscopy would be an advantage.

The appointment is for three years starting at point 3 on the 1A salary scale.

Applications, enclosing a curriculum vitae and the names of two referees, should be sent to Dr M A D Fluendy, Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh 9. Please quote reference 5047. 2922(A)

THE UNIVERSITY
OF MELBOURNE

CHAIR OF VETERINARY
BIOLOGY

Applications are invited for appointment to the Chair of Veterinary Biology. The Chair has been established to give academic leadership in the Department of Veterinary Preclinical Sciences and the University will consider applications from persons appropriately qualified in Veterinary Anatomy, Physiology or Pharmacology.

Salary $A34,119 per annum.

Further information, including details of application procedure, superannuation, travel and removal expenses, housing assistance, and conditions of appointment is available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on 26 October, 1979. 2892(A)

FACULTY POSITION IN PHYSIOLOGY

The Department of Physiological Sciences in the School of Veterinary Medicine at the University of California at Davis, invites applications from qualified individuals for a position in Ruminant Physiology. Appointment level depends on qualifications (Assistant Professor preferred). Qualifications: PhD degree in physiology or related field. A veterinary degree strongly preferred. Competence and advanced training in ruminant physiology. The candidates must have demonstrated interest, aptitude and experience in classroom teaching and demonstrated interest and achievement in research.

Teaching: Professional — lecture and laboratory instruction in the area of ruminant physiology. Lecture and laboratory instruction in other fields of systemic physiology in accordance with departmental teaching responsibilities in the professional curriculum. Graduate — Opportunity to instruct graduate students in formal courses and/or individual study in the area of the candidate's interest and competence.

Research: The development of a creative productive research program in the field of ruminant physiology is a fundamental and indispensable requirement of the position. Send curriculum vitae, a list of publications and names of three referees to:

Dr. J. R. Gillespie, Search Committee Chair, Department of Physiological Sciences, School of Veterinary Medicine, University of California, Davis, CA 95616. This position will remain open until filled. University of California is an Equal Opportunity/Affirmative Action Employer.

W301(A)

PERGAMON PRESS
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If you have just finished your science A-levels and are looking for a career in international scientific publishing, why not apply to Pergamon who currently have a number of vacancies as trainee Journal Production Editors.
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Experienced Copy and Style Editors, who would be able, either full or part time, in Oxford or in their own home, to prepare book manuscripts for typesetting. These tasks are particularly suited to men or women who have done, or are doing similar work in printing or publishing houses or Learned Societies

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Experienced and accurate typists to operate IBM or other electric typewriters either at Pergamon or working in their own home. Pergamon also need experienced operators for Compugraphic Typesetting machines, setting both book and journal manuscripts. Either full or part-time staff will be considered.
Full-time salary, for copy typists in the range of £2700-£3500 +

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Should you be interested in any of these vacancies please write or telephone in confidence to:

The Publisher,
Pergamon Press Limited
Headington Hill Hall
OXFORD
OX3 0BW Oxford 64881 ext: 279

2919(A)

ELECTRON MICROSCOPY TECHNICIAN

The National Institute for Biological Standards and Control is an independant multi-disciplinary establishment concerned with testing and control of biological products used in medicine. A technician is required to assist in the development of electron microscopical methods for standardisation and control of virus vaccines and cell cultures and will be expected to undertake some routine photographic work.

Applicants should have experience in thin sectioning techniques, and some knowledge of scanning electron microscopy would be an advantage. Applicants should have HNC, HND or a suitable degree.

Further details and application forms are available from the Personnel Officer, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3. Telephone 01-435 2232 Extension 200, quoting reference VP/109.

NIBSC

National Institute for Biological Standards and Control

2899(A)

UNIVERSITY OF LONDON
GOLDSMITHS' COLLEGE
New Cross, London, SE14 6NW
SCHOOL OF SCIENCE
AND MATHEMATICS
DEMONSTRATORSHIPS (2)

Applications are invited for one full-time Demonstratorship in each of the following fields:

1) Crystallography, Mineralogy and Petrology (one post)

2) Palaeontology, Stratigraphy and Map Interpretation (one post)

Candidates should have a good honours degree and postgraduate experience would be an advantage. The posts are available from 1st September, 1979 or as soon as possible thereafter.
Salary £4,038 to £4,542 inclusive of London Allowance and supplement.
Write for further particulars to the Personnel Officer to whom applications should be sent by 31st August, 1979.

2935(A)

THE INSTITUTE FOR
PHYSICAL SCIENCE
AND TECHNOLOGY
of the
UNIVERSITY
OF MARYLAND
College Park

invites applications for a tenure-tra appointment in experimental aspects either statistical physics or plasma physi Preference will be given to those applicar who offer greatest promise of clo interaction with the Institute's stro theoretical groups in these areas.
Within statistical physics our princip interests focus on non-equilibrium proce fluids, such as non linear phenomer hydrodynamic instabilities, fluctuatior etc. Within plasma physics, our princip interests focus on the physics of waves a instabilities in space plasma, Q-machi studies of waves and instabilities, or optic plasma diagnostics in the ultraviolet.
A detailed résumé and list of referenc should be sent to: Professor Josej Silverman, Institute for Physical Scien and Technology, Space Science Buildin University of Maryland, College Par Maryland 20742. The University Maryland is an affirmative action/equ opportunity employer. W303(A)

CERRO TOLOLO INTER-AMERICAN OBSERVATORY
LA SERENA, CHILE

May have an opening on 1 October, 1979 for an Experimental Physicist or Instrument Astronomer for the Observatory's Infrared Program. The applicant should have a master or PhD degree or the equivalent experience. The primary responsibility will be tl development of modern infrared observational instrumentation for CTIO's telescope Primary place of work will be at the Observatory's headquarters in La Serena, Chile. Tl minimum salary will be $18,940 per year including an overseas allowance, but the actu salary is negotiable according to experience. Generous fringe benefits include the usu medical insurance coverage and retirement, annual family trips to point of origin ar special duty-free importation privileges. W289(A)

Graduate in Biochemistry or Biochemistry / Physiology

To work in Reproduction Research. The successful applicant will join a research team engaged in the search for novel contraceptive agents, in particular drugs which interfere with ovarian steroidogenesis.

Experience of hormone/receptor binding and radioimmunoassay techniques will be an advantage.

The Division's research laboratories are situated in pleasant surroundings but within easy reach of main road and rail routes. Conditions of service, salary and assistance given to married staff in moving house are designed to attract and retain staff of high calibre.

Applications in writing should be addressed to:

ICI

Mrs D. Bass,
Assistant Personnel Officer,
ICI Pharmaceuticals Division,
Mereside Research Laboratories,
Alderley Park, Macclesfield, Cheshire.

2925(A)

MAX-PLANCK-INSTITUTE
Tübingen, Germany

POST-DOCTORAL RESEARCH POSITION IN TUMOR VIROLOGY

en, beginning October 1979 or later. plicants with PhD or MD degrees ould have some experience in munology, (tumour) virology or protein emistry to join a research group working cell surface and nuclear alternations of us transformed cells.
Salary between 24 to 36,000 German arks per year, depending on alification. Some knowledge of German lesirable, but not absolutely necessary.
Applications should be sent to: Dr R. rth, Friedrich-Miescher-Laboratorium, ax-Planck-Institutes, Spemannstr. 39, 7400 Tübingen, West Germany.
W293(A)

UNIVERSITY OF LIVERPOOL
EPARTMENT OF MICROBIOLOGY

plications are invited for the post of search Assistant/Senior Research sistant in the Department of Micro-logy. The project is funded by the MRC a period of three years. The research ncerns the uptake and establishment of smid DNA during transformation of *herichia coli* and other Gram negative teria, in collaboration with Dr G O mphreys and Dr J R Saunders.
The initial salary will be £3,689, £3,961 £4,232 per annum plus USS benefits. appointment is from 1st October 1979, as soon as possible thereafter.
Applications, together with the names of e referees should be received not later n 30th August, 1979, by The Registrar, University, PO Box 147, Liverpool 3BX, from whom further particulars be obtained. Quote Ref: RV/740/N.
2920(A)

UNIVERSITY OF BIRMINGHAM
FACULTY OF MEDICINE AND DENTISTRY
DEPARTMENT OF CLINICAL GENETICS
(Maternity Hospital)

Applications are invited from suitably qualified graduates in Biological Science for two posts of Research Associate 3/5 time to work in the cultivation and *in vitro* examination of human fetal tissue using various techniques, including cloning, O'Farrell electrophoresis and the banking of cells.

Salary will be on the scale £3,775 to £5,488 × 3/5 (October scales) with super-annuation benefits. Maximum starting salary will be £4,910 × 3/5.

Further particulars are available from the Assistant Registrar, The Medical School, Birmingham B15 2TJ, to whom applications should be sent by 1st September, 1979. 2918(A)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
University of London
DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY
POSTDOCTORAL RESEARCH ASSISTANT

Bio-physical chemist required to join research group working on membrane structure and function. Experience of surface techniques or material science would be a particular advantage. The post is tenable for up to 3 years, supported by the Science Research Council. Initial salary within range £4,261 to £4,805 plus £502 London Allowance.

Applications, naming two referees, to the School Secretary, RFHSM, 8 Hunter Street, London WC1N 1BP, as soon as possible. 2882(A)

UNIVERSITY OF NEW BRUNSWICK

FACULTY OF FORESTRY

CHAIR IN FOREST TREE IMPROVEMENT

Through the generosity of a group of New Brunswick forest industries, funds have been provided to create and support a new Chair in Forest Tree Improvement in the Department of Forest Resources.

Applications are invited from highly qualified and experienced forest scientists. The incumbent will be expected to initiate and develop a program of teaching, research and collaboration with industry in the area of tree breeding and genetics as applied to forest tree improvement, stand improvement and long term enhancement of wood quality.

It is expected that the appointment will be made at a senior level. Appropriate technical support and facilities will be provided. The appointment will be made as soon as a suitable candidate can be located.

Applications or enquiries should be directed to:

Dr. A. J. Kayll
Chairman, Department of Forest Resources
University of New Brunswick
PO Box 4400, Fredericton,
New Brunswick E3B 5A3, Canada

[REPLY INFORMATION HERE]

302(A)

UNIVERSITY OF NEW BRUNSWICK

We have the following vacancies in our Biochemistry Section:

Experimental Officer — Physical Chemist/ Electrochemist

A graduate biochemist to play a part in the discovery of new insecticides. An understanding of neurobiology would be desirable, but practical experience in insect biochemistry is not essential. However, candidates must have considerable experience of modern biochemical techniques.
The successful applicant will join a small team composed of one PhD and one graduate, responsible for initiating research in areas of insect biochemistry that could lead to a programme of chemical synthesis or produce *in vitro* methods for detecting active chemicals. Complementary work will be concerned with evaluating the mode of action of promising new insecticides and gaining an early understanding of the factors that may contribute to the development of resistance.

Experimental Officer — Biochemist

A Physical Chemist to work on the electrochemistry and reaction kinetics of exploratory organic compounds being synthesised as potential pesticides. A knowledge of chemistry at least up to HNC level is required, and experience of polarography or related techniques is desirable but not essential. The successful candidate will work closely with organic and bio-chemists, and will be in a small team headed by a PhD physical chemist. There will be scope for initiative in developing techniques and helping to design structures with the desired electrochemical properties. Jealott's Hill is the chemical and biological research centre for the crop protection interests of ICI. It is situated in open country 30 miles west of London, between Bracknell and Maidenhead. We offer competitive salaries and fringe benefits including membership of the ICI Profit Sharing and Pension Fund. Coaches run between Jealott's Hill, Bracknell and Maidenhead.

If you are interested in this vacancy please write or telephone for an application to:

Mr S R Stephenson
ICI Plant Protection Division
Jealott's Hill Research Station
Bracknell, Berks. *Ref no 79/Biochem*

ICI Plant Protection Division

2889(A)

INSTITUT CURIE ORSAY, PARIS

POSTDOCTORAL RESEARCH FELLOWS

Applications are invited for two positions as postdoctoral research fellows to work with Dr E De Maeyer on aspects of the REGULATION OF INTERFERON MRNA SYNTHESIS. It is expected that the successful candidates will have experience in one or more of the following areas: animal virology, cell biology, protein biochemistry, immunology, nucleic acid isolation, molecular hybridisation, cell-free translation.
The appointments will be for two years and the starting salary will be in the range 65,000 ffr to 85,000 ffr p.a. depending on experience (9.73 ffr = £1.00 sterling). Knowledge of the french language is not necessary.
Applications, including curriculum vitae and the names and addresses of two referees should be sent as soon as possible to:

Dr E De Maeyer,
Section De Biologie, Laboratoires 110-111-112,
Institut Curie, Université de Paris Sud,
Campus d'Orsay, 91405 Orsay, Paris, France.

2896(A)

GUY'S HOSPITAL MEDICAL AND DENTAL SCHOOLS

Two Laboratory Scientific Officers required for 3½ and 2 years respectively in the Department of Oral Immunology and Microbiology for research into cellular or humoral immune responses to streptococci and other antigens. Experience in immunological, bacteriological or biochemical techniques essential.

Salary for qualified Laboratory Scientific Officer not less than £3,261 (under review) plus £354 London Weighting and superannuation.

Apply in writing, stating age and giving details of qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. IM5. 2937(A)

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF BOTANY AND MICROBIOLOGY

A vacancy exists for a

POSTDOCTORAL RESEARCH ASSISTANT

commencing 1 October, 1979. Experience in electron microscopy and related cytochemical techniques essential. The research will be concerned with the differentiation of the reproductive cells of land plants. Salary Range £4,261 to £5,332 (subject to review) plus £502 London Allowance.

Applications, together with names of two referees, should be submitted by 31 August next to the Assistant Secretary (Personnel), University College London, Gower St, London WC1E 6BT. 2906(A)

MASSEY UNIVERSITY
Palmerston North
New Zealand

LECTURESHIP IN ZOOLOGY

Applications are invited for a lectureship in Zoology in the Department of Botany and Zoology, tenable from January 1980. Appointees will have a higher degree in zoology, preferably ethology, and will be expected to teach an introductory and an advanced course in ethology, to supervise graduate students, and to carry out research.

Salary NZ$11,894 to $14,615.

Further details of this position and of the University, together with the general conditions of appointment may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or the Registrar of the University. Applications close on 15 September, 1979. 2939(A)

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

TECHNICIAN GRADE 3 (PHYSIOLOGY)

required from 1st October for a 3 year Wellcome project on the role of purines in the central nervous system. Applicants should have a background in biochemistry, physiology or pharmacology and experience of handling animals. Telephone Dr T W Stone (01-672 1255 ext 4657) for details.

Application forms available from the Establishment Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. Closing date 5th September. 2938(A)

UNIVERSITY OF WARWICK

RESEARCH ASSOCIATE

In Biological Sciences

To work with Dr. A. Colman in Developmental Research Group on project concerned with the secretion foreign proteins from oocytes and embr of *Xenopus laevis*. Applicants should h a first class or upper second class hono degree in biochemistry or a relev biological science, or an MSc if their f degree was a lower second honours degr The successful applicant will not be allow to register for a high degree. Starting sal on the IB scale: £3,775 to £5,488 pa. T post is funded by the Cancer Resea Campaign from 1st October 19 renewable on an annual basis. Furt particulars may be obtained from Dr Colman in the Department of Biologi Sciences.

Application forms from the Acade Registrar, University of Warwi Coventry CV4 7AL quoting ref 1/2A/79. 2901(A

CORNELL UNIVERSITY

POSTDOCTORAL RESEARCH ASSOCIATE I PEST MANAGEMENT MODELING

Opening for scientists or engineers v experience in systems analysis and in management or population dynam Prior training in optimization meth computer science, and in economic desirable. Openings available in fo insect management and in alfalfa management.

Applications should be sent to Christine A. Shoemaker, Departmen Environmental Engineering, Cor University, Ithaca, New York 14 U.S.A. W294(A

Queen Charlotte's Maternity Hospital

Institute of Obstetrics and Gynaecology (University of London)
Goldhawk Road, London W6 0XG

Post Doctoral Research Biochemist

required for joint project with the Institute of Psychiatry, on tetrahydroxyisoquinolines in alcoholism, supported by the Medical Council on Alcoholism. Previous experience of high performance liquid chromatography desirable but not essential. Appointment for one year in the first instance.

Applications, with c.v. and the names of two referees should be made to Prof. M. Sandler, Department of Chemical Pathology. 2917(A)

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOCHEMISTRY

here is a vacancy for a

POST-DOCTORAL BIOCHEMIST OR CHEMIST

work with Dr Grahame Hardie on an RC-supported study of the structure of ammalian fatty acid synthase, an enzyme ith seven active sites on a single olypeptide chain (see FEBS Letters 978), **94**, 33-37). The project involves chniques of enzymology and protein emistry and will be carried out in sociation with the laboratory of Dr Philip ohen.

The appointment is available for up to ree years from 1 October 1979 or another reed date. Initial salary within range ,333 to £4,910 (under review).

Applications, with curriculum vitae, mes of two referees, and an indication of te of availability, should be sent to The cretary, The University, Dundee DD1 IN by 6/9/79. Please quote reference ST/60/79J. 2885(A)

THE MEDICAL COLLEGE OF WISCONSIN, INC.

BIOCHEMIST OR CELL BIOLOGIST

I am looking for 2 imaginative scientists with interest and enthusiasm to study interaction of interferon with cell membranes. This project is funded by a three-year grant from NIH and provides for two positions, one at a postdoctoral level (appr. $13,000 per annum), the other at a lower level, which will pay somewhat less. Thus, persons with PhD, MD, MSc or equivalent training or experience will be considered. The College provides fringe benefits and the atmosphere in a diversified biochemistry department is stimulating and cordial. We recently moved into brand-new and well-equipped facilities.

Please send enquiries to Dr Helmut Ankel, Department of Biochemistry, Medical College of Wisconsin, PO Box 26509, Milwaukee, Wisconsin 53226. W288(A)

UNIVERSITY OF LONDON KING'S COLLEGE

DEPARTMENT OF PHYSICS

A POSTDOCTORAL RESEARCH ASSISTANT (Physicist or Electrical Engineer)

required to work with Dr D Ll Jones on SRC sponsored project in terrestrial low equency radio wave propagation. The oject is experimental and involves the plication of a computer-controlled FFT alyser to transient electromagnetic lses. Experience in digital and analogue chniques is desirable. There will be an ement of field work.

The appointment will initially be for one ar with possible extension for a further o years. The starting salary will be £4,333 £502 London Allowance).

Applications to Dr D Ll Jones, Physics partment, King's College, Strand, ndon WC2R 2LS, by September 24, 79, from whom full details are available, oting ref 191687/N. 2891(A)

UNIVERSITY OF BRISTOL

Department of Biochemistry

Applications are invited for a

POSTDOCTORAL ASSISTANT

post (Range 1A) funded by an MRC project grant to study the biosynthesis of the erythrocyte membrane anion transport protein. The project will investigate the mechanism of incorporation of this protein into membranes, both in intact cells and in an mRNA-dependent cell free protein synthetic system. Applicants with previous experience in the area of nucleic acids and protein biosynthesis preferred. The post is for 3 years at a salary of £4,776 to £5,048 — £5,321 (scale subject to revision with effect from 1st October 1979), and will be available from October, 1979.

Applicants should send their curriculum vitae and the names of two academic referees as soon as possible to Dr M J A Tanner, Department of Biochemistry, University of Bristol, Bristol, BS8 1TD. 2886(A)

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university college of swansea

SENIOR RESEARCH ASSISTANTS/ RESEARCH ASSISTANT

Applications are invited for two posts of Senior Research Assistant and one post of Research Assistant in the Department of Chemistry, all supported by the Science Research Council.

Senior Research Assistant in Organic Chemistry. Applicants should have a PhD, or be about to submit for such a degree. The vacancy is to work with Dr K Smith on "Studies on Organoboranes".

Senior Research Assistant in Analytical Chemistry. The work will be to study the molecular origins of acoustic emissions from polymers under stress, under Dr D Betteridge. This work is interdisciplinary depending to a large extent on signal analysis, and applications from post-doctoral chemists, electrical engineers, material scientists, mathematicians and physicists will be considered.

Research Assistant in Organic Chemistry. Applicants should have a good honours degree (or equivalent) in Chemistry and be interested in synthetic organic chemistry. The successful applicant will work with Dr J S Davies on "Chiral Resins for Synthesis of Amino-acids" and will be expected to register for a higher degree.

The appointments which will be for one year in the first instance, from 1 October, 1979, with the possibility of renewal for a further year, will be on the following scales:

Senior Research Assistant: Commencing salary up to £4,910 per annum plus USS/USDPS benefits.

Research Assistant: Commencing salary up to £4,333 per annum plus USS/USDPS benefits.

Informal enquiries and names of two referees may be sent to the supervisors of the grants, but further particulars and application forms (2 copies) *must* be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea, SA2 8PP.

SENIOR RESEARCH ASSISTANT

Applications are invited for the vacancy of Senior Research Assistant in **Dynamical Sedimentology** in the **Department of Oceanography**. Applicants should be graduates in Oceanography, Physics or Civil Engineering and a postgraduate qualification, an MSc or PhD would be preferred. Mathematical skills and experience of the use of Computers, together with practical ability in the design and/or construction of equipment would be advantageous. The successful candidate will work on a programme determining the threshold of transport of heavy mineral particles under the combined influence of unidirectional and oscillatory flows, under the direction of Dr M. B. Collins.

The appointment will be for two years from October 1, 1979 and the commencing salary will be on a scale up to £4,910 per annum plus USS/USDPS benefits.

Informal enquiries may be made to Dr. M. B. Collins, to whom a short report or published papers exemplifying original work should be sent, together with the names and addresses of two referees, but application forms (2 copies) and further particulars *must* be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea, SA2 8PP. Closing date for all applications, Friday, September 7, 1979. 2927(A)

GERMAN CANCER RESEARCH CENTRE

INSTITUTE OF EXPERIMENTAL PATHOLOGY

Applications are invited for the post of a

POSTDOCTORAL RESEARCH ASSISTANT (M.D.)

to work on a project on the "Development and Application of Automated Procedures for the Early Detection of Cancer". Knowledge in Cytology would be an advantage.

The post is tenable from 1.8.1979 with an initial salary on Range IIa of the German Salary Scale (BAT).

For further details please contact German Cancer Research Center, Administration, Im Neuenheimer Feld 280, 6900 Heidelberg. W295(A)

MEDICAL RESEARCH COUNCIL
TOXICOLOGY UNIT
SCIENTIST

Scientist required to carry out research on the action of cadmium and other metals on mammalian systems. The aim will be to understand the mechanisms of the toxic action of such metals and the studies can range from a molecular to a whole animal approach.

A suitable candidate should have at least two years postdoctoral experience and will be eligible for a limited appointment for five years. Salary according to age and experience in the range of £4,776 to £6,108.

Applications should be sent to Dr W. N. Aldridge, Toxicology Unit, Woodmansterne Road, Carshalton, Surrey SM5 4EF by 15th September, 1979.

2947(A)

MRC Medical Research Council

See Page xxvi for a further APPOINTMENTS VACANT

UNIVERSITY OF NOTTINGHAM

CANCER RESEARCH CAMPAIGN LABORATORIES

Applications are invited for a post-doctoral research appointment to join a group studying the biology of chemical carcinogenesis. The project under the direction of Professor R. W. Baldwin is concerned with immunological studies in the early phases of chemical carcinogenesis and is supported by the Cancer Research Campaign.

Salary in range £4,333 to £6,644 (under review) and the starting date after September 1st by mutual agreement.

Applications in writing with full curriculum vitae and names of two academic referees to the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD, Notts. 2883(A)

UNIVERSITY OF LEICESTER/ICI JOINT LABORATORY

RESEARCH TECHNICIANS

Two vacancies exist for suitably qualified candidates to work as technicians on projects in eukaryotic molecular genetics. The techniques to be used include mRNA isolation, copy DNA synthesis and cloning in bacteria, *in vitro* translation and nucleic acid sequencing.

Appointments will be made at Grade 3 or Grade 5 according to qualifications and experience. Salary scales (under review) are £2,931 to £3,336 for Grade 3, £3,474 to £4,056 for Grade 5.

Applications, in writing, including a detailed curriculum vitae and naming two referees, should be sent to Miss S N Dilks, Chief Technician, ICI Joint Laboratory, Adrian Building, University of Leicester, Leicester, LE1 7RH. 2936(A)

SOUTH GLAMORGAN HEALTH AUTHORITY (T)
VELINDRE HOSPITAL, CARDIFF

Graduate Research Assistant (Radiobiology)
(Scientific Officer (PTA/A)

Applications are invited for the above post in the Department of Radiobiology at Velindre Hospital. The post is funded for three years by the Welsh Scheme for Development of Health and Social Research. The successful candidate will help develop *in vitro* systems for assaying the effects of cytotoxic drugs.

Previous experience with mammalian tissue culture or organ culture, or a background in Microbiology, Biochemistry or Pharmacy will be an advantage but is not essential. The starting salary is £3,486 (under review).

For further details and a visit to the laboratories, contact: Dr. J. L. Moore or Mr. J. Court. Telephone Cardiff 63325, Ext. 32.

Application forms are obtainable from the Unit Administrator, Velindre Hospital, Whitchurch, Cardiff CF4 7XL and should be returned by 7th September, 1979.

2923(A)

MEDICAL RESEARCH COUNCIL

CYCLOTRON UNIT

A medically qualified Pulmonary Physiologist is required for a programme of research into regional lung function. The position entails being involved in the development of technique to measure regional lung function using cyclotron-produced radioisotopes and a Positron Emission Computerised Axial Tomograph. It is intended that the methods developed be used to study regional lung function in various pulmonary disorders.

The appointment will be for one year. Salary according to age and experience, in the range £5,576 to £7,129 (inclusive of London Weighting).

Please write, giving qualifications and curriculum vitae to: Miss A Pires, MRC Cyclotron Unit, Hammersmith Hospital, Ducane Road, London W12 0HS. (Tel: 01-743 4594, extn 103). 2908(A)

GRADUATE REQUIRED for Nolan Laboratories, St. Mary Abbots Hospital, Marloes Road, London W8. Duties mainly tissue typing for bone marrow transplant programme. Knowledge of immunology and experience of tissue typing an advantage. Remuneration according to age and experience. Please apply in writing to Dr Ann White, Nolan Laboratories at the above address. 2914(A)

RESEARCH IN PROTEIN METABOLISM

Applications are invited from graduates (equivalent) with relevant experience for t post of Medical Laboratory Scienti Officer/Senior MLSO to assist with project on the regulation of prote synthesis and breakdown in animal tissu carried out at the Clinical Nutrition Metabolism Unit, St Pancras Hospit The appointment will be for up to two yea on the salary scales £3,261 to £3,9 (MLSO)/£3,813 to £4,680 (Senior) (bo under review) plus £354 Lond Weighting.

Applications, consisting of f education and career details and nami two referees, should be sent to Secreta (A1), London School of Hygiene Tropical Medicine, Keppel Street, Lond WC1E 7HT.

2912(A)

COURSES

THE HATFIELD POLYTECHNIC

MSc in ASTRONOMY and ASTRONAUTICS

(part-time — two evenings per week three years).

Induction course starts 24th Septemb 1979. MSc course starts 11th Februa 1980.

For further details, telephone Hatfie 68100 Ext 324 or write to The Secreta (MSc), The Hatfield Polytechn Observatory, Bayfordbury, Hertfor Herts SG13 8LD. 2890(D)

FELLOWSHIPS

STAFF FELLOWSHIP

The Clinical Branch, National Eye Institute, offers a Staff Fellowship in the Clinical Eye Pathology Section. Applicants may be PhDs or MDs and must have between four and six years' postdoctoral experience in immunology and immunochemistry. In addition, applicants must be U.S. citizens or resident aliens who would be eligible for citizenship within four years. The initial appointment will be made for two years with possible extension. Salary ranges from $20,000 to $37,500 and would be commensurate with the level of training and experience.

Interested persons should send curriculum vitae no later than September 14, 1979 to Karen M. Wright, Deputy Personnel Officer, National Eye Institute, National Institutes of Health, Public Health Service, Bethesda, MD 20205. Phone (301) 496 4274 for further information. Equal Opportunity Employer.

NATIONAL INSTITUTES OF HEALTH

W291(E)

ELLOWSHIPS continued

RESEARCH FELLOWSHIP

Imperial Cancer Research Fund Mill Hill Laboratories London NW7 1AD

A post-doctoral Fellow is sought to oin a group working on the control of lifferentiation of stalk and spore cells n Dictyostelium discoideum. The Fellow would be expected to employ genetical and biochemical techniques o analyse the cell interactions involved n differentiation in this organism.

Appointment will be for three years. Salary with entry according to qualifications and experience within ange £5,823 to £7,129.

Further information from Dr J Gross (01 959 3236). Application with urriculum vitae and names of two eferees, should be sent to The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, by 30th September, 1979, quoting reference 345/79.

2862(E)

UNIVERSITY OF EDINBURGH

EPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH FELLOWSHIP

plications are invited for this post to rk on the mode of action and uptake of drug Suramin in *Trypanosoma brucei* l the cause of resistance to that drug. Salary £4,333 per annum (plus eerannuation), with effect from 1st tober 1979.

The post, financed by the World Health ganisation's Special Programme for search and Training in Tropical eases, will be for one year initially with sible extension for a further two years. Applications with curriculum vitae and names of two referees should be sent to I B R Bowman, Department of chemistry, University of Edinburgh dical School, Teviot Place, Edinburgh, 8 9AG, not later than 30th August, 9. Please quote reference 5046.

2909(E)

UNIVERSITY OF GLASGOW

DEPARTMENT OF MICROBIOLOGY

POSTDOCTORAL RESEARCH FELLOWSHIP IN PROTEIN-LIPID INTERACTIONS

plications are invited for a three year C-financed post-doctoral appointment work with Dr J H Freer and Dr T H kbeck on the interaction of bacterial tolytic toxins with mixed lipid nolayers, liposomes and cell mbranes.

The appointment is for three years mmencing October 1979 (or as soon as ssible thereafter) in the salary range 1A, 333 to £7,521 (under review) plus eerannuation.

Applicants should preferably have a kground in biochemistry or chemistry h an interest in surface chemistry or terial toxins.

Applications, with the names of two erees, should be submitted to Dr J H er, Department of Microbiology, iversity of Glasgow, The Alexander ne Building, Garscube Estate, arsden, Glasgow G61 1QH from whom ther particulars may be obtained. In ly please quote Ref No. 4500M.

2931(E)

RESEARCH FELLOWSHIPS

at Continental Universities

Applications are invited for awards by the CIBA-GEIGY Fellowship Trust for the academic year 1980-81.

Senior CIBA-GEIGY Fellowships

Senior CIBA-GEIGY Fellowships will be awarded to start from an agreed date during the academic year 1980/81. These will be available normally to lecturers, senior lecturers, readers or those who have held comparable permanent teaching positions for a period of at least five years in the field of chemistry, biochemistry, chemical technology (including chemical engineering) and biology, particularly in its relation to chemistry, at United Kingdom or Republic of Ireland universities, polytechnics, or comparable teaching institutions, who wish to undertake special study at an agreed continental European university or technological university.

Duration of the award: minimum period of six months and up to two years.

Value of the award: £8,000 per annum plus travelling expenses. CIBA-GEIGY Fellowship awards are intended to supplement Fellows' salaries for the period of tenure.

No CIBA-GEIGY Fellowships for graduates will be made for the academic year 1980-81.

Further details and application forms, which should be submitted before 1 November 1979, can be obtained from The Secretary, The CIBA-GEIGY Fellowship Trust, 30 Buckingham Gate, London SW1E 6LH (Telephone: 01-828 5676).

CIBA–GEIGY

2898(E)

POST DOCTORAL RESEARCH FELLOWSHIPS

Applications are invited from organic or polymer chemists for two SRC Post-doctoral Fellowships to work in collaboration with Dr. P. Hodge. The topics are "Asymmetric syntheses using chiral polymer-supported catalysts or reagents" and "Kinetic studies of some polymer-supported reactions".

The appointments will be for one year in the first instance renewable for a second year. Commencing date 1st October 1979 or as soon as possible thereafter. Initial salaries £4,910 p.a. plus USS Benefits.

Applications, giving the names of two referees, should be made to Dr. P. Hodge, Chemistry Department, University of Lancaster, Bailrigg, Lancaster LA1 4YA.

2910(E)

To place your advertisement in these pages Phone: 01-831-6901

FRIEDRICH MIESCHER-INSTITUT:

A Postdoctoral Fellowship

in Biochemistry is available for the area of DNA synthesis in cultured mammalian cells. Experience in purification of DNA enzymes, DNA binding proteins or *in vitro* DNA replication is desired. The fellowship will run for two to three years.

Candidates should send c.v. and three letters of reference to Dr. L. Jimenez de Asua, Friedrich Miescher-Institut, PO Box 273, CH-4002, Basel, Switzerland.

W292(E)

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2904(L)

STUDENTSHIPS

UNIVERSITY OF OTTAWA

DEPARTMENT OF BIOCHEMISTRY

PLANT PROTEIN UTILIZATION

Applications are invited from graduates in chemistry, biology, or biochemistry to work on several projects investigating (a) protein-phenol interactions in oilseeds (b) lipolytic enzymes in high-protein oats (c) oat storage proteins (d) microchemical localization of phytate, glucosinolates, protein, etc. in rapeseed (e) Fraction-1 protein from hybrid poplar foliage. Research in the department's Nutrition Program is in collaboration with the food industry, the Food Research Institute of Agriculture Canada and the Ontario Ministry of Natural Resources. Graduate Research Scholarships ($6,500) are available.

Additional financial assistance is available from research stipends and demonstratorships (up to $2,000).

Please apply to Dr I Altosaar, Biochem Dept, Univ of Ottawa, 40 Somerset St E, Ottawa, Ontario K1N 6N5 Canada.

W299(F)

ASSISTANTSHIPS

UNIVERISITY
OF BRISTOL

Post-Doctoral Research in Theoretical Chemistry

Applications are invited for an SRC funded post-doctoral research assistantship to study the theory of photodissociation processes of small molecules. These processes are being studied in sophisticated experiments and the present work is aimed at using theoretical methods to predict the outcome of such experiments and to provide a better understanding of the photodissociation process itself.'

Applicants should send a curriculum vitae and the names of two referees to Dr G G Balint-Kurti, School of Chemistry, University of Bristol, Bristol BS8 1TS.

2887(P)

UNIVERSITY
OF DURHAM
DEPARTMENT OF BOTANY

Applications are invited for a

POSTDOCTORAL
SENIOR RESEARCH
ASSISTANTSHIP

tenable for one or two years from 1 September, 1979 or as soon as possible thereafter in the Glycoprotein Research Unit under the direction of Dr P W Kent. Preference will be given to candidates with experience of cell culture techniques. Initial salary in range £4,232 to £5,048 pa (£4,333 to £5,199 pa from 1 October) plus superannuation.

Applications (3 copies) naming three referees should be sent by 31 August, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham, DH1 3LE, from whom further particulars may be obtained. 2884(P)

CONFERENCES

A TWO-DAY CONFERENCE

Advances in Microbiological Techniques, Foods, Pharmaceuticals and Cosmetics

To be held at the:

Sudbury Conference Centre, London EC1

Tuesday 25th September, 1979

Plenary Lecture: Microbiological techniques in the 1980's *Professor G W Gould,* Unilever Research, Sharnbrook, Beds.

Survey of methods for the identification of medically important micro-organisms *Dr Elizabeth J Shaw,* St Bartholomew's Hospital, London.

The enumeration of micro-organisms in cosmetic products *Professor H Beerens, Ch Romond and C Lepage,* Universite du Droit et de la Sante, Lille, France.

Bioassay and enumeration by flow microcalorimetry *Dr A E Beezer,* Chelsea College, London.

The enumeration of micro-organisms using the spiral plate maker *V Lach,* Public Health Laboratory Service Centre for Applied Microbiological Research, Porton, Wilts.

Optical microscopy in microbiology *Dr K T Holland,* The University of Leeds, Leeds.

Enumeration of micro-organisms using bioluminescence techniques *Dr H H Johnston,* The Radcliffe Infirmary, Oxford.

The fluorescent labelled antibody technique *Irene Batty,* The Wellcome Research Laboratories, Beckenham, Kent.

Wednesday 26th September, 1979

Identification of micro-organisms by measurement of enzyme activity *Dr S Bascome,* St Mary's Hospital Medical School, London.

Kits for the identification of micro-organisms *Maggie Duke,* Elizabeth Arden Ltd., London.

The use of chromatography in food microbiology *M Ackland,* Metal Box Ltd., London

Computer-based bacterial identification systems *M Stevens,* University of Leicester Medical School, Leicester.

The uses of microbiological standards for foods *Dr R H G Charles,* Department of Health and Social Security, London.

Pharmacopeial and regulatory body standards for sterilization and preservation *Dr H S Bean,* Chelsea College, London.

European microbiological cosmetic standards *P E Harbord,* Johnson & Johnson Ltd., Portsmouth, Hants.

Concluding remarks *Professor D A A Mossel,* University of Utrecht, Holland.

For registration forms contact
Beverly Humphrey, Scientific Symposia Ltd., 33/35 Bowling Green Lane, London EC1R 0DA.

Please send me further details on the conference *ADVANCES IN MICROBIOLOGICAL TECHNIQUES, FOODS, PHARMACEUTICALS AND COSMETICS*

Name .....

Company/Organisation .....

Address .....

.....

Signed ..... Date .....

W305(C)

APPOINTMENTS VACANT continued

MEDICAL RESEARCH COUNCIL
TOXICOLOGY UNIT

Scientific staff member (non-clinical, with at least two years of postdoctoral experience) required to continue studies on the mechanism by which chlorinated compounds disturb the regulation of haem and haemoprotein metabolism in the liver.

A suitable candidate will be offered a limited appointment of five years. Salary in the range £4,776 to £6,108 according to age and experience; London Allowance; Superannuation provision and six weeks annual leave.

Applications, complete with curriculum vitae should be sent to Dr F De Matteis, Toxicology Unit, MRC Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF by 15th September, 1979.

2946(A)

MRC
Medical Research Council

GRANTS and SCHOLARSHIPS

EUROPEAN SCIENCE FOUNDATION

EUROPEAN TRAINING PROGRAMME IN BRAIN AND BEHAVIOUR RESEARCH

TWINNING GRANTS

ETP twinning grants are aimed at the promotion of contacts and collaboration between European institutes working in the field of brain and behaviour. Proposals can be submitted by 2 or 3 European laboratories, which should be located in different countries and be either working in the same field of research or in different disciplines which complement each other. Applicants should be submitted by only one of the participating institutes and be accompanied by (a) letter(s) of concurrence from the other(s).

Twinning grants are not paid in advance. Instead, credits of up to Ff10,000 are granted for projects selected by the International Scientific Committee. The costs incurred during the reciprocal visits of the teams involved are reimbursed upon termination of the project within the limits of the original grant.

The deadlines for completed applications is *15th March and 15th September.* Further information and application forms may be obtained from:

Dr. Stephanie ZOBRIST
European Science Foundation

European Training Programme in Brain and Behaviour Research

1, quai Lezay Marnésia
F-67000 STRASBOURG

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W297(H)

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Cover picture

A false-colour IR aerial view of Great Sippewissett Marsh, Massachusetts. The features shown are described on page 652 in an article which gives a complete nitrogen budget for this salt marsh ecosystem.

Vol.280 No.5724 23 August 1979

nature

Volume 280 23 August 1979

UK's wasted opportunity at UNCSTD 619

Science, nonsense and responsibility 619

The poor meet the rich — UNCSTD opens:
- **Third World aims to negotiate** 620
- **Arab group rejects Egypt** 620
- **Technology transfer benefits all — Jordan** 621
- **UK rejects new mechanisms** 621
- **Waldheim criticises 'wasteful consumerism'** 621
- **US supports 'reasonable ventures'** 622
- **Danes urge the need for compromise** 622
- **Canada pledges major increase in joint projects** 622

Press banned from carcinogen meeting 623

Nitrosamines found in Scotch Whisky 623

Hazards at work will kill 100,000 a year 623

In brief 624

India: the struggle for useful science 625

Correspondence 630

NEWS & VIEWS

Primitive model for cell cycle control/Immunotherapeutic T cells/Conserving resources of animal species/The impact of climatic change/Life before birth/ Mars volatiles/Ice on Mars again/New routes to polypeptide hormones 631

REVIEW ARTICLE

Nuclear vibrations — *G.F. Bertsch* 639

ARTICLES

A 30,000-yr isotope climatic record from Antarctic ice — *C. Lorius, L. Merlivat, J. Jouzel and M. Pourchet* 644

Shelf sea fronts' adjustments revealed by satellite IR imagery — *J.H. Simpson and D. Bowers* 648

The nitrogen budget of a salt marsh ecosystem — *I. Valiela and J.M. Teal* 652

Phenotype stabilisation and integration of transferred material in chromosome-mediated gene transfer — *L.A. Klobutcher and F.H. Ruddle* 657

LETTERS

IUE observations of an active region of HD206860 — *C. Blanco, S. Catalano and E. Marilli* 661

Variations of the Sun's radius and temperature due to magnetic buoyancy — *J.H. Thomas* 662

Silicalite-2, a silica analogue of the aluminosilicate zeolite ZSM-11 — *D.M. Bibby, N.B. Milestone and L.P. Aldridge* 664

Extraction of uranium from seawater by polymer-bound macrocyclic hexaketone — *I. Tabushi, Y. Kobuke and T. Nishiya* 665

Anodic coal reaction lowers energy consumption of metal electrowinning — *M. Farooque and R.W. Coughlin* 666

Impact of CO_2 on cooling of snow and water surfaces — *B. Choudhury and G. Kukla* 668

An apparent periodicity in an index of volcanic activity — *A. Chance and P.M. Kelly* 671

The Tweeddale Granite — a newly discovered batholith in the Southern Uplands — *E. Lagios and R.G. Hipkin* 673

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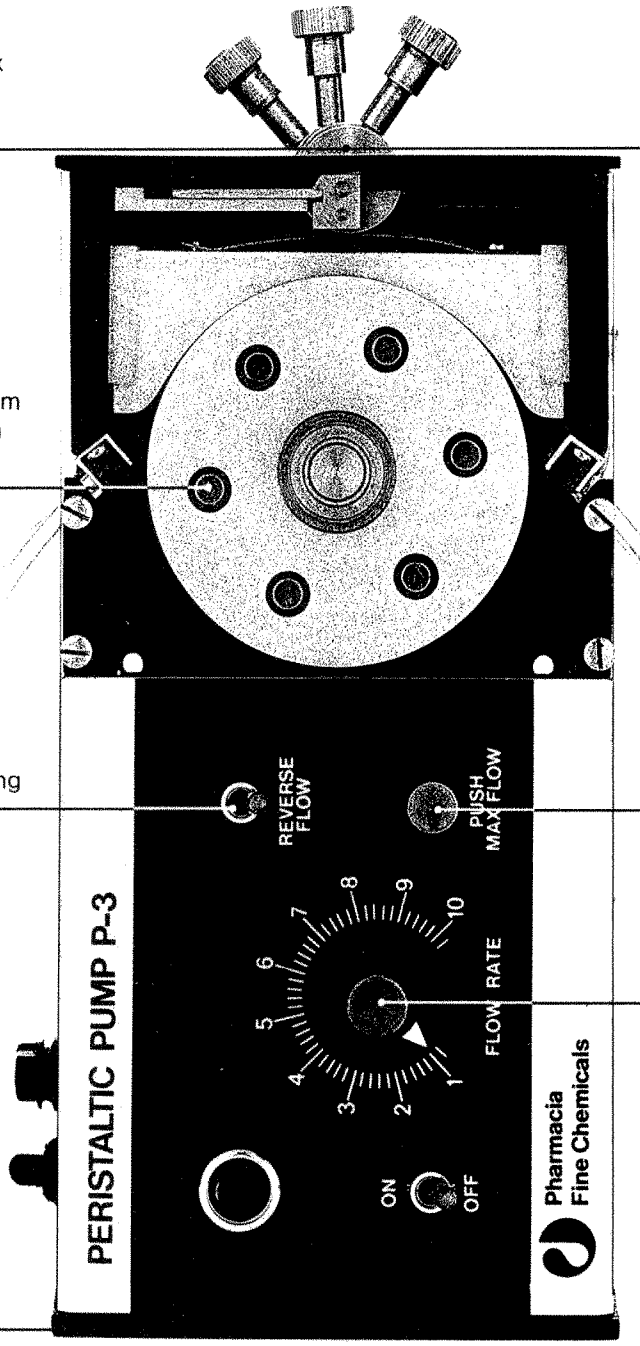
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Lichonometry and earthquake age determination in central Asia	*A.A. Nikonov and T. Yu. Shebalina*	675
Do circadian oscillators ever stop in constant light?	*E.L. Peterson and M.D.R. Jones*	677
Relative 'trophic' influences of excitatory and inhibitory innervation of locust skeletal muscle fibres	*R.B. Clark, K.A.F. Gration and P.N.R. Usherwood*	679
Differential labelling of retinal neurones by ^{3}H-2-deoxyglucose	*S.F. Basinger, W.C. Gordon and D.M.K. Lam*	682
Glucocorticoids inhibit endorphin synthesis by pituitary cells	*R. Simantov*	684
Continuous culture of T cells cytotoxic for autologous human leukaemia cells	*J.M. Zarling and F.H. Bach*	685
Thy-1 antigen on astrocytes in long-term cultures of rat central nervous system	*R.M. Pruss*	688
Characteristics of *Aedes albopictus* cells persistently infected with dengue viruses	*A. Igarashi*	690
The X-linked α-chain gene of *Drosophila* LSP-1 does not show dosage compensation	*D.B. Roberts and S. Evans-Roberts*	691
Cellular protein synthesis and inhibition of cell division are independent of butyrate-induced histone hyperacetylation	*P. Rubenstein, L. Sealy, S. Marshall and R. Chalkley*	692
Micro- and macro-stabilities of globular proteins	*P.L. Privalov and T.N. Tsalkova*	693
Membrane crystals of ubiquinone:cytochrome *c* reductase from *Neurospora* mitochondria	*P. Wingfield, T. Arad, K. Leonard and H. Weiss*	696
Adenine N^6-substituent of agrocin 84 determines its bacteriocin-like specificity	*M.E. Tate, P.J. Murphy, W.P. Roberts and A. Kerr*	697
Erratum		669

MATTERS ARISING

Dihydroergocryptine is a non-selective antagonist for human platelet α-adrenoreceptors	*M.C. Scrutton and J.A. Grant*	700
Are cardiac muscle cells skinned by EGTA or EDTA?	*J.P. Reuben and D.S. Wood*	700
Reply	*D.J. Miller*	701
Response	*S. Winegrad*	701
Reply	*D.J. Miller*	702

BOOK REVIEWS

General Relativity: An Einstein Centenary Survey (S. W. Hawking and W. Israel, editors)	*Robert Wald*	703
The Operon (J.H. Miller and W.S. Reznikoff, editors)	*Richard Calender*	704
Sex, Hormones and Behaviour (Ciba Foundation)	*Alan F. Dixson*	705
Atomic Absorption Spectroscopy (M. Slavin)	*Allan M. Ure*	705
Neural Modelling: Electrical Signal Processing in the Nervous System (R. J. McGregor and E.R. Lewis)	*Jack Cowan*	706
Laboratory Handbook of Chromatographic and Allied Methods (O. Mikes, editor)	*R.L.M. Synge*	706

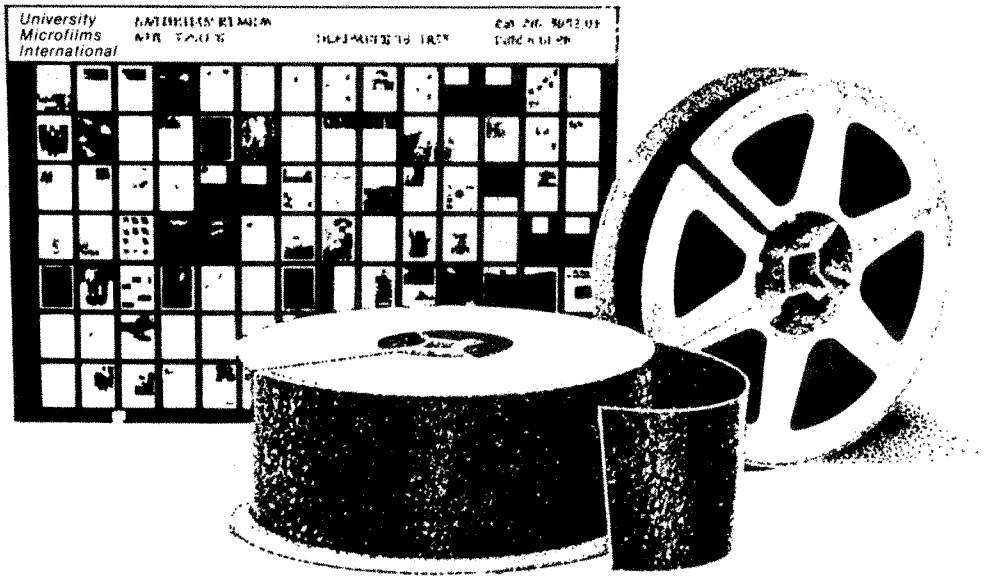
OBITUARY

Giulio Natta	*G. Lindeberg*	707
Scott R. Mazzur	*G.A. Jamieson*	708

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Nature Vol. 280 23 August 1979

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UK's wasted opportunity at UNCSTD

Whatever may or may not be decided at UNCSTD in Vienna these two weeks, it is perfectly clear that the world scientific and technological community is experiencing strong pressures for change, for instance through proposals for the establishment of a massive global research and development fund, and through demands that the legal framework for the transfer of technology be restructured. It is thus vitally important that all those who attend UNCSTD take back to their home country a clear impression of the forces at work and alert their colleagues in every possible way to the need for new thinking. To a degree press reports help, but a very important ingredient is what individual people who attend do.

The constitution of the British delegation to UNCSTD is — for whatever reason — such as to make this job very difficult to perform for the scientific and technological community. A delegation of twenty or so could have included some well connected scientists, some influential industrialists, some representatives of education, and some non-governmental development experts in addition to the necessary complement of civil servants. Instead the delegation comprises a minister (for two days), eight civil servants, five representatives of the Vienna embassy, three private or personal secretaries and only two people who could in any sense be regarded as outsiders. The burden on these two to inform the broadest community is intolerable. It will be interesting to see what help they get from their civil-servant colleagues in the months ahead.

Science, nonsense and responsibility

THE right — and responsibility — of the layman to exert an influence on the actions of scientists and technologists is becoming firmly established, as is evidenced this week by the controversy over the exclusion of journalists from an international meeting on short-term testing for carcinogens (see p.623). But is there not an equal right and responsibility for the scientist to guide the layman? Guidance is surely needed. The misuse of science by those often lacking in scientific training and understanding, but never lacking either conviction or commercial acumen, is now commonplace. It is commonplace because there are profits to be made from it.

For example, it is there even in the bookshops. A new form of escapism has been packaged as an alternative to science fiction, romantic fiction and thrillers. The new genre treats as fact a wide range of spurious topics. Themes include the artefacts left by our extragalactic forefathers, the possibility of disappearing through triangular holes in the sky over the Caribbean, the everyday lives of UFO-flying folk, paranormal fork-bending and outrageous claims about the influence that this additive-free food or that celestial body might have on our lives. A factor common to all these books, it seems, is the need for belief. A recent example of the extent to which strongly held but totally unfounded belief can resist the weight of fact is the saga of the alleged anti-cancer drug laetrile.

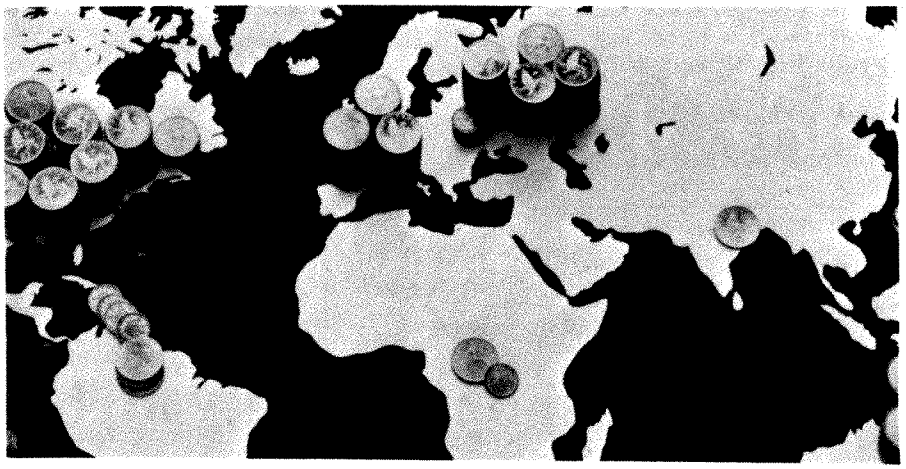
Can this need for belief be satisfied by something more sober than UFOs and cancer cures? By, say, a belief that an analytical and scientific approach to a physical problem will lead to as near as we can get to a solution? This will be possible only if genuine critical science, with its clarities *and* uncertainties, is sold to the public with the determination that sells pseudo-science. It does not help to hold closed meetings and so increase the public's natural distrust of 'the expert'. The exercise is difficult, but it must be undertaken, or the voice of reason will not be heard at all.

True science does answer back sometimes. The BBC *Horizon* series carried an excellent critique of von Daniken's books. But many thousands more of his books will have been sold since then. Science should answer back more loudly and more often. One medium which invites such a response is the relatively new one (in the UK) of local radio, which thrives on the cheap-to-run 'phone-in' programme. In these programmes all forms of fringe and suspect forms of pseudo-science are often discussed by 'experts', and the more far-fetched the thesis proposed the more enthusiastic the response from the listeners. Just as harmful are 'open-line' programmes in which callers attempt to discuss subjects which neither the caller nor the programme presenter comprehends. Presenters are generally conscientious and, especially on medical matters, make pleas for calls from more qualified listeners. The pleas are often unanswered. This seems a pity because, although discussion of this type are certain to be shallow and are often not conducted very well, a shallow discussion with some sense in it must be better than one with no sense.

It may be true that 'truth will out', as it did eventually for laetrile. But scientific truth will 'out' much faster if it is sold with more enthusiasm, and if this means descending to the level of the market place, then perhaps that's as it should be.

The poor meet the rich

'Nature' correspondents report from Vienna on the opening sessions of the United Nations Conference on Science and Technology for Development



Third World: we will negotiate

DELEGATES to UNCSTD from a wide range of Third World countries agreed at a meeting in Bucharest last week that, at least at the start of the conference, they will bury economic and ideological differences behind a common negotiating strategy. They also agreed to stand firm behind the developing countries' proposals for a draft plan of action to emerge from UNCSTD — although they are prepared to negotiate on the plan's main demands rather than invite confrontation by sticking to a hard line.

The meeting was organised by the Group of 77 which has been negotiating on behalf of over 120 developing countries during the UNCSTD preparatory meetings. Representatives from the developed countries were relieved to hear that the Group of 77 would not seek initial confrontation. Although the developed countries are not prepared to accept all the demands that the developing countries have included in the proposed plan of action, they are keen, for political and economic reasons, to engineer a successful outcome to the conference.

Before the Bucharest meeting, some developing countries had complained that the proposed draft plan did not reflect their particular concerns. (The draft is the basis for the negotiations currently taking place in Vienna). Some African countries, for example, had complained that the proposals were too heavily weighted towards measures to benefit the more advanced of the Group of 77 countries — for example, with conditions governing technology transfer — and would not do enough to meet the technical needs of the poorest countries.

The Bucharest meeting agreed to recommend that the plenary session in Vienna establish a special working group to look at proposals for areas in which greater scientific and technical effort should be focused. In return the African groups agreed to support the Group of 77's proposals for a new funding system and new institutional arrangements for science and technology with the UN. Both proposals will be examined by UNCSTD's two main committees.

Members of the Group of 77 still differ, however, over how the new institutional arrangements would operate to assure the politically weaker countries adequate control over research funds and their application.

A statement summarising the demands of the plan of action was issued at the end of the meeting. It says that inequalities in all fields of international economic relations are especially actue in science and technology. Despite the efforts of the developing nations there has been no real progress towards setting up a New International Economic Order with science and technology forming an intergral part. The statement expresses alarm at the large fraction of global research expenditure devoted to defence and asks for "free and full access to scientific and technical knowledge, discoveries and innovations".

International cooperation should also play an important part in harnessing science and technology to the social objectives of the developing countries, the statement says. "For this, global and fundamental structural changes are required in the existing distribution of scientific and technological capacities in the world, in order to ensure increased participation by the developing countries in the quest for new scientific and technological knowledge".

In line with the suggested plan of action, the statement urges that international law governing technology transfer should be "restructured" in line with the goals of the developing countries who should be able to participate in financing mechanisms set up for scientific and technological development. The statement also urges support for measures to increase scientific and technical cooperation between developing countries. It says that practical measures "should be undertaken to support the cause of national liberation of peoples and territories under foreign occupation, control and domination, racism and racial discrimination, to enable them also to benefit from the application of science and technology for their development".

David Dickson

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Arab group expel Egypt and establish Libya as spokesman

The decisions taken at the Tenth Conference of Islamic Foreign Ministers, held in Fez during April, are now being implemented by a strong 'Arab group' that has emerged at UNCSTD. The Islamic conference decided to boycott Egypt on all issues and support the Palestine Liberation Organisation.

In the past Egypt has assumed a natural leadership on Arab issues, in the field of science and technology, and this is the first time it has faced a determined and joint opposition to its efforts to lead the Arab group. Libya was unanimously elected to lead the group last week-end despite the fact that, nominated as a vice-president for North Africa, it was absent from its first meeting.

Alienation of Egypt from the Arab group has provided even stronger Arab support for the PLO delegation to UNCSTD. "We are determined to get a nation-state status for the PLO" a member of the Syrian delegation declared. At their first meeting, the group decided not to articulate an OPEC (or more appropriately, AOPEC) position. OPEC Arab countries will play a low-key role where we are allowed to do so" and will merge their identities within the Arab group.

The group also decided to give full support to the announced position of the Group of 77. Although the stance taken by the Group of 77 is a little too radical for some of the more conservative Arab states, like Saudi Arabia, Kuwait and the UAE, these states have been pursuaded to support the Group of 77 as much as possible. Similarly Syria, which has shown considerable opposition to some declarations of the Group of 77, has decided to overlook its objections and support the decisions of the Arab group. Dr Shakir Fahm, Syrian Minister of Higher Education and leader of the Syrian delegation to UNCSTD says, "now is the time for the developing countries to show

their strength. This strength lies in unity".

What the Arab group did not discuss is the financing of a development fund to help scientific and technological activities in the developing countries. Whether Saudi Arabia and other oil-rich Arab states will back such a fund to the tune of $65 million a year for the first two years of the fund, as some suggested, they did not decide — though there is some indication that Saudi Arabia will give in to the US pressure. The Saudi aid to developing countries granted through the Saudi Development Fund, far exceeds this sum in an average year. The Saudi decision to support the fund, if it is forthcoming, will therefore be in line with their aid policies.

In addition to national efforts in such countries, international cooperation can and should play an important part, the statement says. "For this global and fundamental structural changes are required in the existing distribution of scientific and technological capacities in the world, in order to ensure increase participation by the developing countries in the quest for scientific and technological knowledge."

Ziauddin Sardar

'In everyone's interest' to transfer technology

The transfer of technology is not an issue of necessary conflict between developed and the developing countries, according to Crown Prince Hassan of Jordan, who shoulders the responsibility for social and economic planning in Jordan. Speaking during the general debate of the second plenary meeting at UNCSTD, Prince Hassan said that "despite short term disagreements and apparent disparities, in the long term, technology transfer is in everybody's interest". He warned, however, that technology "is not a commodity which can be copied to exaction. The transferred technology has to be appropriate to the needs of the receipient country".

Many developing countries, said the Prince, lack the basic conditions for the establishment of agencies that promote and enhance technology. Furthermore, technology in the developed countries is based on a particular concept of entrepreneurship — that motivated by economic and commercial considerations. The Prince questioned the desirability of the developing countries adopting such a concept and argued for a "spirit of resourcefulness" or "appropriate entrepreneurship".

The Prince pointed out that at present much aid is channeled towards the poorest members of the Third World, the decisions for granting aid being made on the basis of gross national product (GNP). But there is hardly any relationship, argued the prince, between GNP per capita and the levels of development.

Talking of the brain drain, the Prince emphasised that the human dimensions of the problem, "which have a direct bearing on technological and scientific development in both labour-exporting and labour-importing countries", are often overlooked.

Crown Prince Hassan suggested that the labour exporting countries should turn the situation to their advantage through policies that aim to utilise the experience of their nationals working abroad. "These should take the form of annual feedback programmes, where highly trained scientists and technologists working abroad would come back to their countries for varying lengths of time".

Making an obvious reference to the proposed global development fund, the prince said that "we have to be careful before we decide to create yet another international agency providing yet another variation on the existing manner of solving an already identified problem".

Ziauddin Sardar

Waldheim attacks 'wasteful consumerism'

A major task of UNCSTD is to help generate universal acceptance of the need to direct the use of humanity's scientific and technological potential to constructive ends, Dr Kurt Waldheim, Secretary General of the United Nations, told the opening session of the conference on Monday morning.

In a speech which reflected many of the ideas put forward by the conference secretariat during the preparatory process, Dr Kurt Waldheim singled out three areas in which he claimed much of human ingenuity and innovative ability had been misdirected into areas not beneficial for mankind: military technology; a type of economic growth which ignored damage to the environment or the social and cultural fabric; and the support of "wasteful consumerism in a world where famine and malnutrition are tragically present".

Another motivating factor behind the conference was the enormous imbalance in research and development being undertaken internationally, he said, resulting in developing countries having to depend excessively on imported technology. This hampered the growth of indigenous skills more harmonious with local conditions and socials or economic needs.

"This becomes a self-generating process and breeds over-all dependence. The starting point for breaking this circle is to enable the developing countries to gather and share the scientific knowledge so as to enhance their technological capabilities and accelerate their development", he said.

Three elements were crucial in designing a successful programme of action: a conscious political will, increased funding for science and technology at the national and international levels, and the institution of an efficient mechanism for this purpose. Finally, directly echoing words used on many occasions by conference Secretary-General Mr Frank da Costa, Dr Waldheim said that the real divisions in the world were not between the north and south or east and west, but between those in favour of a passive continuation of the status quo, "which is prejudicial to all" and those in favour of dynamism, changes and innovation. "Let this conference signal the fact that science and technology can unite the developed and the developing countries in the common cause of the world's future as a whole", Dr Waldheim said. □



Britain opposes creation of new mechanisms

A greater proportion of national and international aid budgets should be devoted to scientific and technological aspects of development, but there is no need at present for new financing mechanisms or institutional arrangements to do this. Such was the main message of the British delegation's contribution to the UNCSTD plenary debate on Wednesday morning.

Mr Neil Marten, UK Minister for Overseas Development, told the assembled delegates that, if developing countries so wished, Britain would be prepared to devote an increasing share of its bilateral aid programme to scientific and technological activities in the Third World. He also announced that Britain is considering plans to set up a technology transfer centre to act as a general switch-

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board for enquries about British technology.

However on the two main issues which are likely to dominate negotiations during UNCSTD — the Group of 77's proposal for a new international financing system for science and technology, and for a new co-ordinating body within the UN — the British response was lukewarm at best, reflecting the position of other EEC countries.

Nor did the British show any keenness to discuss the heated topic of technology transfer or the conduct of multinational companies, and area where encouragement and support of existing channels and procedures is preferred to greater regulation and control.

"Experience shows the advisabilty of making progress through continuing change based on tested practices and procedures, rather than embarking on abrupt changes of direction. We have serious doubts about the value of any new financial mechanisms or the separation of funds for science and technology for development", Mr Marten said.

"Given that aid budgets are limited, the separation of funds is likely to drain off the resources available for development into different pockets. This would make it difficult for individual recipient governments to set their own priorities clearly and would lead to inflexible and inefficient programmes." □

Danes urge the need for compromise

In one of the most direct statements made on the opening afternoon of the conference, the head of the Danish delegation said that it was "imperative" that a compromise be reached on various proposals for financial resources to support greater scientific and technological efforts in developing countries, if the conference was to be made a success.

"My country is definitely prepared to carry its fair and proportionate share of the financing of science and technology" the Danish representative, Mme Lise Ostergaard told the conference, an indirect reference to proposals for a new fund largely financed by the developed countries.

● A further pledge for additional financial support came from the Swedish delegation. Pointing out that Sweden is already one of the few countries which contributes more than the 0.7% of its gross national product to international development previously agreed as a global target, the head of the Swedish delegation said that agreement had already been reached on a number of recommendations which, for their implementation, demanded increased financial resources.

Sweden was prepared "together with other countries and on the basis of a fair burden-sharing formula, to make a substantial increase of our contributions in support of international cooperation in the field of science and technology.

US supports 'reasonable ventures'

Speaking on the opening afternoon of UNCSTD, the chairman of the US delegation announced that the US was prepared to join "reasonable ventures" to strengthen worldwide scientific and technological cooperation.

This theme was echoed in a message from President Carter to the conference, in which he said that the US was willing to support "all practical endeavours" to help overcome problems such as food scarcity, the energy crisis, and the population explosion.

Although no direct comment was made in either pronouncement, the choice of words was partly a response to some of the proposals contained in the draft plan of action submitted to the conference by the Group of 77 — such as the setting up of a new international financing mechanism totalling $2 billion by 1985 and $2 billion by 1990 — which has previously been characterised by US negotiators as being "unrealistic".

US officials have not made it clear what they would support as a more "practical" alternative. However they indicate that the executive branch would be prepared to look favourably in the order of $1.50 million over the next two years, although pointing out that no firm commitment to such a contribution can be made without the support of Congress.

In his speech to the conference plenary session, Father Theodore Hesburg, president of Notre Dame Univeristy and chairman of the US delegation, said that the global economy was not working as well as it should for either the poor or the rich countries, and that the patters of worldwide technology generation, diffusion and utilisation lacked the cohesion that would incorporate and benefit the majority of people. "We have not yet found the right mix between scientific excellence and needed technologies" he said.

It was an imperfect global economic order that did not fully benefit from the "robust and dynamic role" of international business and industry, and had not yet found "the right balance between the interests of private enterprise and of the developing countries" he said — an implicit recognition of the need to negotiate on issues such as the conditions for the international transfer of technology.

"The task of this conference is not one of restating the errors of the past but of weaving science and technology into the fabric of the future, the fabric of development. We need collaboration, not confrontation", Father Hessburgh said.

Among the specific proposals were that the US would take the initiative to bring together the operators of remote sensing satellites as systems. In addition, it was necessary for developing countries to have access to the leverage provided by scientific and technological information, if more just and equitable patterns of scientific and technological cooperation were to be achieved.

Referring to President Carter's description of science and technology for development as a "joint venture", Father Hessburgh said that the challenges faced by developed and developing countries alike made such a joint venture a global imperative. "The US notes, therefore, with pleasure the declaration of Bucharest (see page 620) in which the developing countries reaffirmed their willingness to work with a sense of urgency to assure the success of this conference", he said.

Science and technology should be used to help bring forth a threefold harvest: in the realm of reason, the realm of reality and the realm of rights. Efforts made in the real of reason were not a zero-sum game, in which the gains of those who seek equality and parity would automatically register as a loss for those who possess more. "In this realm we can prove the mutual benefits thesis — that advances in any part of the world are for the benefit of all", he said.

David Dickson

Canada pledges increase in joint projects

The Canadian government has agreed substantially to increase the budget of the International Development Research Center (IDRC) to allow it so support a joint projects between scientists in Canada and research groups in developing countries.

Addressing the UNCSTD plenary session on Tuesday, M. Martial Asselin, recently-appointed secretary of state for the Canadian International Development Agency (CIDA), said that although the precise amount of funds allocated to this purpose is yet to be decided, it would be in the order of the one-third of IDRC's current budget — ie about $12 to $14 million, an amount equivalent to 1% of Canada's total foreign aid.

Although under its initial terms of reference, IDRC is permitted to fund joint ventures of this nature — which from the Canadian side could involve university research groups or scientists from federal or provincial laboratories — the vast majority of the center's current funds are spent solely on research efforts in the developing countries.

The new commitment will, according to the Canadian Government, provide a direct way of linking up the needs of developing countries with Canada's own scientific and technological capacities. It has yet to be decided whether there will be an expansion Canada's foreign aid budget new year. □



Time to drown your sorrows?

US scientists find carcinogen traces in Scotch whisky

US scientists have found traces of nitrosamines, one of the most potent known carcinogens, in six brands of Scotch whisky. A few months ago, similar traces were found in various types of beer.

In both cases it appears that the nitrosamines are formed during the drying of sprouted barley malt. The levels detected are small, less than two parts per billion, according to Dr David H. Fine and Dr E. Ulku Goff, who carried out a study as part of a project funded by the National Science Foundation.

However levels as low as 10 parts per billion of nitrosamines are known to increase the incidence of tumours in laboratory animals. The beer industry has already said that, although little is known about the effects of such low doses on humans, it is taking steps to eliminate the presence of nitrosamines. It expects to have a solution "within a matter of months".

A spokesman for the Distilled Spirits Council of the US said last week that there was a marked discrepancy between the results of the NSF study and measurements reported last year by the World Health Organisation in Geneva, which had found nitrosamine levels of less than 0.2 parts per billion in Scotch whiskies. The council said this difference "may raise questions about the accuracy" of the new measurements.

But Dr Lawrence Garfinckel of the American Cancer Society doubted that such small amounts of nitrosamines would cause cancer. The most significant factor for him was that many drinkers were also smokers; a synergistic effect could account for the increased death rate from mouth and throat cancers that had shown up in studies of beer, wine and whisky drinkers.

The brands of Scotch whisky said by the NSF study to contain nitrosamines are: Chivas Regal, Black and White, J & B, Ballantine's Sandy Scot and Cutty Sark. Nitrosamines were not discovered in White Label whisky, nor in 46 other alcoholic beverages that were also studied.

Press banned from conference

The press is to be excluded from a meeting to evaluate short term, *in vitro* tests for carcinogenicity, following a ruling by the conference's organising committee. A decision not to invite journalists to attend the October, 1979 meeting in Atlanta, Georgia was taken when some of the participants voiced fears that the attendance of the press at the event would have an inhibitory effect and restrict the usual scientific discussions on such occasions. It has been decided, however, that in view of media interest in the subject, information on the conclusions of the conference will eventually be issued in December, at a press conference to be held in Washington.

Decisions to exclude the press from important scientific meetings are not without precedent. Recently, COGENE, the Committee on Genetic Experimentations of the International Council of Scientific Unions attempted to ban the reporting of the proceedings of a meeting on genetic engineering at Wye College in Kent. (See *Nature*, **278**, 496, 1979). The directive announcing the ban was greeted with a storm of protest and COGENE were forced to reverse their decision. The criticism of a reporting ban could perhaps have been predicted, given the public interest in genetic engineering.

Carcinogens are also a subject which are much in the public eye, a fact given emphasis by the current proposals for their control being discussed by Government Regulatory Authorities in Europe and the United States. European regulatory authorities appear to be far more in favour of *in vitro* tests as a screening net for carcinogens than do their counterpart organisations in the US. The Environmental Protection Agency, for example, views these tests with some doubt.

In view of the importance of the short term tests, and concern about the variability in results reported from different laboratories testing the same chemicals, two scientists — Dr John Ashby of ICI's Central Toxicology Laboratory, Alderley Edge, Cheshire, UK, and Dr F.J. Serres, NIEHS, Research Triangle Park, North Carolina, U.S., decided to organise a carefully controlled study to evaluate short term tests. Ashby, with the resources and expertise of ICI to assist him, was responsible for the synthesis, coding and distribution of some 42 chemicals to be tested by 25 *in vitro* assays in various laboratories throughout the world (see *Nature*, **274**, 740; 1978). The results of this valuable collaborative programme will be discussed at Atlanta in October.

The one notable ommission in the list of participants in Atlanta is Dr Bruce Ames of the University of California. Ames, who developed the famous *in vitro* test for carcinogenicity which bears his name declined to take part in the Ashby/Serres study. The Ames test has, however, been evaluated in several laboratories along with other tests.

Few scientists involved with short term tests are unaware of the significance of the Atlanta meeting; Government Regulatory Authorities are similarly aware. Dr David Douglas, Head of the UK Health and Safety Executives unit for collating information on toxic substances referred to the Atlanta meeting as the one which would "take the lid off" the subject of short term tests.

The importance of the Atlanta meeting cannot be denied. It is this which makes the intended exclusion of journalists so controversial. It remains to be seen whether the organisers will have second thoughts and reverse their decision.

Alastair Hay

Hazards at work 'kill 100,000 a year'

In a draft report to the President, the Toxic Substances Strategy Committee of the US Council on Environmental Quality claims that "more than 100,000 workers are believed to die each year as a result of physical and chemical hazards at work". The committee estimates that between 20% and 38% of all cancer may be caused, in part, by occupational exposure to carcinogens.

The report says prevention is the major key to controlling these environmental hazards and calls for the rigorous application of more than two dozen statutes for the regulation of toxic chemicals already on the books. It also advocated more legislation, including setting up a federal authority responsible for cleaning up hazardous waste sites.

More than 43,000 chemicals are listed by the Environmental Protection Agency as being subject to regulation. It may take years to determine whether or not a substance is hazardous and the complexities in reaching such a judgement are enormous. But the committee feels that the difficulties can be mitigated, to some extent, by greater cooperation between agencies involved in toxics-related research. The Government is to spend around $1 billion on toxics research next year.

The report has drawn some criticism from the chemical industry. The Chemical Manufacturers Association called it a "hodgepodge of concepts and recommendations that have been either disputed or discredited since the committe was created in 1977."

Anne Norman

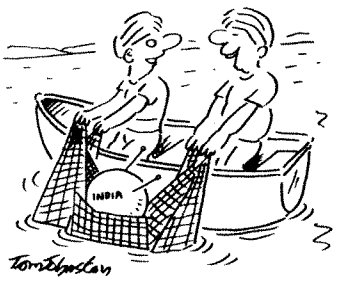
news in brief

Windscale issues emergency advice: Residents living within a mile of the UK Windscale nuclear fuel reprocessing plant in Cumbria have been issued with leaflets explaining emergency procedures in the event of any serious incident there. Similar booklets have also been sent to farmers within 10 miles of the plant. The move follows a recommendation by Justice Parker — whose inquiry report last year gave the go-ahead for expansion at the plant — that local residents be informed of all emergency procedures. At first explantory leaflets were sent to local libraries and the media. Now these have been issued to the residents nearest to the plant to keep for reference. The booklets give details of evacuation plans, information broadcast procedures and medical arrangements in case of serious radiation leaks.

Harrisburg re-opening depends on residents' mental health: The mental health of residents living near the damaged Three Mile Island nuclear plant is a matter of "real and substantial concern" and should be carefully considered before allowing even the unaffected reactor on the site to be restarted, the US Nuclear Regulatory Commission has announced. Leonard Bickwit, NRC general counsel, said the commission felt that psychological, sociological and economic distress should all be considered in hearing the licensee's application to restart the plant, a decision unlikely to be taken by the NRC until the middle of next year. He added that a ruling on the mental health of residents could be a landmark in regulatory legal process.

This pronouncement contrasts with the recent publication by the operating company, General Public Utilities, of a plan to resume operations at the plant. This would involve venting into the air some of the radioactivity now contaminating the reactor chamber. However, although the quantities proposed would only involve releasing one tenth of a millirem of gamma radiation, compared with the 83 released at the height of the station emergency, it is unlikely the proposal will be given the go-ahead without strong legal and political opposition. And even if approved, it is unlikely that the plant could resume operations before June 1983.

Draft code to protect asbestos workers: A consultative document has been produced by the UK Health and Safety Commission on a proposed code of practice for workers dealing with asbestos insulation. The proposals are an extension and clarification of existing legislation. They include proposals for improving asbestos dust containment, waste disposal, personal protection and training for insulation workers. Industry and trade unions have until 30 November to give their view before a final code of practice is drawn up.



"Perhaps we'll have some benefit from our own technology now!"

Indian rocket crash: India's first rocket designed to put a satellite into orbit crashed into the Indian Ocean during a test flight. The launcher flew for only 15 minutes after blasting off from Sriharikotta space research centre in South India. The test failure is a setback for the Indian space programme which has been preparing for greater independence from the Soviet Union, whose launchers it uses at present. It was intended to put a satellite, Rohini Satellite-1, into orbit later this year using the SLV-3 rocket — which is similar to the US Scout rocket and uses solid propellants in all its four stages. Once the SLV-3 is successfully completed, India intends to launch a series of Rohini satellites — to be followed by a more ambitious programme of communications satellites.

Ariane delay: The first flight of Europe's independent space launcher, Ariane, has been postponed by about a month. The launch was scheduled for 3 November but because of problems with ball-bearing mounts for first and second stage engines, this has now been put back until the end of November or the beginning of December. However, a European Space Agency said the difficulty had not been a major one and had now been overcome.

African venereology conference opens: The first African International Conference on Sexually Transmitted Diseases was due to open at Ibadan University, Nigeria yesterday. More than 200 doctors and researchers were expected to attend and the opening speaker, Dr Duncan Catteral, described the event as "an historic day in African medicine". It had taken three years' campaigning to arrange the conference he said, and the agreement to stage it indicated that the African governments now recognised that sexually transmitted diseases were a major problem. Although no statistics were available, Dr Catterall believed the diseases were now widespread throughout the continent and there was an urgent need to develop a common policy towards treatment and contact tracing. In particular, the uncontrolled use of penicillin in many African countries was leading to the development of resistant strains which in future could prove very difficult and expensive to cure.

Two sponsors required for animal research under new Bill: Lord Halsbury's Bill on the protection of laboratory animals, due for its second reading in the House of Lords on 25 October (16 August, page 534), will require an applicant for a licence to do research on animals to have two sponsors, Lord Halsbury announced last week. One must be the applicant's supervisor, and the other an independent expert in the procedures to be used, who will also advise on alternative techniques. After the licence has been granted, the new licensee will have to work for a probationary period under the supervision of an experienced licensee. These rules will apply to those working with animals to produce vaccine and serums as well as those "experimenting" with animals.

The licensing of institutions where "procedures" on animals are carried out should ensure that stolen pets do not end up in laboratories, Lord Halsbury explained at a press conference on the Bill last week. The conference came to an abrupt end after interuptions from members of the Animal Welfare Society and the National Anti-vivisection Society.

Lord Halsbury's is not the only Bill on laboratory animals shortly to be considered by Parliament. Peter Fry MP will be putting another Bill to the House of Commons for its first reading shortly after the start of the next session.

US climate programme sets goals: Three major areas of activity — climate impact assessment, climate system research, and climate data, information and services — have been earmarked for the newly-established National Climate Programme, it was announced in the US. The programme was established by Congress last year and will coordinate the work of various federal, state, private, academic and international organisations. It is also planned that several experimental climate forecast groups should be set up as part of the programme, which will cost a total $114.2 million next year. These will develop, evaluate and test new climate forecasting methods.

Soviet cosmonauts land: Cosmonauts Vladimir Lyakhov and Valerii Ryumin have returned to Earth after a record 175-day flight aboard the Salyut-6 space station. First reports say they are healthy owing to an "exceptionally conscientious" attitude to exercise — unlike the ground crew, who according to Flight Controller A.S. Eliseev "have been suffering a certain amount of weariness".

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India: the struggle for useful science

Are India's scientists "spoilt brats" who need to be put in their place? Or are they being manipulated by power-hungry politicians? *Anil Agarwal* reviews the beleagured state of science in India at the end of the two-year government of Mr Moraji Desai, who resigned as Prime Minister last month.

NEVER before was there more tension between the Indian government and the country's scientific establishment than during Prime Minister Morarji Desai's administration, which was catapulted into power in April, 1977, after the unexpected defeat of Mrs Gandhi at the polls. In October 1978, Mr Y.B. Chavan, the Opposition Leader in Parliament, took the unprecedented step of writing a protest letter to Mr Desai urging him to call an all-party meeting at the very earliest to discuss the problems created by the Janata Government's science policy. Mr Chavan said: "Science is a major national concern. It is not a party matter . . . our national commitment to science has been an agreed component of our national development policy right from independence". However, he alleged that since the Janata Party came to power, "several very disturbing developments have taken place . . . which have resulted in rapid demoralisation of the scientific community."

Mr Chavan criticised Mr Desai for permitting "foreign powers to make major inroads" into the country's nuclear programme and for unilaterally abjuring nuclear explosions even for peaceful purposes; for his totally unwarranted attack on the Council of Scientific and Industrial Research (CSIR) which in the face of a strong and unanimous reaction in the Parliament and the press, he had "to practically reverse" within barely three months; and, for mortgaging such vital sectors of the Indian economy as the aircraft and heavy electrical industries to Western and multinational interests.

Mr Chavan informed the Prime Minister that "we in the Congress Party believe that India's scientific and technological capability is a national resource, which any government whatever its political predilections, should cherish and develop to give strength to our country in a grimly competitive world."

For full thirty years, India was ruled by the Congress Party, first, under the leadership of Prime Minister Jawaharlal Nehur, and then, except for a brief period in the mid-sixties, under his daughter, Mrs Indira Gandhi. Both these leaders closely identified themselves with the scientific community. They made it a point to inaugurate every annual meeting of the Indian Science Congress; they would seldom miss an opportunity to visit a scientific laboratory or inaugurate a scientific seminar; and, they always kept in their Prime-ministerial portfolio such scientific departments as atomic energy, space and electronics to ensure that the department did not suffer from any bureaucratic constraints. Mr Nehru also set the tradition that the President of the CSIR would be the Prime Minister.

He believed that science was the only tool that could help India to eradicate poverty without a violent revolution, and therefore, tried to give the scientific community enormous prestige. Mrs Gandhi would always describe Indian S&T capabilities as the bulwark of India's self-reliance. During their combined regimes, R&D funding increased 353-fold — from Rs. 11 million in 1948-49 to Rs. 3887 million in 1976-77 (1 Rs = £ at 1979 prices) — and giant organisations like the CSIR (with 44 scientific institutions), Defence Research and Development Organisation (with 31), Indian Council of Agricultural Research (29), Atomic Energy Commission (7), Indian Council of Medical Research (8), Department of Science and Technology (18), Space Commission (5) and Electronics Commission (a funding and coordinating agency) grew up from scratch.

In 1977, the Central Ministries, public sector companies and State governments had another 327 R&D units; the private sector, 348; and 120 universities and institutes of technology were also conducting a variety of R&D projects. India had by then the world's third largest S&T manpower (only behind the US and USSR) consisting of 2.33 million, doctors, engineers, technicians and scientists. Over 53,700 professional were directly working on R&D projects in 1976-77.

Yet as far back as the mid-sixties, a feeling had started growing that India was not getting sufficient returns from this massive investment, particularly in industrial research. By the mid-seventies, Mrs Gandhi too occasionally began to chide Indian scientists for not conducting more relevant research. But the criticism of the Congress leadership was negligible compared to its praise. In a sense, it was a matter of vested interest for it to praise



Moraji Desai: some accused him of being anti-science

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Indian R&D efforts, which, after all, as it rightly claimed, were there because of its own foresight. The vast urban intelligentsia, which grew up in the three decades of independence, (and which helped to sustain and drew sustenance from the Congress leadership) also came to see in these giant R&D establishments a sense of national achievement — a matter of pride for a developing country in a world where both political power and economic status essentially come out of the twin barrels of science and technology.

The new Janata Government was, however, not bound by this traditional association with Indian science, and, therefore, saw no reason to evaluate its achievements in muted tones. It immediately raised the question: have these organisations provided the country with benefits commensurate to the investments made in them? Dr Atma Ram, a former Director-General of CSIR, who was appointed by Prime Minister Desai as the chairman of the country's major science policy body, the National Committee on Science and Technology (NCST), explained in a recent lecture: "There is a vital difference between Morarji Desai's period and the periods of his predecessors. During the period of his predecessors, most of the expenditure in S&T was devoted to building up the institutional infrastructure . . . This phase has now given place to the next phase of assessing benefits from science. People want to know how far they have benefited from the science in which they have invested their hard-earned money. It is natural that the Prime Minister should pose such questions before the scientists."

But why should there be any basis for asking such questions? In thirty years, Indian industry has grown substantially. India today manufactures computers. nuclear reactors, textile machinery, pharmaceutical plants, heavy engineering goods, tractors and other sophisticated industrial products. But the bulk of this manufacturing activity is based upon imported technology, obtained through foreign collaboration agreements which by 1977 totalled about 5200. Direct foreign investment has remained restricted as a government policy but know-how has been imported from Eastern Europe and the USSR as much as from Western multinationals. India's own R&D institutions — even the national paper to the UN Conference on Science and Technology for Development admits — have played a very minor role in supplying the technology for this industrial development. The scientists and engineers in industrial establishments have themselves played a major and praiseworthy role in absorbing this imported expertise.

Take the case of Indian engineering goods for which the export trade has grown more than one hundred-fold — from Rs 52 million in 1956-57 to Rs 5517 million in 1976-77. These goods now constitute the most dynamic element of India's exports. India has also developed a series of its own mini-multinationals which have established several joint ventures with local enterprises in other developing countries. As the Indian government does not easily allow Indian companies to make foreign investments in cash, they have had to capitalise almost entirely on their know-how and equipment produced in India.

But why has the output of Indian R&D institutions been so poor? There are several reasons for this, and the main one is that Indian research institutions behave more like science laboratories than technology development institutions. They have failed to develop good links with prospective users of their know-how; they seldom carry their work beyond laboratory scale plants; most of them don't have adequate design and engineering capabilities; they seldom provide users with the software (raw materials procurement, credit, organisation, management, marketing, etc.) which is required to commercialise their hardware.

The Indian paper to UNCSTD admits in a milder language: "most Indian R&D agencies are much stronger in the generation of technology than in its delivery." But why is this so? Dr Atma Ram explains that "in those early days of the beginning of organised research efforts, there may not have been much understanding about the problems that would have to be dealt with in relation to the overall economic development of the country. There was perhaps a fascination for science, maybe a feeling of romanticism about it but not necessarily a clear and precise understanding of the mechanics of the application of science to economic growth and other spheres of national development. In other words, the necessary structure for research had been built with a noble but perhaps an undefined goal."

In March 1958, the Indian parliament had adopted a unique "Scientific Policy Resolution" (SPR) which was a declaration of intent by the people of India, as opposed to a resolution adopted by a particular government, to give sustained support to science. No other Third World country has ever made such a public commitment to science. The SPR was Prime Minister Nehru himself with some



T for Tata: assembly line in western India

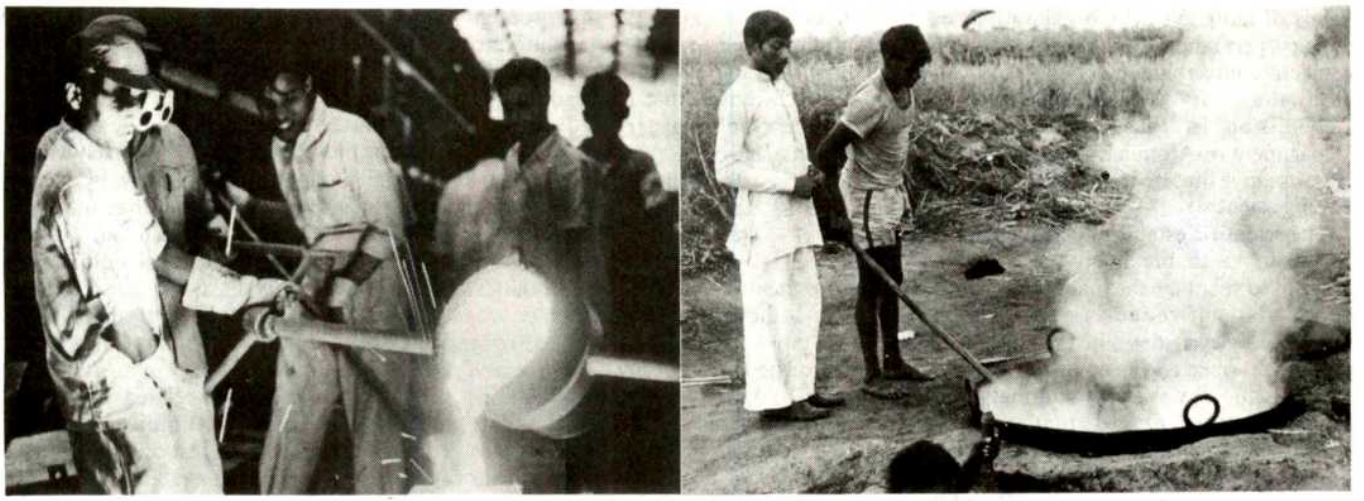
Imported technology . . . exported trucks

One good example of an Indian mini-multinational is the Tata Engineering and Locomotive Company (TELCO), which is probably the first real Third World automobile multinational. TELCO even uses its own brand name. TELCO's joint venture in Malaysia assembles 7-ton 'TATA' trucks with components made by the parent TELCO in India. TELCO originally imported its technology from Daimler-Benz but now sells more than that company in Malaysia. It still does not have the capability to develop a totally new engine or a new vehicle. To manufacture a 13-ton truck in India, it has purchased a fresh licence from abroad. But through a well-organised technology assimilation programme, it extensively modified the 7-ton truck, concentrating on increasing its ruggedness and reliability — important attributes for bad road conditions in developing countries. Western companies meanwhile paid more attention to speed, driver comfort and emission control, which are of greater concern only in the developed countries. TATA trucks are, therefore, in demand in the Third World.

Apart from its Malaysian joint venture, TELCO has also licensed assembly of its trucks to companies in Indonesia, Egypt, Guyana and United Arab Emirates. Assembly of scooters (of a design originally imported into India from Italy) has been licensed by another private sector company to Taiwan and Indonesia. Meanwhile, the premier public sector company, Bharat Heavy Electricals Ltd (BHEL), which in the past obtained its technology from USSR, Czechoslovakia, UK and West Germany, is now independently setting up power stations, boilers and transmission systems in Malaysia, Libya, New Zealand, Saudi Arabia and Jordan, and competing successfully with big companies abroad.

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Should technology be backing heavy industry or traditional rural processes like sugar making (right)?

help from the Chairman of the Atomic Energy Commission, Homi Bhabha. But it is noteworthy that the word 'technology' does not appear anywhere in the text of the SPR, nor does it reflect the distinction between science and technology and the need for technological innovation.

It is very difficult to make a cost-benefit analysis of an R&D organisation like the CSIR which also conducts basic research and occassionally plays an important role in increasing the bargaining strength of Indian enterprises while purchasing foreign know-how. Out of the total number of processes developed in Indian R&D institutions, up to 1974-75, 1293 out of 1726 were from CSIR and out of the total number of 729 inventions, CSIR had 607 to its credit. (Incidentally, defence laboratories which remain outside the realm of public scrutiny, had only 46.) But many CSIR processes remain unused. The royalty earned from processes released for commercialisation does throw some light on the return made from investments in R&D. Total royalties earned by the National Research and Development Corporation, which offers technology developed in Indian R&D institutions for commercialisation, came to Rs 5.2 million in 1976-77. The CSIR alone spent Rs. 413 million in that year on R&D.

Entrepreneurs, both in the public and private sector, have generally claimed that they are afraid of investing in 'half-baked' CSIR technology. They, therefore, exert pressure on the government to allow import of know-how from established foreign sources. R&D institutions, on the other hand, have always complained that the technology they generate is not used by industrial establishments because of vested interests in importing from abroad. Often these entrepreneurs have even been allowed to re-import the same technology. This results in unnecessary remittances of scarce foreign exchange, breeds technological dependence, and reduces the demand for indigeneous technology. In this way, both the country and its scientific community suffer. In a bid to stop such repetitive imports and encourage the use of local know-how, successive governments have allowed representatives of scientific establishments to participate in the various licensing committees, where they have the votes to stop any import of technology if it is already locally available, or it can be made available within reasonable time. But this practice has often led to delays in starting industrial establishments and is, therefore, not very appreciated by Indian entrepreneurs.

The Electronics Corporation of India Ltd, a public sector company that takes strong pride in indigeneous technology, was some years ago manufacturing and selling an outdated computer which was several times dearer than an equivalent computer abroad. The few components that had to be imported to manufacture it locally, were said to be higher than the total cost of a similar computer abroad. The Electronics Commission had to protest to the company about its price, as Indian users had no other alternative but to buy these computers because of a protected market. There is another case where a national laboratory offered to develop the technology for a public sector company to manufacture ferrites. The laboratory took several years to develop it, during which the country had to import the finished product in scarce foreign exchange. Finally, when the laboratory did supply the know-how, the company found that it still had to import the capital equipment.

Dr Atma Ram is a strong critic of unnecessary restrictions on import of know-how. It is "Made in India" and not "Invented in India" that matters ultimately for economic development. Indigeneous R&D, and import of technology, are not mutually exclusive, he says. "They are complementary. We need far more intensive efforts in R&D in areas in which technology is borrowed so as to adopt, modify and improve with the least possible delay."

Dr Atma Ram strongly criticised the restrictive approach to technology imports by the Electronics Commission arguing that the Indian electronics industry does not even compare with that of far smaller countries like South Korea, Taiwan and Hong Kong. "We are not a technologically illiterate nation," says Dr Atma Ram. "Our experts can be depended upon to make the best available choice and build upon it, provided they are suitably utilised." Japan, USSR, South Korea and now even China, are all buying technology. "My fear is," he told me, "that it is not a militarised China but an industrially powerful China that will soon begin to threaten India." Professor M G K Menon, a close confidant of Mrs Gandhi, has since relinquished his position as Chairman of the Electronics Commission and is now Director-General of CSIR.

Desai's battle to reorganise research

But it is not the transfer of Professor Menon as much as the earlier attempt by Prime Minister Desai to reorganise the CSIR that has drawn the real ire towards the Janata Government. A few months after he had assumed office, Mr Desai suggested that the CSIR had become too large for one Director-General to manage. Without any semblance of consultations with the scientific community at large, he got the Indian cabinet to accept the reorganisation of the CSIR. Under this plan, 28 CSIR institutions were to be split off and attached directly to their main user ministries. Each R&D institution would have become a separate registered society with the relevant Minister as its President to ensure the autonomy of the institution. Some senior officials of the Ministry would also become members of its governing body. However, when the news leaked out, the reaction was hostile. The scientific establishment immediately saw in it an attempt to erode its autonomy and make it subordinate to interfering bureaucrats. Indian scientists have traditionally despised generalist bureaucrats for their power in decision-making. To circumvent bureaucrats and ensure direct access to Ministers, Mrs Gandhi's government started the practise of making

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heads of most scientific organisations ex-officio permanent secretaries of their respective government departments. The Chairman of the Atomic Energy Commission is, thus, secretary to the Department of Atomic Energy as well. Mr Desai's move thus revived a traditional fear among scientists.

The scientific establishment further saw in the move to break up the CSIR, an attempt to sabotage the main source of the country's self-reliance. Several left intellectuals even described it as a move that would open up the country's industry to domination by Western multinationals. Some scientists argued that the new government was trying to "cut scientists down to size" because of the good relations they had enjoyed with Mrs Gandhi's government — which indeed was true even during the authoritarian emergency days, when the scientific establishment rushed to show its loyalty to its political benefactors, probably more than any other professional group. Others dismissed the new government as basically anti-science, and conveniently used the government's interest in simple rural technologies and Mr Desai's own interest in traditional medical systems like auto-urine therapy, to illustrate their point.

It was soon clear that Mr Desai's government had not only ineptly handled the reorganisation proposal but also totally misjudged the symbolic national value that the CSIR held in the minds of the urban intelligentsia. It was a rare newspaper that supported the government. Several members of parliament also protested. Headlines screeched: "CSIR has been an eyesore to the multinationals" and "CSIR is the first martyr to the Janata Government's ill-concealed solicitude for the international giants."

As is usual in Indian politics, such charges are seldom accompanied by proof. While it is possible that the proposal could result in more harm than good — through greater bureaucratisation of the laboratories linked directly to Ministries and thus greater stifling of their scientists — it is difficult to imagine that Prime Minister Desai could have deliberately taken a decision to sabotage the country's self-reliance. I interviewed a series of senior scientists, many strongly opposed to the government's proposal, but none would say that Desai had wrong intentions. All agreed that the output of organisations like the CSIR needs to be both increased and improved.

However, the proposal was strongly supported by the bureaucracy because they could have surreptitiously helped those Indian interests who prefer foreign technologies and thus increase the influence of Western multinationals, explained a senior scientist working in one of the government departments. Once scientists came under the direct control of bureaucrats, they could no longer oppose their wishes. And it is among the bureaucracy, he alleged, that foreign technology-oriented industrial interests have considerable influence. The case of the research centre, that has traditionally been under the Minister of Posts and Telegraphs is cited. This centre has apparently done less to generate indigeneous technology and more to "sanctify the import of foreign technologies".

In the face of the unexpected political clout that the scientific community was able to muster, the proposal had to be scaled down. Finally, only four research institutes and, ten research associations were split off from the CSIR. The three museums under it were also transferred to the Ministry of Education. And it was decided that the reorganisation would be treated as an experimental measure, the performance of the separate institutions watched, and if so required, the decision could be reversed.

Getting more out of India's labs

But whatever may have been the outcome of Mr Desai's proposal, it is important to note that his original question remains: how can Indian laboratories be coaxed to produce more? His was not the first proposals for re-organisation. Several committees like the Administrative Reforms Commission and Sarkar Committee of Enquiry into CSIR have also considered reorganisation. But none of the proposals have ever met with success. The scientific establishment itself has produced no proposals, nor even made any effort to come up with some.

The Indian scientific organisations are themselves extremely hierarchial and bureaucratic establishments, where success is often the result of patronage. Within these organisations, it is hard to find any semblance of enquiry or debate. Whatever may happen, the working scientists remain quiet. In fact, there is no evidence to show clearly how the scientific community (as distinct from the top establishment) reacted to Mr Desai's proposal. "Must scientists keep mum?", asked Surendr Jha, editor of *Science Today*, India's leading science journal, in an editorial. The government today provides nearly 90 per cent of Indian R&D funds. These funds have brought with them a financial and administrative culture, that has resulted in a stifling bureaucratisation of the R&D institutions. Scientists have turned into scientist-bureaucrats, appendages of a vast system of patronage and hierarchy. It is as if these big laboratories are like the Taj Mahals of Indian science, built to glorify and bury it.

In this bureaucratic system, the director-generals of the various councils and chairmen of the various commissions enjoy vast power and they take decisions without any reference to their own area of specialisation. Ward Morehouse, a US social scientist who has extensively studied Indian science policy, claims that these seven or eight scientists take virtually all the decisions in the world of Indian science. Committees are rampant in the decision-making procedure, and they are freely used to legitimise any whim or fancy by simply ensuring proper selection of their members — a practice that the Indian administration, in general, has imbibed in more than a full measure from Britain. Taking all the members of the NCST, directors of various laboratories, etc., decision-making of any national significance in science-related matters is restricted to about 200 individuals. The decisions made by the rest of the scientific community, if they make any, are of, at best, very local significance only.

It is not surprising that in this sytem, power and prestige usually comes to an Indian scientist when he leaves his laboratory, stops any R&D work directly, and turns into a science administrator. A study of Indian scientific and technical manpower engaged in R&D recently released by the Institute of Applied Manpower Research, New Delhi, reveals that only nine scientists out of 23,700 surveyed had a salary above Rs2,500 and 108 were in the scale between Rs2,250-2,500. Almost all of them were administrators. The apex for personnel engaged in full-time research lay below Rs2,500.

It is, therefore, easy to appreciate that such powerful and heirarchical establishments will strongly resist any reorganisation, whittling down of their empires, or even any effective science planning, for that matter. The best example of resistance to the latter is provided by the work of the first National Committee on Science and Technology, which was constituted in 1971 as a small and compact body of 10 members chaired by Mr C Subramaniam, then Minister of Planning and Science and Technology. The principal task of the NCST was, in the words of Mr Ashok Parthasarathi, then Mrs Gandhi's special assistant on science and technology, "to prepare a comprehensive science and technolocy plan, derived from and closely integrated with the fifth Development Plan (1974-79). This was the first time that a deliberate political committment had been made to integrate the economic, scientific and technological and educational dimensions of the development strategy into a coherent whole."

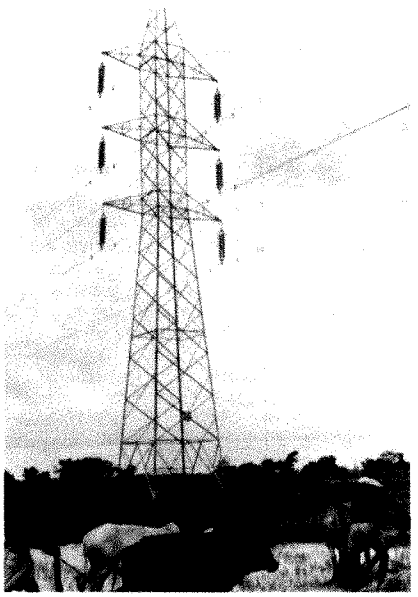
The members of this NCST were all young, middle-rank scientists, deliberately chosen to bring a breath of fresh air into science planning. But the giant organisations controlling agricultural research, defense research, atomic energy and space research, which consume the bulk of institutional R&D funds, made no serious attempt to co-operate. None of them wanted their areas of competence to be subjected to scrutiny by any committee outside. The NCST, too, recognising that it

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Power plant deal that sparked off protests

One outstanding incident that attracted considerable public attention during Mr Desai's administration was the deal of the Bharat Heavy Electricals Ltd (BHEL) with the West German multinational, Siemens, to acquire know-how for a comprehensive range of heavy electrical equipment, including 200-500 MW coal-based power plants. The deal was expected to last 15 years, an unusually long period as the government generally frowns upon long-term collaboration agreements. Over 100 MPs and virtually every newspaper columnist again protested that this deal would make the country's electrical industry totally subservient to the foreign company. Part of the criticism emanated from pro-USSR MPs, who are piqued that BHEL was dumping technology that was originally acquired at easy terms from USSR. But many Indian public sector companies, who initially obtained know-how from socialist countries (often at critical moments when they had been totally denied access to know-how by Western sources) have in recent years sought collaboration with Western suppliers. This is because their technology demands have become more sophisticated, they themselves have



gained more bargaining strength, and because they are now looking for competitive export markets.

The Minister of Industries, George Fernandes, replied to the criticisms by pointing out that it was the Janata Government which sent IBM and Coca-Cola out of the country. His Ministry had promoted a drink developed by the Central Food Technology Research Institute, a CSIR constituent, to replace Coca-Cola. Moreover, the negotiations for the BHEL-Siemens deal had begun when Janata Ministers were behind bars, and therefore his government should not be described as "selling the country out to multinationals". He himself supported the deal as one that would be useful to BHEL. The BHEL board approved it and so did the Ministry of Industry and the inter-ministerial Foreign Investment Board. But when public pressure mounted up, he had to seek the approval of the Indian Cabinet. And when the Cabinet couldn't reach a decision, it was referred to a specially constituted committee of five Cabinet Ministers for further consideration. It is not certain now whether the deal will go through.

The BHEL-Siemens deal can certainly be criticised for its comprehensiveness as it does appear to leave little to challenge BHEL's own engineers. It could have been more selective and BHEL's know-how demands could have been tempered by the company first seeking to fully supply the Indian market instead of being excessively worried about gaining more export muscle. Many vital industrial areas like West Bengal and Maharastra are facing crippling power shortages. But the manner in which the deal has been questioned publicly gives the impression that many people believe Indian companies should be totally self-reliant for their technological needs. Except USA, there is no country in the world which generates itself even 10 per cent of the technology it uses. It is ironical that the atomic energy programme, which is held up as India's finest example of self-reliance, is really an example of a programme that is based on assiduously assimilated foreign know-how.

did not possess the muscle to take them on, concentrated mainly on the areas of R&D for which the CSIR is responsible. This too became possible apparently because the chairman of NCST was also then Minister in-charge of CSIR. As soon as the plan was prepared, the NCST was reconstituted and the young scientists replaced by the heads of the giant organisations. The simple explanation given was that the new NCST should rightly consist of the organisational heads as they are the people responsible for implementing the plan. But was that really the case? As the paper to UNCSTD puts it, the plan soon became "a useful check-list of programmes and projects", and that "suitable mechanisms for their implementation, monitoring and review could not be devised till the middle of the fifth plan (1974-79)." Some 2000 scientists had been involved in the preparation of the NCST plan, which was so easily, more or less, scuttled.

The next chairman of the NCST, Mr P N Haksar, seldom convened the reconstituted NCST, and, in fact, after a long delay, when he did, he asked it to discuss for a whole day whether there was a need for such a committee. As he put it, there were already so many barons to take care of Indian science. The NCST was, thus, soon reduced to a dormant state. Until, of course, Mr Desai appointed Dr Atma Ram, a close confidant, as the third chairman, and then unpleasant decisions like the reorganisation of the NCST and review of the Electronics Commission began to be taken.

Prime Minister Desai certainly refused to acknowledge the need for the all-party meeting suggested by Mr Chavan and challenged "anyone to prove that the transferred institutions are not closely and intimately connected with the policies and programmes of the Department concerned." "I would go so far as to say," he pointed out, "that the scheme of reorganisation promotes the country's commitment to using science for national development." But despite this bold face, the Janata Government became increasingly defensive. Both Prime Minister Desai and Dr Atma Ram, chairman, NCST repeatedly pointed out that the Janata Government was neither anti-science or anti-scientists, and that actually increased the R&D budget from Rs3,887 million in 1976-77 to an estimated Rs4,767 million in 1978-79. The Prime Minister's speech to the Indian Science Congress in 1979 is reported to have been largely drafted by Professor Menon instead of Dr Atma Ram.

The lack of communications between the government and the Indian R&D community has come at an inopportune time. Something that Indian scientists have always wanted, an R&D culture in the industrial establishments themselves is now steadily growing. Many large public and private sector companies have set up their own R&D units. Science organisations like the CSIR now not only need to increase and improve their output, but also begin to co-ordinate their work with these units and even slowly begin to reconsider their long-term role in Indian R&D. As industries begin to undertake routine industrial R&D themselves, R&D institutions could possibly move into more sophisticated and forward-looking basic and applied research that is required to support industrial R&D. In the present state of disarray, there appear to be very few science planners thinking at all, particularly in the scientific establishments.

The incidents during the Desai administration show that the scientific establishment can muster enormous political clout in a developing country. But, unfortunately, in the Indian case, it is behaving more like a pampered and spoilt super-brat, who immediately turns nasty when its ears are tweaked. But questions of public accountability in India will certainly arise again. □

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correspondence

Fast reactor safety

SIR, — In his article on fast reactor safety (26 July, page 270) Norman Dombey claims to introduce to non-specialists some features of fast reactors that are not available outside the technical literature. The non-specialist would do well to treat this article with caution as it contains a number of errors (some of them elementary) and is more likely to mislead than to inform. The following is a list of some of the more outstanding errors:

- "Thermal reactors are designed to be in their most reactive nuclear configuration". This is incorrect and some examples were given by Professor F.R. Farmer (12 April, page 593). Some reactivity coefficients in thermal reactors are positive and are not all negative as stated — indeed gas-cooled Magnox reactors have operated safely for over 20 years with strongly positive moderator temperature coefficients.
- "Thermal reactor has less than one critical mass" This is incorrect: all reactors have to have at lest one critical mass.
- "So in a fast reactor of the design envisaged in CFR1 . . . there will be no overall negative Doppler coefficient" The reasoning and the conclusions are both incorrect and there is in fact a strong negative Doppler coefficient which is a major contributor to the safety of the reactor. (I have now seen the erratum (9 August, page 444) which corrects this point.)
- "For example a coolant channel gets blocked, sodium boils . . . pieces of plutonium fall and form a molten mass which if large enough can become prompt critical!" This is incorrect. The reactivity effect of voiding a single channel is so small that there would be little effect on the power level in the rest of the core. There is insufficient fuel in a single fuel sub-assembly to produce criticality even if all the fuel pins in it were melted and compacted.

There are other incorrect technical statements: for instance, sodium is more easily contained than high pressure water or steam and is not as corrosive to reactor constructional materials; and the delayed neutron fraction, on which the normal control behaviour of thermal reactors as well as fast reactors depends, is nearly twice as great as stated (and is always greater in a fast than in a thermal reactor with the same fissile material).

These errors are important in that they convey the impression that fast reactors are difficult to control, unsafe and not well understood. On the contrary the fast reactor in normal operation has no tendency to become unstable and can be left for hours without any adjustment of the controls. Temperature and power control is simple, straightforward and easy to engineer to very safe standards. Their safety has been the subject of intense study for very many years and it is well understood. Clearly there will continue to be work related to the detailed choice of safety features until licensing rules have been formulated and as with other reactor types the preferred selection of intrinsic and engineered safety features will be clarified during negotiation of the licence for the first commercial reactor. What is important is that fast reactor designers are confident that they can meet safety standards at least as stringent as those applied to other reactor types. Furthermore any design features incorporated for safety reasons are not likely to increase the expected cost by more than a few per cent.

The economic statements are equally ill-founded. The increases in the capital cost of SNR 300 were due largely to late changes in the design requested during the complex licensing process which was being developed using SNR 300 as a guinea pig: even if the changes incorporated in this way were thought to be necessary for later reactors the cost of including them *ab initio* would be small compared with the costs of SNR 300.

Whilst the capital cost for a fast reactor appears to be inherently higher than for a thermal reactor — estimates range from as low as 1.1 times to about 1.5 times or perhaps approaching 2 times for the first-off commercial sized demonstration plants — there is little doubt that the lower fuel cycle costs will more than compensate as uranium prices increase, due to pressures in the next few decades on the limited supplies of uranium ore. The breakeven point could well be before the end of the century, which is the timescale for planning exploitation of the fast reactor system. Remember the fast reactor will produce about 60 times more energy than a thermal reactor for the same quantity of uranium. Commercial designs are of course being developed to meet safety requirements which are included in cost estimates, and the implications by Dr Dombey that designers have to suddenly take them into account after the rest of the design is complete shows a lack of understanding of the attitude of design engineers to safety.

Despite his failure to reach it by logical argument, Dr Dombey's general conclusion that the UK should continue to collaborate as closely as possible with other countries in developing the fast reactor is commendable and has been stated on many occasions to be current policy. Articles which undervalue the extent of the UK's possible contribution to such a collaboration are not likely to be of assistance in such collaborations.

It is disturbing that the article contains so many inaccuracies, which, compounded with the error about the Doppler Coefficient, gave such an unbalanced view of fast reactor safety at a time when an impartial academic view would have been particularly valuable.

Yours faithfully,

R.D. SMITH

Fast Reactor Development Directorate, UKAEA, Risley, UK.

Domestic animals are not inferior

SIR, — Mellanby (*Nature* 275, 82; 1978) attacks opponents of 'factory farming' for their errors in animal behaviour, as well as for their own behaviour at his talk. First, while not wishing to justify the human behaviour he describes, it should be borne in mind that many in the environment movements have suffered far worse behaviour at the hands of establishment figures — treatment not all that different from what is meted out to dissidents in the socialist world, well detailed in the columns of *Nature*.

Second, there are scientific, practical and humane reasons for objecting to some aspects of intensive husbandry. Those who are critical of such practices do not get their views published — in contrast to the regular columns in *Nature* by such strong advocates of 'factory farming' as T. H. Jukes and Mellanby.

Third, since Mellanby frequently (and rightly) complains of occasional errors and oversimplifications made by environmentalists may we have the opportunity of correcting some of his.

The statement by Mellanby, "Domestic animals, cattle and sheep, and pets such as dogs and cats only exist because man breeds them . . . but if man disappeared from the world, so would most domestic animals" seems at variance with his later comment about the feral cat " . . . endangering the native fauna . . .". Both statements are misleading.

The feral cat in Australia largely has the rabbit as its major prey (Coman, B. J. and Brunner, H., *J. Wildlife Management* 36, 848; 1972). Habitat destruction by man is the major threat to Australian native fauna (Marshall, A.J., *The Great Extermination;* Hienemann, Melbourne 1966).

In reviewing literature on the success and failure of introduced animals, we find that there is no statistically significant difference between domestic and wild species in regard to successful establishment in the absence of human care. Indeed, some major animal domesticates, notably goats, pigs, cats, dogs, and rabbits become successful in a variety of habitats and resist sustained human efforts at control or eradication.

The idea that domestic animals are inferior and degenerate — and thus less deserving of humane attention — is widespread. It is dangerous to confuse science and value judgements, however, and it is worth bearing in mind that Konrad Lorenz justified his support for the German 'eugenics programme' on the basis that man was becoming domesticated (Haldane, J.B.S. in *Culture and the Evolution of Man;* Galaxy Books, Oxford University Press, New York, 1962 and Nisbett, A., *Konrad Lorenz;* J.M. Dent, London 1976).

Yours faithfully,

C. M. ANN BAKER
CLYDE MANWELL

Selby, Yorkshire, UK.

Brazilian official was illegally dismissed

SIR — I wish to congratulate you for the story on the Brazilian exiled scientists (26 July page 268). I would only like to rectify a misquotation concerning my husband, an ex-official of the Brazilian Parliament who was illegally dismissed for political reasons when the military took over in 1964, and was not "an ex-deputy whose mandate was annulled", as published.

Yours faithfully,

ALICE RIVERA

CNRS, Gyf sur Yvette, France.

What use is scientific awareness?

SIR — your concern for the lack of scientific awareness of the British public is comendable (9 August, page 435), but may I ask to what end? As an unemployed maths teacher who has never held any scientific or technical position, I am probably as close to the "general public" as your regular readership extends. I try to keep myself informed on all branches of science, and my general scientific awareness is far greater than could ever be reasonably expected as a norm. I think it is worth noting that as far as I know this awareness has never been of the slightest benefit to anyone.

Yours faithfully,

R.J. SALISBURY

Anglesey, Wales, UK.

news and views

Primitive model for cell cycle control

from Clive Lloyd

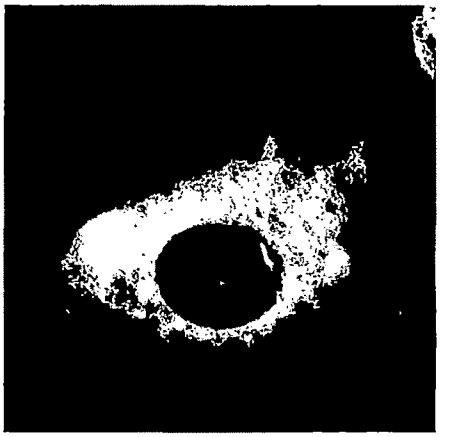
IN non-motile cells of multicellular organisms atypical, internal cilia form from the centriole. The role of these so-called 'primary' cilia in such cells is unclear but recent work shows that nevertheless they may provide a useful indicator of the state of the cell cycle.

The centrioles appear in different guises at different stages in the eukaryotic cell cycle. During the division of most animal cells for instance, they are associated with each pole of the mitotic apparatus. They may, however, merely be passengers during this process, since amorphous pericentriolar material seems to be the major nucleation site for those microtubules formed at the poles of the mitotic apparatus (Gould & Borisey *J. Cell Biol.* **73**, 601; 1977). By contrast, the role of centrioles in non-dividing cells is less ambiguous for they act there as pre-formed templates (basal bodies) for the outgrowth of the microtubules which form cilia and flagella. Cilia and flagella are generally thought of as organelles of motility; propelling unicellular organisms through the environment or moving the environment over the surfaces of tissues (see *Cell Motility* (eds Stebbings & Hyams) Longmans, 1979). However, atypical, internal cilia are also seen associated with the centrioles in non-motile cells of multicellular organisms.

These internal cilia differ from the motile variety in several ways. Motile cilia/flagella generally consist of a bundle of nine outer doublet microtubules surrounding two singlet microtubules, but the internal cilia have a 9+0 instead of a 9+2 arrangement (Barnes *J. ultrastruct. Res.* **5**, 453; 1961). In addition, the 9+2 cilia possess a basal organisation involving only one centriole or basal body, whereas cilia possessing twinned basal structures generally display a 9+0 pattern (although the converse may not necessarily be true). The 9+0 cilia have been referred to as 'primary' since Sorokin (*J. Cell Sci.* **3**, 207; 1968) first noted that they were produced in bronchial epithelial cells before the 9+2 type which make up the ciliated border.

As for their function, primary cilia have been observed at the site of regrowth of cytoplasmic microtubules in 3T3 fibroblasts (Osborn & Weber *Proc. natn. Acad. Sci. U.S.A.* **73**, 867; 1976) (see figure) and are aligned with the current track of migration in such cells (Albrecht-Buehler *Cell* **12**, 333; 1977). This could imply some function for the primary cilium in influencing cell polarity, but Albrecht-Buehler is careful to point out the difficulty of sorting out cause from effect in such observations. Perhaps primary cilia serve no useful purpose. Found in unicellular and multicellular organisms alike, the centriole may be part of a primitive motile system and in differentiated, non-motile tissue cells, the 9+0 cilium on the centriole may be vestigial — representing a throw-back to a unicellular, free-swimming mode of existence.

However, even if it is now redundant, the cyclic formation and disappearance of the primary cilium still provides clues to cell cycle control. As long ago as 1898, it was suggested that the transformation of centrioles into ciliated or flagellum-bearing basal bodies could be one of the control mechanisms for the regulation of mitosis and differentiation (Henneguy *Arch. Anat. Microscop. Morphol. Exp.* **1**, 481; Von Lenhossek *Verh. Anat. Ges. Kiel.* **12**, 106). This theme was revived by Rash *et al.* (*J. ultrastruct. Res.* **29**, 470; 1966) who, studying cardiac differentiation, reasoned that "the blockage of mitosis usually associated with the initiation of cytodifferentiation can be correlated with the formation of cilia and may be mediated by the transformation of the mitotic centrioles into ciliary basal bodies." Several reports agree with this: primary cilia do not occur in mitotic BHK fibroblasts, but reappear soon after division (Archer & Wheatley *J. Anat.* **109**, 277; 1971); they are more frequently observed in differentiated, non-dividing brain tissue than in dividing tumours or juvenile tissue (Dingemans *J. Cell Biol.* **43**, 361; 1969); primary cilia are observed in rat liver cell cultures only after their growth has been arrested in the post-mitotic phase of G_1, and not before (Mori *et al. Expl. Cell Res.* **120**, 435; 1979).



Immunofluorescence micrograph of 3T3 cell stained with antibodies against tubulin. The cell has been treated with colchicine to destroy the microtubule network and the primary cilium is clearly visible. Magnification × 900. (From Osborn & Weber *op. cit.*)

Now, in a recent paper (*Cell* **17**, 527; 1979) Tucker, Pardee and Fujiwara map out the timetable of centriole ciliation and deciliation in 3T3 fibroblasts and suggest that a correlation exists between the centriole cycle and the cycle of DNA replication and chromatid separation which is a more familiar feature of the cell cycle. Using indirect immunofluorescence, Tucker *et al.* confirm that centriole ciliation occurs when cells are arrested by conditions of low serum or high cell density in the post-mitotic phase of G_1. When stimulated to divide by the addition of fresh serum, this unduplicated centriole deciliates within 1–2 hours, reciliates by 6–8 hours and undergoes a further deciliation between 12–24 hours which roughly coincides with the initiation of DNA synthesis. The microtubules of the mitotic spindle form part of an apparatus for dividing genetic material between sister cells and so it may not be surprising that microtubule and nucleic acid cycle are somehow coordinated. As these investigators point out, microtubule-

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organising centres (for example the centriole and, probably, its associated material) seem to be diverted from alternative activities in preparation for mitosis at about the time that DNA replication is initiated. This is reminiscent of the sequence of events which occurs in the division cycle of the yeast *Saccharomyces cerevisiae* (Hartwell *Bact. Rev.* **38**, 164; 1974) where mutation of the gene controlling the replication of the spindle pole body (a microtubule-organising centre analogous to the centriolar complex) blocks the initiation of DNA synthesis and hence of other steps leading to mitosis. The timing of centriole duplication in mammalian cells is not yet determined, but may occur in late G_1 or early S (see also Rattner & Phillips *J. Cell Biol.* **57**, 359; 1973).

Although a useful marker for some cells, primary cilia are absent from others and it should not be forgotten that higher plant cells may not possess centrioles (see *News and Views* **277**, 515; 1979). Higher plants do, however, possess amorphous microtubule-organising centres analogous to the amorphous type which surrounds the centriole in animal cells and plays a more direct part in forming the mitotic apparatus. Telzer and Rosenbaum (*J. Cell Biol.* **81**, 484; 1979) have isolated such pericentriolar material from HeLa cells and demonstrate that when isolated from mitotic cells it supports reassembly of microtubule protein into microtubules, but is ineffective when obtained from cells in interphase. Considered together, recent evidence points to a close association between the activity of microtubule-organising centres (both structured and amorphous) and the mitotic cycle. In view of this it may be useful to consider even the cells of multicellular organisms as 'differentiated flagellates' which undergo a metamorphosis to the reproductive stage driven by signals affecting microtubule organising centres. □

Immunotherapeutic T cells?

from Peter Beverley

TWENTY years ago Thomas proposed the theory of immune surveillance. Since then it has been a hope of cancer immunologists that the immune system might be persuaded to do the work of physicians and surgeons and destroy the tumours of cancer patients.

Initially, because of their role in allograft rejection, thymus-derived lymphocytes (T cells) were thought to be the main agent of surveillance. Later data have thrown up other candidates for the role of 'surveyor' (Baldwin *Nature* **270**, 557; 1977) and the very idea of surveillance has been challenged (Moller & Moller *J. Natn. Cancer Inst.* **55**, 755; 1975). Nevertheless T cells remain an attractive possibility for immunotherapy because of their extreme specificity of response and because high levels of activity can be generated by stimulation *in vitro*.

The practicality of immunotherapy rests on a number of assumptions. First that tumours carry surface antigens distinct from those of normal cells; second that lymphoid cells of the tumour-bearing host can be immunised against the tumour antigens; and third that immune cells (or antibodies) when administered to the tumour-bearing animal will be effective in reaching and destroying the tumour.

Experimental tumours often provoke an immune response, but in many cases it has been shown that immunity is directed against antigens coded by RNA tumour viruses carried in the tumour cells, while in general the evidence for immunogenic antigens on spontaneous tumours of animals is far weaker. In man apparently, tumour-associated antigens have been defined using heteroantisera but it is not clear whether these antigens provoke a T cell response by the tumour bearer. Similarly, although there have been many reports of cell-mediated immune responses to tumours in man, their exact nature and specificity have been difficult to define because the responses are weak and inconstant. Thus the two findings that T cells cytotoxic for human leukaemias could be generated (Sondel *et al. J. Immun.* **117**, 2197; 1976) and that specific cytotoxic cells could be propagated indefinitely *in vitro* (Gillis & Smith *Nature* **268**, 154; 1977) raised the hope that some of these problems might be resolved by the production of large numbers of highly active cytotoxic T cells directed at human tumour antigens.

This has now been achieved by Joyce Zarling and Fritz Bach, (this issue of *Nature*, page 685) and the power of the new methodology is certainly demonstrated. Previously, not all leukaemia patients could be shown to respond to their own leukaemia cells (Lee & Oliver *J. exp. Med.* **147**, 1334; 1978) but a combination of stimulation *in vitro* followed by expansion of the cytotoxic T cell population *in vitro* (up to 10 million-fold) reveals that virtually all patients can make an immune response to their own leukaemia cells. Furthermore, since stimulation with a pool of allogeneic lymphocytes was effective in generating kill against autologous leukaemia cells, it may be possible to generate cytotoxicity against a tumour even if no tumour material is available (important for immunotherapy of solid tumours where stimulator cells may be hard to obtain). That pooled allogeneic cells are effective reinforces the immunologists' dogma that transplantation antigens (HLA) in some way determine or are related to the T cell repertoire.

On the face of it the finding that T cells can be sensitised to autologous leukaemia cells and that other cells are not killed would seem to be strong evidence for tumour-specific antigens on leukaemia cells. However, this is a conclusion which should be regarded with considerable suspicion. Recent studies of leukaemia cells from many laboratories suggest that at least the majority of leukaemias have surface membrane phenotypes characteristic of normal, though sometimes rare, cells. A cautionary tale is provided by the acute lymphoblastic leukaemia (ALL) antigen which, although first discovered on leukaemia cells and thought to be ALL specific is in fact found on bone marrow stem cells in fetal and regenerating bone marrow (Greaves & Janossy *Biochim. biophys. Acta* **516**, 193; 1978). As yet no controls carried out in studies of autologous anti-leukaemia cytotoxic T cells have proved that the target antigen(s) for the killer cells are not just the same sort of normal differentiation antigen. If immunotherapy involved elimination of a stem cell population along with the leukaemic cells it might not be so attractive a proposition!

Delivery of immunotherapy is an additional problem. In animal experiments immunised cells have been most effective when administered mixed with tumour cells in local tumour growth assays. The relative inefficiency of systemically administered cells may be due either to incorrect migration *in vivo* of cultured lymphocytes or because more than one subset of lymphocytes is required for optimal elimination of tumour cells.

Even if there remain doubts about the use of T cells amplified *in vitro* for therapy, there can be no doubt that cultured T cells will be extremely useful tools. Not the least important of the questions which will surely soon be answered, doubtless using cloned sublines of killers, is what is the nature of human leukaemia antigens? If they turn out to be exclusively tumour-associated, the way to T cell immunotherapy may be open. □

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Conserving genetic resources of animal species

The idea of the botanical gene bank, where seeds of rare and endangered plants are kept dormant in the deep freeze, to be revived when required, is now widely accepted. Here Dr B.N. Veprintsev of the Institute of Biological Physics and Dr Natalia Rott of the Institute of Developmental Biology of the USSR Academy of Sciences, argue that similar gene banks should be started immediately for rare and endangered animal species and speculate on how it might be possible in the future to reconstruct extinct animals from their germ cells stored in such a bank.

WE are witnesses today to radical changes in the environment — the disappearance of virgin forest and prairie and the exploitation of hitherto undisturbed oceans, lakes and rivers. The disappearance of many animals and plants is an inevitable consequence of the loss of their natural habitats. Their loss would not only produce an aesthetically impoverished landscape but, more practically, eliminate important genetic resources and disrupt the stability of fragile ecosystems which have evolved over the centuries. The rate of change is accelerating rapidly. If we do nothing to save rare and endangered species now it will soon be too late.

Current efforts to preserve rare animals and plants in natural reservations, zoos and botanic gardens may not provide sufficient security for their future. We believe that we must find ways of conserving the basic genetic material of these species — the gonads, germ cells and somatic cells — so that they can be revived in the future when conditions for their survival may be more favourable.

We should like to propose various ways in which animals might eventually be reconstructed from the conserved genomes (Fig. 1). To put this hypothetical scheme into practice we need:

- to preserve the genetic information of endangered species in as complete a form as possible.
- to find ways of realising this information by obtaining zygotes and rearing animals from them.
- to find ways of changing the sex of some of the reconstructed animals if they are all of the same sex, as would inevitably follow from some of the methods proposed.

Sources of genetic information

The most realistic approach at present is to store frozen gonads, germ or somatic cells for subsequent reconstruction of zygotes. One immediate problem of course is how to obtain such material without further endangering the population of rare animals. In our opinion the most suitable cells for conservation would be the germ cells (oocytes and spermatozoa) and fertilised ova — the zygote itself before reduction cleavage.

For some species somatic cells might be an attractive genetic source as they are easily collected and some at least could be expected to contain all the necessary information for development.

Storage

Deep freezing seems the best method of conservation at present. Already deep frozen sperm are widely used in animal breeding and more recently, methods of storing deep-frozen oocytes and embryos of cattle[1,2] and some laboratory animals[3] have been developed. Oocytes can be stored in gonadal tissue culture and in deep frozen gonads[2-5] and subsequently can be matured either by implantation of the gonad into a female recipient[5] or by hormone treatment *in vitro*[4,6].

Reconstruction of a viable zygote

The means of obtaining a viable zygote from the stored material must of course be the most speculative part of our scheme. In normal development, the interaction of the zygote nucleus with its cytoplasm is necessary for the proper expression of the genetic information. In differentiated somatic cells, however, although the nucleus contains a complete set of genetic instructions, most genes are not expressed[7]. Therefore, in order to regenerate an animal from its conserved genome, it is necessary to provide an environment in which the genetic information is expressed as in normal development.

If we have only been able to conserve spermatozoa, one approach would be to fertilise the ova of a closely related species. The success of such interspecific hybridisation varies in different animals. In birds and fishes, hybrids between different genera and even families are possible. In mammals and amphibia interspecific hybridisation can take place. In cases where spermatozoa cannot fertilise the ova of another species this difficulty might be overcome by injecting the sperm, treated with proteolytic enzymes, directly into the ovum cytoplasm[8]. In some cases hybrid incompatibility might be overcome by obtaining tetraploid and amphiploid hybrids in which two chromosome sets belong to one species and two to the other. Such hybrids have been obtained in frogs and silkworms[9]. In all cases where the genetic material of two species is mingled, the required species would have to be re-established by repeated backcrossing of hybrid females with male sperm of the original species.

Another possibility might be androgenesis. This would avoid the problem of backcrossing to eliminate unwanted genetic material. Just as complete plants can be regenerated from male pollen alone, it might be possible to fertilise an ovum of another species, in which the nucleus had been inactivated, with the sperm of the desired animal. In this case one would obtain an, admittedly haploid, zygote, in which the genes were those of the required species but the supporting cytoplasm was of a different species. To get a diploid zygote two male

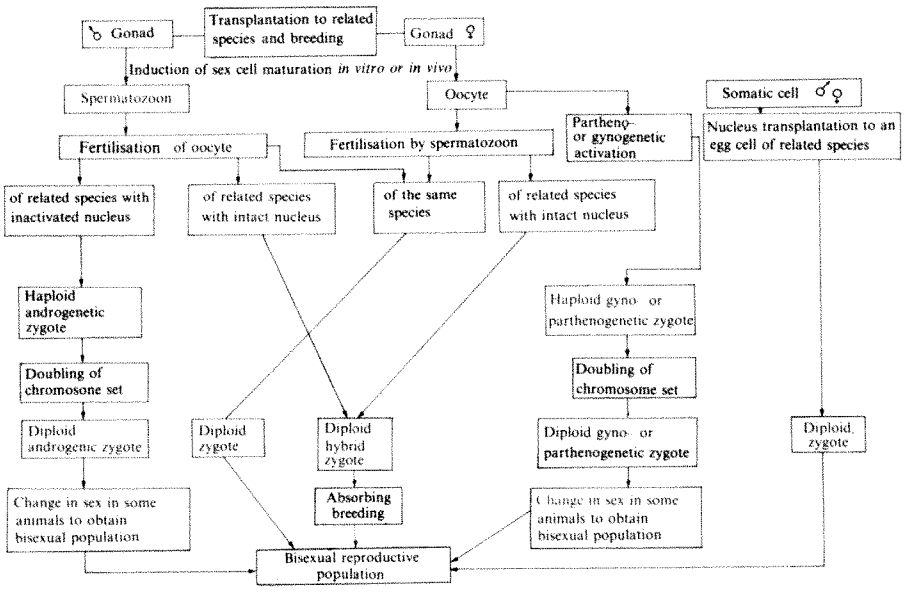


Fig. 1

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nuclei would have to fuse (dispermic androgenesis), and unfortunately so far this is known only in silk-worms[10] and axolotls[11]. Alternatively zygotes might be made diploid by inhibiting one of the primary cleavage divisions[12], but the resultant homozygous embryo might reveal lethal or undesirable recessive genes[13,14].

If only oocytes were available these might be induced to undergo parthenogenesis by chemical activation or by pseudo-fertilisation with sperm of a different species which cannot subsequently take part in development. A diploid embryo could again be obtained by inhibiting either one of the maturation divisions or one of the first cleavage divisions[14]; the problem of homozygosity remains.

If only somatic cells were available, perhaps the nuclei could be transplanted into the fertilised zygote of a closely related species, in which the nucleus had been inactivated. Experiments with amphibia of the same species have shown that in such cases normally inactive genes are reactivated and normal development proceeds. Microsurgical nuclear transplantation is succesful in amphibia, fish and insects, and some limited success has been achieved with mammalian material[15]. Nuclei for one species can also be introduced into cells of another by cell fusion, brought about by Sendai virus or polyethylene glycol[2].

But nuclear transplation from one species to another has proved very difficult. In the many experiments which have been done, hybrids have been obtained in only four cases, and in two of them the oocyte nucleus remained in the recipient cell[15]. In most cases the embryos did not develop. Producing chimaeric embryos may help to overcome this difficulty. The cells of lethal hybrids were shown to have increased vitality when fused with the embryos of a normal parental species[17]. But although chimaeric mice can be produced routinely by aggregating two very young embryos or by injecting cells from one into the other, attempts to obtain interspecific chimaeras present more problems.

Zygote development

How might reconstructed zygotes be reared to produce living animals? In insects, fish and amphibia this is not particularly difficult but for reptiles, birds and mammals the problems are enormous. No-one has yet achieved the complete development of such an embryo *in vitro*. In every case in which manipulated embryos have been successfully reared, they have been reimplanted into the mother or into a fostermother of the same species.

The most reasonable course in our scheme would be to transplant embryos 'conceived' *in vitro* into foster mothers of a closely-related species. However, attempts to do this — mouse into rat for example — have so far failed. The reasons for failure are unknown. There may be immunological rejection of the embryo; in this case it might be possible to induce tolerance. This would need suitable antigens from the 'preserved' species so we need to conserve not only gonads and germ cells but other tissue as well. For birds and reptiles interspecific ovarian transplantation seems very attractive.

Sex determination

Some of the methods outlined above (parthenogenesis and gynogenesis for example) would produce animals all of the same sex, so it would be necessary to look for some way of converting some of them into the other sex. Mechanisms of sex determination differ from species to species so any treatment would have to be individually tailored. This may be less daunting than it seems, for there are species, for example among the fishes and amphibia[16], that change sex spontaneously. And in other species hormones and other factors can influence sexual development.

Although most of the methods we have discussed are not yet practicable, research is proceeding at such a rate that they are beginning to look feasible in the not-too-distant future. Semen, blood and skin tissue from rare animals in zoos could be collected and stored without any danger to the animal itself. So we urge that collection and storage of suitable material should start now. But we need to be very cautious and not think that this approach can save the animal kingdom. It must be only one necessary step among many measures needed to meet the present emergency. □

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The impact of climatic change

from Martin Parry

OUR knowledge of fluctuations of climate has exhibited a precocious flowering in the past 5 years and the time has come to consolidate the progress already made along many different paths and to point a way forward. This was the goal that 250 climatologists and historians from 33 countries set themselves recently at the conference on Climate and History at the Climatic Research Unit, University of East Anglia*.

The issues that emerged from this meeting touched on many facets of contemporary economics and society, in addition to the more specific problem of the impact of climate in history. The latter will be reported in detail elsewhere, but a number of points that were raised in papers and highlighted in discussions had far-reaching implications and deserve immediate debate. The points can broadly be divided into those that derive from a greatly increased understanding of the scale, direction and timing of climatic fluctuations, and those that are related to the economic and social impact of such fluctuations.

The reconstruction of past climates has benefited from the recent advances made both in the quarrying of new data sources and the improved analysis of existing ones. At the University of Bern, Christian Pfister has recently developed a new set of procedures for standardising and storing a great variety of data, from weather descriptions in farming diaries to dates of lake freezing and the size of vine harvests. The system could provide the basis for an integrated and international pool of data — a pool to which information from new sources could continually be added. Several new data sources were mentioned at the conference, including tax records in Norway and meteorological records from China. At the same time, the improved 'calibration' of tree-ring data by Harold Fritts and his colleagues at the University of Tucson is giving us a vital bridge between the beginning of the instrumental records (about AD 1600 in Europe and AD 1800 in the New World) and the more distant record provided by studies of pollen, ice and sediments. In short, there is the exciting prospect of the construction of a climatic chronology of temperature and rainfall for much of the Northern Hemisphere over the last millennium that will be sufficiently accurate for us to evaluate the economic impact of the

*Held on 8-14 July, 1979 and organised by the Climatic Research Unit.

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fluctuations. As well as establishing our present climatic pattern quite firmly in the context of past changes, we can now say something about the range of alternative patterns of climate and weather we should be prepared for in the future. In particular, several papers reflected a significantly improved understanding of the nature of short-term climatic fluctuations, and the factors that distinguish them from long-term changes of the climatic pattern. The contributions from China were particularly welcome here because of the remarkably long and detailed run of records available.

The distinction between minor fluctuations and major changes may be arbitrary in terms of the mechanisms of climatic change, but it is important in comprehending their contrasting economic consequences. The ability of a rural economy, whether peasant or commercial, to absorb the shock of year-to-year changes in weather — or, especially, runs of adverse weather — is a critical factor in determining its economic success and its social harmony. Studies of climatic change and agricultural response strongly indicate the need for farming systems which will reduce the risks from uncertain weather by a spread of farming options. The irony is that improvements in technology and organisation enable us to respond more rapidly to drought in, for example, the Sahel or the US Great Plains; but, at the same time, they encourage farmers to adopt specialised and consequently risky farming systems that are more prone to suffer from climate changes. We can thus distinguish between the need for farming communities, especially in marginal areas, to be prepared and able to adapt to long-term changes of climate; and, on the other hand, the necessity of adopting farming systems which can absorb short-term climatic shocks without producing economic or social hardships.

These conclusions are drawn from several studies of marginal areas, where the nature of the climatic impact can more readily be interpreted. One of these is the cold marginal zone around the North Atlantic, whose reponse to climatic fluctuations has now been quite thoroughly researched. Analyses of options lost and gained by societies in Greenland, Iceland, Scotland and Norway were presented at the conference. The climatic constraint on agriculture in these areas is largely temperature; and, because this exhibits less variability than rainfall, any trend in its behaviour can be more readily detected, and suitable measures planned. The dry marginal zones in Africa, the Middle East and the US present a much greater problem, but there are encouraging signs of an increased understanding, particularly through the work of geographers based at Clark University, Massachusetts.

Moreover, we are now beginning to comprehend the enormous social and economic cost of climatic fluctuations. There are now quite precise measures of the relationship between Indian food grain production and monsoon reliability; of changes in the US regional and national house heating bill resulting from the postwar decline in winter temperatures; and of correlations between wheat production and weather patterns on the Soviet steppes. The return on predicting climatic fluctuations is, of course enormous, but it is unfortunately still not in prospect. In the meantime, our increased knowledge of the nature of the fluctuations and their impact should enable us to plan an improved ability to absorb the shocks and to adapt to the changes. In this sense, the Climate and History Conference was a milestone, for it marked the move from the pioneer phase of initial chronology construction to a secondary phase of impact evaluation; and for the pioneers who are witnessing this coming of age — Hermann Flohn, Hubert Lamb and Gordon Manley — this must have been an extraordinarily satisfying experience. □

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Life and death before birth

by Mary Lindley

LIFE before birth is fraught with hazards, and for man at least they often seem to be too much for the fetus, leading to spontaneous abortion and stillbirth, known collectively as fetal wastage. Some of the factors controlling the fate of the fetus were aired recently at the annual symposium of the European Society of Human Genetics*.

The timing of some of the events of fetal wastage is beginning to become clear from studies of developing mice. The fate of XO mouse embryos is being investigated by P. S. Burgoyne (University of Edinburgh). Using female mice heterozygous for a large inversion of the X chromosome, he has obtained XX, XO and XY zygotes from XX mothers. He has found that when XO embryos from such mothers are cultured, they are often abnormal by the morula/blastocyst stage. Up to 11 days of gestation they are normal, but immediately after implantation some are resorbed. Burgoyne suggested that the preim-plantation abnormality leads to the postimplantation loss of XO embryos.

However, those embryos that survive the critical period seem to develop well, providing an intriguing parallel with the human situation. It has long been a puzzle that XO human fetuses have a very high rate of spontaneous abortion in the first three months of gestation, while those that are not lost then will progress quite well by the standards of embryos with chromosome abnormalities. Burgoyne has also compared mouse embryos from the XX mothers possessing the inversion with embryos from XO mothers and found a greater degree of retardation in the latter. He suggests that some maternal effect influences development in addition to the chromosome abnormalities produced in the offspring.

A. Gropp and colleagues (Institute for Pathology, Lübeck) have now looked at all nineteen trisomies of the mouse, using the breeding programme they designed for the purpose. As would be expected from what is known of the fate of abnormal human embryos, different trisomies survive for different times. Three critical periods of development can be recognised. Some of the embryos, including trisomies for chromosome 17, do not survive a first phase of organogenesis up to days 11 and 12 of gestation. Days 13 and 14 constitute the second critical period and trisomic fetuses surviving this far may have gross malformations, for example of the heart. These include trisomies 12, 14 and 16. The third critical period, days 15 to 18, seems to be associated with impaired placental function and oxygen supply. Some trisomic mice survive birth, but their subsequent life span is limited by general hypoplasia.

The human fetus is of course much less convenient for study, and although fetal wastage is known to be extensive, its full extent can only be estimated. According to A. Boué (Centre for the Study of Prenatal Biology, Paris) the accepted dogma is that 15% of all conceptions end in recognisable fetal wastage, while 31% survive to birth. The other 54% are presumed to be lost very early in gestation, but they cannot be detected.

E. Williamson and J. Miller (Southampton General Hospital) have come up with a value of about 40% early post-implantation loss in their prospective study of 195 women who were trying to conceive. By taking regular measurements of human choriogonadotropin in the urine, Williamson and Miller were able to recognise when pregnancies began and then follow their course, some ending in early loss and others proceeding further. It has still not been possible to recognise the time of fertilisation, and figures of the sort produced in this study require a generous measure of speculation. It seems that there is no substitute for specimens, but at the very early stages of gestation the problems of collection are too great at present.

Some of the earliest hazards, preceding even conception, arise during meiosis. This

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*The symposium, on Genetic Aspects of Fertility and Fetal Wastage, was held in Southampton on 19-21 July 1979 and organised by Dr. M. Seabright, Wessex Regional Cytogenetics Unit, Salisbury General Hospital. The abstracts will be published in the *Journal of Clinical Genetics*.

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is still to a large extent a mysterious process, but clues to its dangers are beginning to emerge. A. G. Searle (MRC Radiobiology Unit, Harwell) pointed out that in male mice X-autosome translocations and a substantial number of autosomal reciprocal translocations (those with break points near one end of the chromosome) are particularly associated with a breakdown of meiosis, often due to failure of pairing. This leads to the impairment of spermatogenesis and almost complete male sterility. It is not clear why this should happen, and the puzzle is increased because similar translocations have no such drastic consequences in female mice.

During a session on sex determination A. Jost (College de France, Paris) pointed out some inadequacies in current thinking about the role of H-Y surface antigen in this process. The antigen has been widely discussed as the agent responsible for the differentiation of testes in mammals, in which the male is the heterogametic sex (XY), and of ovaries in birds and other vertebrates in which the female is the heterogametic sex (ZW). Jost pointed out that such a scheme is not compatible with the results of his own and earlier work on the development of freemartins. He said that the course of development is similar either when a female calf fetus is masculinised before birth in the presence of a male twin, or when a female axolotl or *Xenopus* is induced to become a male by having a testis grafted onto its ovary. In each case there is first a phase of no change, then profound retardation of ovarian development and finally, masculinisation, leading to complete sex reversal in the two amphibians. According to current theory, the role of H-Y in mammals is to masculinise rather than to suppress female organs, and Jost pointed out that this does not account for the early and prolonged ovarian inhibition. Also according to current theory, the heterogametic sex influences the homogametic sex, and this cannot explain why the heterogametic female amphibians were masculinised by the homogametic males. Some other explanatory scheme is needed, Jost said.

U. Wolf (University of Freiberg), the leading exponent of this work in Europe, explained his view of the role of H-Y antigen in development. From studies in his laboratory with gonads of rat and chicken, he concludes that H-Y acts as a signal inducing the undifferentiated gonad of the early embryo to become a testis in the XX/XY system or an ovary in the ZZ/ZW system. Thus the gonad begins to develop in the direction of one sex or the other according to whether H-Y is present or absent. Sex determination therefore involves control of the expression of a putative *H-Y* gene or genes specifying the production of H-Y antigen. Wolf believes that, although the *H-Y* gene is usually active only in the heterogametic sex, it is carried in the genome of both sexes. He said that its presence in the homogametic sex is demonstrated when embryonic chicken testis is treated with oestrogen and converted to ovotestis which will then produce H-Y antigen. This may offer comfort to those who never believed that the *H-Y* gene was on the Y chromosome, and perhaps encourage rumours that it is really on the X chromosome.

More insights into sex determination and the role of sex chromosomes are promised by the Scandinavian wood lemming — billed as 'The incredible *Myopus*' and as usual, enthusiastically championed by K. Fredga (University of Lund). Wild populations of this small rodent (*Myopus schisticolor*) contain unexpectedly large proportions of females, some of which produce only daughters. Breeding data are bearing out the hypothesis that an X-linked mutation (designated X*) prevents the expression of masculinity in the presence of a Y chromosome, so that X*Y, as well as XX and X*X will be phenotypically female. Fredga said that the chromosomes X and X* can be distinguished by their banding patterns. Most X*Y females produce only daughters because a unique form of non-disjunction gives rise only to eggs carrying the X* chromosome. A consequence of the failure of this mechanism is the production of an unusually high proportion of offspring with abnormal sex chromosome constitutions. Fredga and his colleagues in Lübeck, A. Gropp and H. Winking, have bred X*YY fertile females, X*XY sterile males and an X*XY true hermaphrodite with an ovary on one side of its body and a testis on the other. They believe this indicates that the normal X chromosome is necessary for male development, with at least two genes involved, possibly one structural and the other regulatory. Fredga suggested that the development of X*XY individuals is influenced by the pattern of X chromosome inactivation. Thus in the hermaphrodite, inactivation of the normal X would have resulted in the development of an ovary, while inactivation of X* would have resulted in a testis. Further news of *Myopus* will be awaited eagerly. □

Mars volatiles

from C. T. Pillinger

IT is now almost 8 years since Mariner 9 went into orbit around Mars and found the planet shrouded by a gigantic dust storm to such an extent that only the very largest topographical features were visible. The storm abated and no doubt everyone remembers those tremendous first pictures of our nearest planetary neighbour. Later, in 1976, two Viking spacecraft, both soft landers and orbiters, continued the exploration of Mars. During the primary mission (June to November) little or no dust activity was observed. However, the scientific groups involved continued to monitor their instruments throughout the extended mission and data for an entire Martian year (approximately two Earth years) have been obtained. In that time 16 local and two global (although not as intense as the 1971 example) storms were observed. 'Mars Volatiles (and Meteorology and Dust)' (*J. Geophys. Res.* **84**, 136, 2793-3007; 1979) is a compilation of 16 papers by members of the Viking Volatile and Meteorology Working Group dealing with the results of the extended mission. Although various atmospheric problems are considered, dust and dust storms provide a common thread. As the working group ceased to have any official standing long ago, its members should be commended for the effort which must have gone into internal editing and cross-referencing so that readers are presented with a continuous story. All the papers are interesting and informative even to a casual reader looking for some general knowledge about Mars. The complete volume or high quality reprints of any of the papers may be obtained from the American Geophysical Union.

It is not easy to summarise the cumulative efforts of such a diverse study but some of the points of most general intest might be as follows. (All references following are to the volume cited above). It appears that dust storms occur predominantly when Mars is near perihelion and originate mainly in the southern hemisphere possibly near the ice cap (Briggs *et al.*) Local storms are up to 8 km high and have abrupt tops (Peterfreund & Kieffer). Surface pressure oscillations indicate that thermally driven global atmospheric tides are involved with the major storms (Leory & Zirek). Perhaps not surprisingly, measurements of wind, surface and atmospheric temperature and pressure reveal that the arrival of storms is accompanied by significant changes in these parameters (Ryan *et al.*; Martin & Kieffer). Infrared thermal mapping may be able to distinguish ground fogs and water ice clouds from dust and to provide information about the structure of the lower atmosphere (Hunt). The observation that water vapour is uniformly distributed in the atmosphere explains the lack of predicted early morning diffuse fogs. Thus, water is not frozen out at night and

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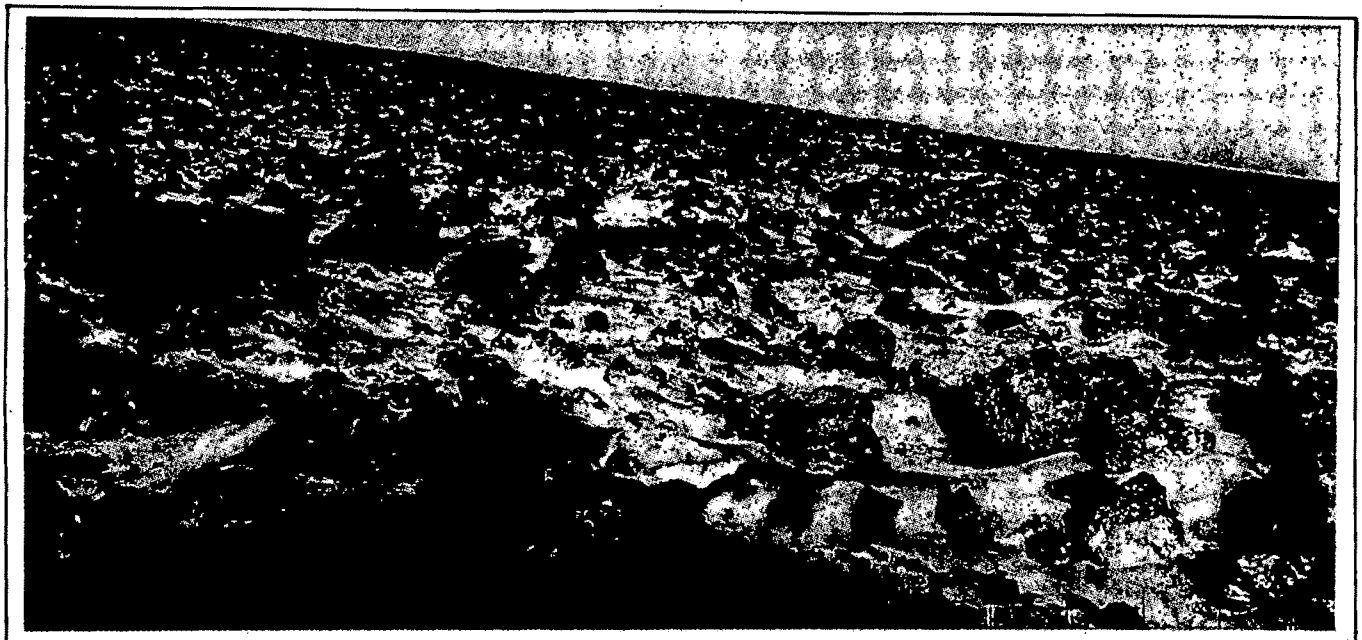


Photo: NASA

ICE on Mars again — This high-resolution photo of the surface of Mars was taken by Viking Lander 2 at its Utopia Planitia landing site on May 18, 1979, and relayed to Earth by Orbiter 1 on June 7. It shows a thin coating of water ice on the rocks and soil. The time the frost appeared corresponds almost exactly with the buildup of frost one Martian year (23 Earth months) ago. Then it remained on the surface for about 100 days. It is thought that dust particles in the atmosphere pick up bits of solid water. That combination is not heavy enough to settle to the ground. But carbon dioxide, which makes up 95% of the Martian atmosphere, freezes and adheres to the particles and they become heavy enough to sink. Warmed by the Sun, the surface evaporates the carbon dioxide and returns it to the atmosphere, leaving behind the water and dust. The ice seen in this picture, like that which formed one Martian year ago, is extremely thin, perhaps no more than one-thousandth of an inch thick.

exchanged between the surface and the atmosphere on a daily basis (Davies). The global and seasonal distribution of water vapour is consistent with a 10 cm to 1 m reservoir of H_2O in the regolith at latitudes above 40°. An asymmetrical transfer of water from south to north at a deposition rate of a few milligrams per square centimetre occurs possibly due to the propagation of storms in the south (Farmer and Doms). The spring–summer recession of the south pole observed by the Viking 2 orbiter was slower than previously reported by Mariner and Earth-based telescopes. Occurrence of a dust storm at the appropriate time might have been responsible (James *et al.*). Seasonal variations of up to 26% in atmospheric pressure are caused by evaporation of the ice caps — approximately 7.9×10^{12} tonnes or a 23 cm thick layer needs to be exchanged (Hess *et al.*). The temperature of the south polar cap in winter is significantly below the expected 148 K. One explanation is that there is a reduction of CO_2 in the atmosphere and a lowering of mean molecular weight near the surface (Hess). Wind speed and direction, pressure, temperature and optical depth measurements indicate the existence of low and high pressure 'weather' systems in the atmosphere (Tillman *et al.*). Optical depth studies also provide information about the shape and imaginary refractive index of the dust; the cross sectional weighted mean radius is estimated to be around 2.5 μm. At the moment, water ice and CO_2 are being preferentially deposited in the north polar regions because of scavenging by the dust. Equatorial and mid latitude regions are probably being eroded at a rate around 7 m per 10^6 yr; it may take 10^5 yr to produce the individual strata observable at the poles (Pollack *et al.*). Thus, cyclic variations in direction over geological time have led to sedimentary deposits of millions of cubic kilometres under both poles as testimony to the effects of dust storms. Parallel undulations of period a few kilometres and height a few tens of metres observed near north polar surfaces may be the intermediate topographic features (Cutts *et al.*).

Perhaps the only unrelated, but nevertheless interesting paper (Carr), describes the formation of the chaotic terrain, thought to be Martian flood features, formed by the release of water from confined aquifers. □

New routes to polypeptide hormones

by Peter Newmark

THE production of polypeptide hormones has, until very recently, started either in the slaughter-house/mortuary or on the solid phase beads of the organic chemist. But at a recent meeting* it was clear that the knife and the spatula are yielding to the restriction enzyme, although not without a struggle.

A particularly elegant example of the bacterial route to the production of a human hormone was recounted by D. V. Goeddel (Genentech, San Francisco). Since the synthesis of the complete nucleotide sequence corresponding to the 191 amino acid human growth hormone (hGH) would have been a monumental task and since the use of DNA copied from natural messenger RNA and then converted to double-stranded form (ds cDNA) would have coded for the precursor to hGH which bacteria would not be able to process to the mature hormone, the following approach was taken. First ds cDNA for hGH was cloned and treated with restriction enzymes to give a DNA sequence corresponding to all but the first 23 amino acids of the hormone. Then the synthetic polynucleotide corresponding to amino acids 1-23 and preceded by an ATG initiation codon was cloned. Finally the two fragments were ligated and cloned 'behind' a pair of *lac* promoters and a ribosome binding site.

*The 12th Annual Miles Symposium on Polypeptide Hormones was held in Baltimore, 11-13 July, 1979.

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This led to a considerable yield of 186,000 copies of radioimmunoassayable hGH activity per *E. coli* cell (2.4 $\mu g\ ml^{-1}$). Furthermore, and as planned, the product was of about the correct molecular size, not being fused to a plasmid-determined protein as has been the case in most previous approaches to the bacterial production of hormones (see for example Martial *et al. Science* **205**, 602; 1979).

Given Genentech's efforts and their plans to market hGH in collaboration with Kabi, it was surprising to hear doubts expressed that more hGH is really needed. In the USA at least, said S. Raiti (National Pituitary Agency), although there were theoretical grounds for believing that several thousand untreated patients existed, in practice the medical demand for hGH was readily met by hGH isolated from cadaveric pituitaries. On the other hand he conceded that lashings of the hormone would allow proper trials of its mooted efficacy on burns, non-healing fractures, ulcers and haemopoiesis.

As yet, however, not even the biological activity of the presumed hGH has been tested. Indeed it remains to be shown that any bacterially-produced hormone has proper biological activity although Lydia Villa-Komaroff (University of Massachusetts, Worcester) reported that the substance released by trypsin treatment of fused β-lactamase–insulin produced by bacteria was somewhat active in a rat fat pad bioassay.

Another polypeptide (?hormone) that is attracting the attention of genetic manipulators is interferon. One only has to read *Omni* these days to discover that interferon is the great white hope of cancer therapy. The problem with both *Omni* and interferon is to separate science fiction from fact. For interferon that problem will only be overcome when it is available in sufficient quantities and in pure enough form for proper trials to be carried out. As yet the few, if encouraging, results that have emerged from clinical tests have relied on interferon that is less than 10% pure.

Greater purity is one of the main aims of both S. Pestka (Roche Institute of Molecular Biology, New Jersey) who is purifying interferon from chronic myeloid leukaemia serum and of C.B. Anfinsen (National Institutes of Health) who will soon be growing 1,000 litres of human lymphoblastoid cells as his source. Purity is important not only for clinical trials but for the determination of interferons's structure. So far it is thought to be a polypeptide of about 154 amino acids. The polypeptide is associated with carbohydrate components which, however, are not essential for activity. It is said (by others) that both at NIH and at Dupont the N-terminal amino acid sequence of interferon has been determined. If true that may give the genetic manipulators their entrée. For with a synthetic polynucleotide complementary to the N-terminal amino acid sequence it may be possible to fish out pure messenger RNA for interferon to make cDNA for subsequent cloning.

Does the bacterial approach leave anything for the synthetic peptide chemist to do? Certainly! In the first place chemical synthesis still makes much sense for the smaller peptides, 'smaller' being less than 20 to 75 amino acids, depending on whose opinion one takes. It should however be noted that even for such a small peptide as the 14-amino acid somatostatin the bacterial route of production may be commercially viable; otherwise why will it become Genentech's first marketed chemical (for research purposes only) by the end of this year? Second, chemical synthesis is inherently 'cleaner' and has been considerably improved by the advent of continuous flow techniques with the prospect of real time monitoring of the products (B. W. Erickson, Rockefeller University). Third, chemical synthesis is well suited to the production of labelled peptides and peptide analogues. That is particularly the case when the analogues contain either modified or D-amino acids.

The synthetic peptide chemists have had, for example, considerable success in producing potent agonists and antagonists of the 10-amino acid peptide, luteinising-hormone-releasing-hormone (LHRH). Chiefly by the substitution of a D-amino acid at the sixth of the ten positions and by N-terminal changes, it has been possible to produce 'super LHRHs' with more than a hundredfold the potency of the natural hormone but also with paradoxical antifertility effects (see for example Ying & Guillemin, *Nature* **280**, 593; 1979). Considerable progress has also been made in producing analogues that are LHRH antagonists. The most active antagonists described by D. H. Coy (Tulane University, New Orleans) have para-halogenated D-phenylalanine at position 2 and acylated D-phenylalanine at position 1. Those substitutions together with a D-tryptophan at position 6 result in an antagonist that blocks ovulation at 0.03 mg per rat. Three years ago the best antagonists were 30-fold less potent.

The peptide chemists have had less success so far in improving on those thymic hormones whose structure has been determined, thymopoietin and FTS ('facteur thymique serique'). Thymopoietin is 49 amino acids long but a constituent pentapeptide has the biological activities of the full molecule (G. Goldstein, Ortho Pharmaceutical Co.). FTS is a nonapeptide first isolated from pig serum, now from human serum, and shown also to be present in thymic epithelium (J. F. Bach, Hôpital Necker, Paris).

Another serum peptide with effects on T cells is likely to be a tetrapeptide, possibly with a methylated amino acid at the N-terminus (A. Astaldi, Netherlands Red Cross Blood Transfusion Service). Ironically the only clinical trials that were reported involved either the relatively crude 'thymosin fraction V' of A. W. Goldstein (George Washington University, Washington) or the unsequenced 'THF' polypeptide of N. Trainin (Weizmann Institute). Thymosin fraction V has recently proved effective in prolonging the survival of patients with oat cell carcinoma. And THF has induced a remarkable recovery in immunosuppressed children with lymphoproliferative diseases who had developed infections. The basis of these clinical successes, the cellular or molecular effects of thymic hormones and the relationship of the various contenders for that title remain mysterious.

The testing and production of polypeptide hormones is clearly a very competitive business. It will not be unprecedented if the clinical optimism evident at this stage has to be moderated when larger scale trials can be completed. Meanwhile amongst the genetic manipulators the perceived returns have led them to adopt Madison Avenue tactics. A sobering-up period will doubtless follow when the products have to be shown to be suited to clinical use. Meanwhile many claims are made to the effect that bacterial production is both cheaper and easier than present techniques. It will be interesting to see whether the eventual price as well as the availability of the hormones reflects current optimism. □

Peter Newmark is Deputy Editor of Nature.



A hundred years ago

But there is, perhaps, nothing which shows more strikingly the identity of the protoplasm in plants and animals, and the absence of any deep-pervading difference between the life of the animal and that of the plant, than the fact that plants may be placed, just like animals, under the influence of anaesthetics.

We owe to Claude Bernard a series of interesting and most instructive experiments on the action of ether and chloroform on plants. He exposed to the vapour of ether a healthy and vigorous sensitive plant, by confining it under a bell-glass into which he introduced a sponge filled with ether. At the end of half an hour the plant was in a state of anaesthesia, all its leaflets remained fully extended, but they showed no tendency to shrink when touched. It was then withdrawn from the influence of the ether, when it gradually recovered its irritability, and finally responded, as before, to the touch.

It is obvious that the irritability of the protoplasm was here arrested by the anaesthetic, so that the plant became unable to give a response to the action of an external stimulus.

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review article

Nuclear vibrations

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Three types of nuclear vibrations have been observed in which a large fraction of the nucleons participate. There is a satisfactory explanation for the vibrational frequencies but not for the damping rates.

NUCLEI, like more familiar mechanical systems, can undergo simple vibratory motions. One mode of motion, in which protons move together in one direction while the neutrons simultaneously move in the opposite direction, has been known for 30 years. In the past decade, several new vibrational modes have been discovered. Our theoretical understanding of the nuclear motion has also considerably advanced. Although the most refined descriptions of the motion require a quantum many-body theory, the main features of the vibrations can be understood using classical physics. The motion of an extended body is described by a field of vectors which give the velocity of the medium at each point. Figure 1 shows the velocity fields for three types of vibration: monopole, dipole and quadrupole. These three modes are exhibited by nuclei as so-called 'giant vibrations', in which a large fraction of the nucleons participate in the motion. In both the monopole and quadrupole modes, all the nucleons move coherently in a direction which depends on their position in the nucleus. In the dipole motion, as mentioned above, protons and neutrons move in opposite directions.

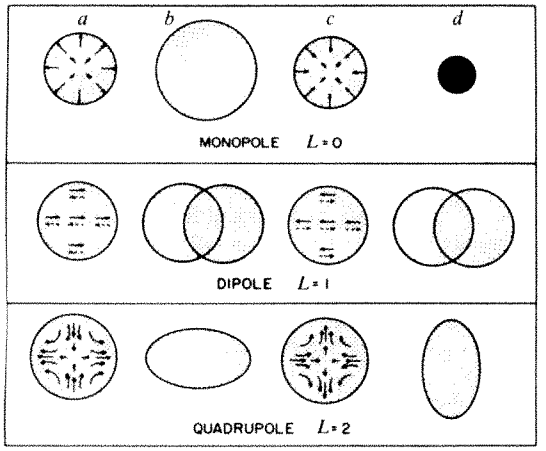


Fig. 1 The cycle of oscillation for simple vibrations. The velocity field at the moment when the nucleus passes through the equilibrium shape is shown in *a* and *c*. The shape at maximum distortion is shown in *b* and *d*. For the dipole, the neutrons and protons move in opposite directions. For other modes, the neutrons move with protons.

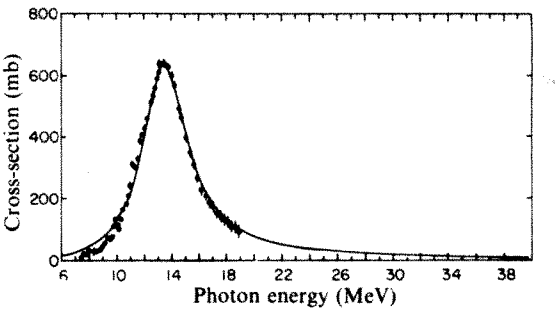


Fig. 2 The giant dipole resonance in ^{208}Pb, as observed by the photon reaction cross section (ref. 3, Fig. 32). The points are the experimental data, and the curve is the lorentzian function.

Dipole vibration

The first nuclear vibration to be observed, the giant dipole, was found in measurements of photon absorption. The reaction cross-section is enhanced by resonance when the photon frequency matches the vibrational frequency. The first indications of the giant dipole resonance were obtained before photon beams with a broad energy range were available. Bothe and Gentner in 1937 had a source of 14 MeV photons and they measured the radioactivity produced in a variety of targets[1]. The cross-sections varied considerably from target to target, indicating that there was a resonant absorption in some of the targets. By the mid-1940s, electron accelerators became available which produced bremsstrahlung photon beams of sufficient energy to observe the resonance. The improved data[2] showed that the absorption was concentrated in a resonance, the frequency of which varied systematically with the size of the nucleus.

One problem with these measurements is that the bremsstrahlung beam is broadly distributed in energy, so that the measurement for a particular energy required careful subtraction techniques. Currently, these experiments are done with photons that are produced by annihilation of positron, and are therefore monoenergetic. An example of the data now available[3] with this improved technique is shown in Fig. 2. The points are experimental data, and the curve is the lorentzian distribution,

$$\sigma(E)=\frac{\sigma_m}{1+[(E^2-E_m^2)^2/E^2\Gamma^2]}$$

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This formula has three parameters: a maximum strength σ_m, a centre frequency E_m, and a damping width Γ, which are adjusted to fit the data. For the heavier nuclei, the frequency depends on nuclear mass number A roughly as $\hbar\omega = 80/A^{1/3}$ MeV. The damping width is 4–5 MeV.

The spatial structure of the vibration, with protons moving against neutrons, is inferred indirectly from the data. Photons at the frequencies of interest have a long wavelength (~100 fm) on the scale of nuclear dimensions $R \sim 1.2\,A^{1/3} \sim 3$–$8$ fm. With a long wavelength, the force field is quite uniform and the photons cannot excite other kinds of motion besides the uniform displacement of protons against neutrons. Thus, as is the case with optical photons interacting with atoms, all but a negligible fraction of the cross-section is due to dipolar motion. It is useful to compare the magnitude of the cross-section with a theoretical limit known as a sum rule. The Thomas–Reiche–Kuhn sum rule for the absorption is given by[4]

$$\int_0^\infty \sigma(\omega)\,\mathrm{d}\omega = \frac{2\pi^2 e^2}{Mc}\,\frac{NZ}{A}$$

where M is the mass of a nucleon, and N, Z are the neutron and proton numbers. The dipole vibration will satisfy this sum rule only if all the protons move coherently with a uniform translation. In the example shown in Fig. 2, the experimental absorption strength from the lorentzian fit is 3,000 MeV mb, which is just the limit from the sum rule. Thus, the motion in the dipole vibration seems to be completely coherent. This conclusion is modified by the presence of charged mesons.[5]

Quadrupole and monopole vibrations

As mentioned above, wavelengths shorter than can be provided by photons are needed to excite the monopole and quadrupole vibrations. The quantum relationship between momentum and wavelength, $p = h/\lambda$, implies that modes with more complex spatial structure can be excited only if enough momentum is given to the nucleus at the same time. This condition can be met by the inelastic scattering of massive projectiles. The first published experiments exhibiting a new vibration were done in 1957 with inelastic proton scattering at 180 MeV (ref. 6). Figure 3 shows these data; a distinct bump is visible at 18 MeV excitation. However, the significance of this bump was not realised until in 1971, when Bertrand and coworkers[7] found the bump in their own data taken at lower energies and concluded that it was the quadrupole vibration of Fig. 1. Others also saw the vibration in electron scattering[8], and offered a quadrupole vibration as one of the possible explanations.

The main evidence that the bump might be a quadrupole vibration was the angular distribution of the scattering cross-section. Each multipolarity has a characteristic shape to the angular distribution, which can be calculated by the Schrödinger equation for the projectile wavefunction. If the wavelength of the projectile is short compared to the dimensions of the target,

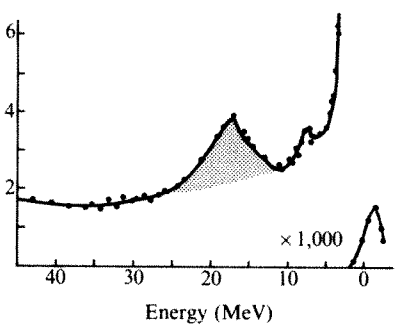


Fig. 3 Inelastic proton scattering spectrum for 180 MeV protons on a Ca target (ref. 5, Fig. 2). The stippled bump is the quadrupole vibration. The rise in cross-section towards zero excitation energy is the elastic scattering.

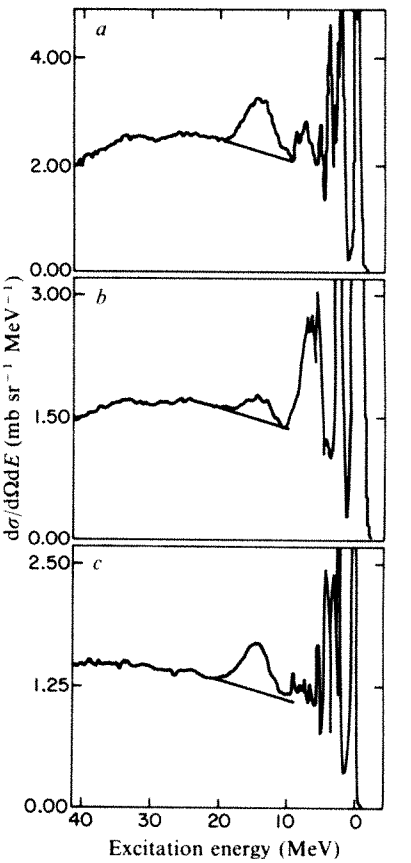


Fig. 4 Inelastic α-particle scattering on ^{90}Zr, taken at three different angles. *a*, $\theta_{lab} = 17.5°$; *b*, $\theta_{lab} = 20.5°$; *c*, $\theta_{lab} = 22.5°$. The giant quadrupole is prominent at 17.5° and 22.5°, but is much reduced at 20.5° (ref. 9).

the angular distribution peaks at an angle given roughly by $\theta = L/\lambda R$, where L is the multipolarity of the excitation and R is the nuclear radius. At larger angles, the cross-section is oscillatory, with interference from the projectile passing opposite sides of the nucleus. Some recent data with angular dependence is shown in Fig. 4[9].

The reaction is induced by α particles scattering on the nucleus ^{90}Zr. A prominent peak is seen at angles of 17.5° and 22.5°, but much reduced at 20.5°. This behaviour is only consistent with the predicted angular distributions for quadrupole or monopole vibrations. To decide between monopole and quadrupole, one must either look at smaller angles, or argue on the basis of the absolute strength of the peak.

As in photon absorption, sum rules can be used to analyse the absolute strength. However, the strength of the interaction for projectiles such as protons and α particles is not as well known *a priori* as it is for electromagnetic probes. To avoid this problem, the interaction between specific projectiles and target nucleons is adjusted by fitting the elastic scattering data. The interaction determined in this way describes inelastic transition quite well, and for many cases can be checked independently by its electromagnetic properties[10].

Defining V as the potential field of the scattering projectile on the target, the inelastic scattering sum rule reads

$$\sum_f (E_f - E_i)\langle i|V|f\rangle^2 = \int \mathrm{d}^3 r n_0(r)\,\frac{(\vec{\nabla}V)^2}{2m}$$

where n_0 is the density of nucleons in the ground state and the excited states are labelled by f. This sum rule is easily understood in classical terms. The left-hand side is the average energy given to the nucleus by the collision. On the right-hand side, $\vec{\nabla}V(r)$ is the impulse given to a target nucleon at position r. Thus $(\vec{\nabla}V(r))^2/2m$ is the average kinetic energy given to a nucleon at r, and the integral is the average total energy given to

the nucleus. Since the integral involves only the ground state density, it can be reliably computed and compared with the actual strength of a given vibrational excitation. The peak in Fig. 4 has about 75% of the strength for a quadrupole vibration; if it were monopole the sum rule would be exceeded.

By now the systematic behaviour of the quadrupole vibration has been thoroughly studied[11]. The mean frequency varies with mass number as $\omega \sim 63/A^{1/3}$ MeV/$\hbar$. The width varies from 8 MeV in light nuclei to 3 MeV in heavy nuclei, with dips at magic nuclei.

In the past two years, experiments have been done at smaller angles, where the monopole and quadrupole have different behaviour. Inelastic scattering at 0° should favour the monopole vibration. The physics behind this is shown in Fig. 5 which shows a projectile wave enveloping a nucleus. The attractive interaction between the projectile and the target pulls the nucleus out along the equatorial region. This outwards motion can be resolved into a major monopole component and a minor quadrupole component. All the wavelets from the projectile will interfere coherently at 0° scattering angle. So, like the Poisson spot seen in optical diffraction around spheres, the scattering will show a pronounced peak at 0°.

The quantum calculations of the scattering support this picture: Fig. 6 shows calculations comparing the angular distributions of monopole and quadrupole at the most forward angles[36]. These calculations treat the excitation of the vibration in the Born approximation, and use the solution of the Schrödinger equation in an optical potential for the projectile wavefunction. The sharp rise of the monpole near 0° is evident.

As the experiments have progressed to smaller angles with shorter wavelength projectiles, evidence has accumulated for a monopole vibration at a frequency slightly higher than the quadrupole. Figure 7 shows recent data (D. Youngblood, personal communication) for 0° inelastic α scattering. The centroid of the peak is at

$$\omega \sim 80/A^{1/3}\ (\mathrm{MeV}/\hbar)$$

Fortuitously, this is the same frequency as the dipole mode. The damping width of the vibration is about the same as for the quadrupole.

Theory

Paralleling the experimental developments, there have been considerable advances in the theory of the vibrations. The general theory, derived from many-body quantum mechanics in the 1950s, is known as RPA (random phase approximation). This theory was originally applied to nuclei[12,13] in models which used an oversimplified description of the wavefunction. These models were instructive in exhibiting qualitative trends for the vibrational frequencies but they had severe deficiencies. The sum rules were not satisfied. The interaction between particles was unrealistic, and in many cases had to be adjusted to fit observed vibrational properties. In the past decade it has become possible to do the theory properly. The availability of

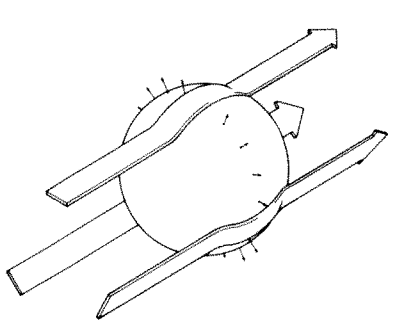


Fig. 5 A projectile wave, depicted with the broad arrows, envelops a nucleus and induces the transverse motion of the nucleus, shown with the small arrows. This motion resolves into a combination of monopole and quadrupole vibrations.

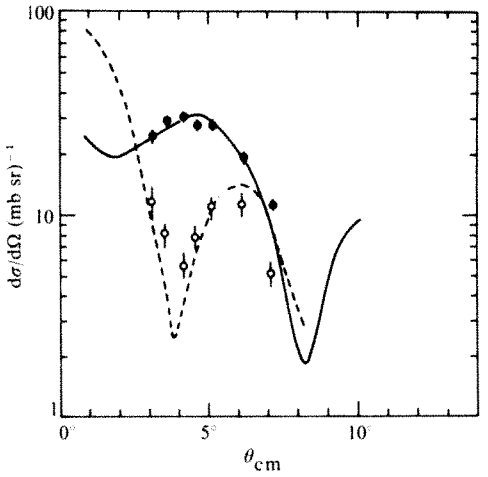


Fig. 6 Angular distribution for inelastic α scattering on ^{208}Pb (ref. 36). The solid line is the theoretical distribution for exciting a quadrupole vibration $E_x = 11.0$ MeV; $L = 2$, and the dashed line is the theoretical distribution for the monopole $E_x = 13.7$ MeV; $L = 0$. Points are data extracted by fitting the peaks like those in Fig. 4.

medium-sized computers means that the description of the wavefunction can be kept sufficiently general. In a configuration representation, the number of configurations for a heavy nucleus is typically several hundred. An example of an RPA calculation of the photon absorption strength is shown in Fig. 8[14]. The other key to a quantitative theory is the use of the same interaction hamiltonian to describe the vibrations as is used for the Hartree–Fock ground state. The important property of the interaction is that it contains a short range attraction which diminishes as the density increases. A simple parameterisation of the hamiltonian introduced by Skyrme has the form,

$$H = \sum_i \frac{p_i^2}{2m} + \sum_{i<j} v(n_0, \mathbf{p}_i, \mathbf{p}_j)\, \delta(r_i - r_j)$$

where v is a polynomial function of density $n_0(r)$ and momentum $\mathbf{p}_i$. Such a hamiltonian can be determined by fitting ground-state sizes and binding energies[16]. More fundamental theories of the interaction, based on the free nucleon–nucleon potential, can be reduced to the Skyrme form.[17] With the zero-range interaction, the Hartree–Fock ground states of nuclei are easily computed, as is the RPA response. With the largest computers, it is feasible to remove the restriction to zero-range interactions in the hamiltonian, and the most sophisticated RPA calculations are based on a more general parametrisation[18]. Using a hamiltonian consistent with ground state properties, the RPA gives an excellent description of the vibrations without any adjusted parameters[19]. The sum rules are automatically satisfied. The giant vibrations, with the exception of the monopole, are found at the proper frequency. The frequencies of low vibrations are generally reproduced to 10%. Figure 9 compares the theoretical[20] and experimental[19] density fluctuation in a low-frequency vibration of ^{208}Pb. Note that the density fluctuation is strongest at a radius ~7 fm, which is at the nuclear surface.

The RPA can be simplified to algebraic formulae when the vibration exhausts the sum rule. These formulae are analogous to the classical formula for a vibrational frequency ω in terms of a mass M and spring constant k, $\omega^2 = k/M$. Before the RPA theory was known, classical models were proposed *ad hoc*. In the case of the dipole vibration, the mass is simply the mass of the protons and neutrons. The restoring force is due to the change in potential energy when the protons and neutrons are displaced with respect to each other. The dipole frequency was first calculated along these lines by Migdal in 1944 (ref. 22; see also refs 23. 24). The dipole motion in Fig. 1 physically separates neutrons from protons in the surface region: Migdal modified

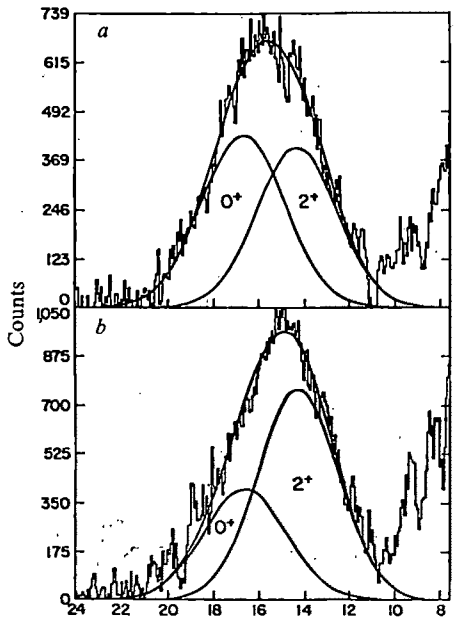


Fig. 7 Comparison of the α scattering at *a*, 0°; and *b*, 4°. The peak is shifted upward in energy at 0°, due to the relative enhancement of the monopole at the forward angle.

the velocity fields to keep the particles together at the surface. The predicted frequency is then found to be

$$\omega \sim \left(\frac{4.3 b_s}{MR^2}\right)^{1/2} \sim 80/A^{1/3}\ (\mathrm{MeV}/\hbar) \qquad (1)$$

where $b_s = 50$ MeV is the symmetry energy of nuclear binding. The reduction of RPA yields a formula that contains additional factors arising from mesons and from a more accurate description of the velocity field[25,26]. These corrections tend to cancel, and equation (1) is in excellent agreement with the empirical vibration frequencies of the heavier nuclei.

Migdal[22] and others[27] also tried to calculate the quadrupole vibrational frequency, but in this case the classical ideas were simply wrong. It was assumed that the surface tension of the nuclear surface provided the restoring force to the spherical shape. The general formula for liquid drop vibrations was derived by Rayleigh, and is

$$\omega^2 = \frac{\sigma n_0}{MR^3} L(L-1)(L+2)$$

where σ is the surface tension. The formula can be evaluated using the nuclear surface tension derived from binding energy systematics. The predicted frequency is found to be ~2 MeV for heavy nuclei, which is much too low[27]. However, as mentioned above, RPA calculations do predict the quadrupole vibration at the correct frequency. In fact, on the basis of such calculations Mottelson in the 1960s urged a search for the mode.

The reason why the classical calculation fails is that the main element in the restoring force is neglected. There is a resistance to shearing motion in any Fermi system caused by the complex spatial structure of the many-Fermion wavefunction. It turns out that the elastic shear modulus is proportional to the quantum kinetic energy of the particles. The proper formula for the vibrational frequency based on this restoring force is

$$\omega^2 = \frac{2\langle T\rangle}{M\langle r^2\rangle} \qquad (2)$$

Here $\langle r^2\rangle$ is the mean square radius of the ground state, and $\langle T\rangle$ is the average kinetic energy per particle in the nucleus. Note that this formula, unlike the liquid drop formula, has the correct $A^{-1/3}$ dependence of frequency on mass number because $\langle r^2\rangle \sim A^{2/3}$. When a simple estimate is made of the kinetic energy per particle, for example, with the Fermi gas model or harmonic oscillator model, the predicted frequency from equation (2) agrees with the empirical model to better than 10%. As in the case of the dipole, equation (2) is somewhat oversimplified, since some of the subtler effects of the nuclear interactions are neglected[28].

We now consider the theory of the most recently discovered giant vibration, the monopole. The frequency of the monopole is especially interesting because it is directly related to the compressibility of nuclear matter. The compressibility is a fundamental property of the medium, but up to now there has been no way to measure it. There have been many calculations of nuclear compressibility beginning in the early 1940s, before the nuclear interaction was very well known. Techniques of calculations have since become very sophisiticated, but we do not yet have a satisfactory understanding of the equilibrium density of nuclear matter[29]. More detailed properties of the nuclear medium, such as its compressibility, are correspondingly more difficult to calculate. However, the existing calculations should serve as a guideline. Predicted values for the compressibility are in the range

$$k = V^2 \frac{\partial^2 E}{\partial V^2} = 2.5\text{–}5\ (\mathrm{MeV\,fm^{-3}})$$

with the higher number being more recent. The classical relationship between the radial vibration frequency and the compressibility of a liquid drop is $\omega^2 = \pi^2(k/Mn_0R^2)$. Applying this formula to finding k from the empirical ω, the compressibility of nuclear matter turns out to be $k \approx 3.5$ MeV fm^{-3}. Having fixed the compressibility coefficient with the information from the monopole, it will now be possible to make more reliable calculations of other phenomena involving high-density nuclear matter. This includes high-energy heavy-ion collisions and the formation of neutron stars.

Damping

The damping of the nuclear vibrations is not well understood, theoretically or experimentally. There are two possible mechanisms for the damping. The vibration may disappear by emitting particles, which could be called evaporation; or the motion may mix with the more complex degrees of freedom in the many body system, which is analogous to friction. This complex state would eventually decay according to statistical considerations, which generally emphasise low-energy neutron emission. The measurement of decays of giant vibrations is a very active area of research. Some published experiments in the heavier nuclei claim that the decays are inconsistent with the statistical picture[30,31]. On the theoretical side, the damping by particle emission is found to be unimportant except in light nuclei. In RPA, the damping rate due to nucleon emission can be

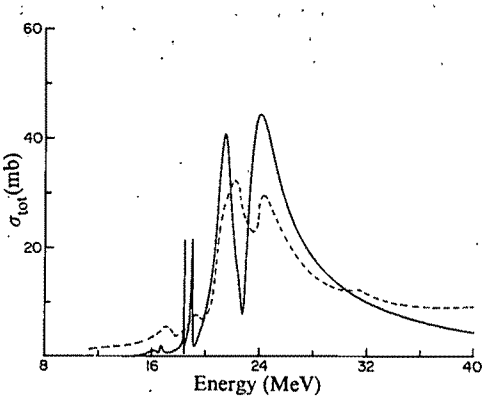


Fig. 8 The photon absorption strength of ^{16}O in the RPA theory (solid line)[14], compared with experiment (dashed line). The resonance is split into five components by the shell structure, and has a width due to the decay by nucleon emission.

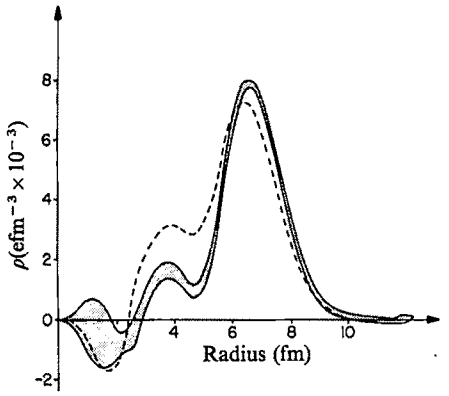


Fig. 9 The transition charge density $\langle 3^-|\rho|0^+\rangle$ for the lowest $L=3$ state in ^{208}Pb. The experimental density[20] (hatched area) is measured by a Fourier–Bessel transform of the electron scattering form factor. The theoretical density[19] is the result of an RPA calculation (dashed line), using a large basis for the wavefunction but a simple interaction.

calculated directly[14,15]. The rate of emission of heavier particles, such as α particles, is more difficult to calculate but is also estimated to be small.

Nevertheless, the study of emitted particles has provided a useful tool for studying the vibrations. By examining a particular final transition, the emitted particle can be forced to carry away the entire angular momentum of the vibration. If a coordinate system is chosen in which there is no angular momentum about the z axis, the angular distribution of the particle then follows a Legendre function, $N(\theta) \simeq (P_L(\theta))^2$.

Such a measurement was made for the giant quadrupole of ^{16}O, decaying by α-particle emission to the states in ^{12}C (ref. 32). The angular distribution for the ground ' tate transition, which satisfies the above condition, is shown ı Fig. 10. The data exhibit a peak at 0° of the right width to be described by $[P_2(\theta)]^2$. However, unlike the Legendre function, the data is not symmetric about 90°. This means there must be coherent mixtures of angular momenta. The fact that the background interferes with the decay of the vibration is not really unexpected. In the classical limit nucleon emission can take place by the projectile's knocking out a nucleon in the direction of the momentum transfer. The quantum description of this process requires intereference from many angular momenta. It remains to be seen what theory predicts about how much interference is required.

The other form of damping is caused by mixing with more complicated modes of motion. A simple example of this is the behaviour of the dipole state in a deformed nucleus. A vibration in an arbitrary direction resolves into vibrations along the principal axes with different frequencies. This is seen in Fig. 11, showing the dipole strength for an ellipsoidal nucleus.

The lineshape can be well described as the sum of two lorentzian curves. The lower frequency and higher frequency components correspond to the vibration along the major and minor axes respectively. The difference in frequencies agrees well with the R dependence in equation (1). Because there are two minor axes, the upper peak is predicted to have twice the strength which is found to be the case empirically. For the quadrupole vibration, there are three distinct frequencies instead of two, and the overall splitting is smaller because the motion has components along both principal axes. Consequently, for the quadrupole the deformation splitting is much more difficult to see.

The major source of damping is mixing with the statistical degrees of freedom of the many body system. In principle, this could be calculated from the shell model representation of nuclear wavefunctions, and several calculations along such lines have been reported[33,34]. However, these calculations are not transparent, and it is more instructive to ask how the damping of the vibrations compares with damping of other kinds of motion.

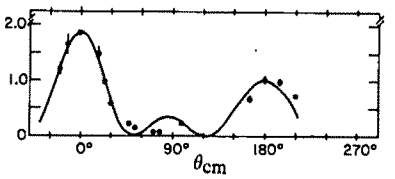


Fig. 10 Angular distribution of decay α particles following excitation of ^{16}O in the giant quadrupole region[32]. The 0° axis is along the momentum transfer direction. The probability distribution peaks in this direction, and also has a smaller peak in the opposite direction. $E_x = 17.9$–19.2 MeV.

The simplest motion is that of a single nucleon across the nucleus. This is described quantum mechanically with an optical potential, and the finite lifetime of the nucleon τ is related to the imaginary part of the optical potential $\langle W\rangle$ by $\hbar/\tau = 2\langle W\rangle$. Optical potentials can be inferred from elastic scattering of low energy nucleons and from the capture of neutrons. The spreading of the single-particle strength can also be seen directly in the reactions in which a nucleon is transferred between the projectile and the target. These methods yield a damping width for nucleons close to the particle emission threshold of about $\hbar/\tau \approx 5$ MeV.

A naive model of the vibrational damping can then be constructed, utilising the representation of the wavefunction in terms of single-particle wavefunctions. The vibration is a superposition of one particle above the Fermi level together with a hole in the Fermi sea. Each of these should have a single particle lifetime so the total damping width would be twice that, hence $\hbar/\tau \approx 10$ MeV.

This estimate ignores a coherence in the vibrational motion: the fact that all of the particles are moving together inhibits the coupling to other degrees of freedom. This coherence is well-known in other quantum systems. For example, in metals the plasmon vibration might only be damped by 1 eV while an electron at the same energy has a damping many times larger. In fact, the detailed calculations of nuclear vibrational damping do show this quenching effect[35].

Although classical ideas are relevant to the description of the vibrational frequencies, they have not yet been helpful in discussions of damping. The classical concept for dissipation in extended systems is viscosity, and attempts have been made to apply this idea to the nuclear vibrations. Unfortunately, there is no fundamental justification for viscosity; it is only valid when particles have short mean free paths. In nuclei, the particles can travel completely across the nucleus with a high probability. The nuclear surface probably plays a more important part in the damping than do collisions between particles in the interior. A simple description of the damping in these circumstances is needed not only for the understanding of nuclear vibrations, but also for interpretation of data on heavy ion collisions.

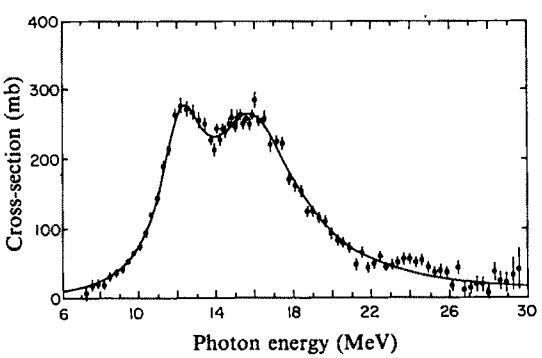


Fig. 11 The giant dipole resonance is ^{150}Gd, an ellipsoidal nucleus (ref. 3, Fig. 28). The curve is a sum of two lorentzian functions, the upper having twice the area of the lower.

Prospects

What is the likelihood of discovering more vibrations? The possibility of more complex motion always exists, and other vibrations can be calculated in RPA. However, all the other modes of particle displacement have a higher mean frequency. The damping is predicted to be much larger, both within RPA and due to the coupling to other degrees of freedom. Thus it is unlikely that a coherent mode, containing most of the sum rule, will be resolved as a distinct peak. In the inelastic scattering experiments, the spectrum contains a smooth background together with the presently known peaks, as may be seen in Figs 3 and 4. Part of this background at least must be due to highly damped vibrations of greater complexity. Without some specific measurable characteristic, however, it is not possible to identify them as such.

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articles

A 30,000-yr isotope climatic record from Antarctic ice

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Simple glaciological conditions at Dome C in east Antarctica have made possible a more detailed and accurate interpretation of an ice core to 950 m depth spanning some 32,000 yr than that obtained from earlier ice cores. Dated events in comparable marine core has enabled the reduction of accumulation rate during the last ice age to be estimated. Climatic events recorded in the ice core indicate that the warmest Holocene period in the Southern Hemisphere occurred at an earlier date than in the Northern Hemisphere.

ALTHOUGH the stable isotopic composition ($^{18}O/^{16}O$, D/H) of polar ice sheets can provide a continuous record of past climatic conditions, the interpretation of the isotope profiles in terms of climatic temperature changes over a time scale ranging back to the last ice age is complicated by several factors including: (1) the determination of the time scale; (2) the influence of the elevation at which the snow was deposited and of ice sheet stability; (3) the establishment of a transfer function from isotopic δ ratio to temperature in past conditions. Despite these limitations the study of ice cores has already provided very useful palaeoclimatic data[1-5]. Similar limitations occur in the interpretation of other indirect sources of climatic data.

As isotopic records from areas near an ice divide are simpler to interpret, thermal core drilling to 906 m depth was carried out at Dome C (74° 39′ S; 124° 10′ E; elevation: 3,240 m, mean annual temperature: −53.5 °C, Fig. 1) during the 1977–78 Antarctic field season as part of the International Antarctic Glaciological project[6].

The isotope profile

The core recovery was about 98%. Sampling was carried out in the field by cutting a continuous slice from along the length of cleaned ice core. The $^{18}O/^{16}O$ ratios were measured with a fully automatic double mass spectrometer with an accuracy of 0.15‰ in the δ scale relative to the V. SMOW. The results averaged for samples of about 4 m length are plotted in Fig. 2 with depths expressed in metres of ice equivalent to take account of the

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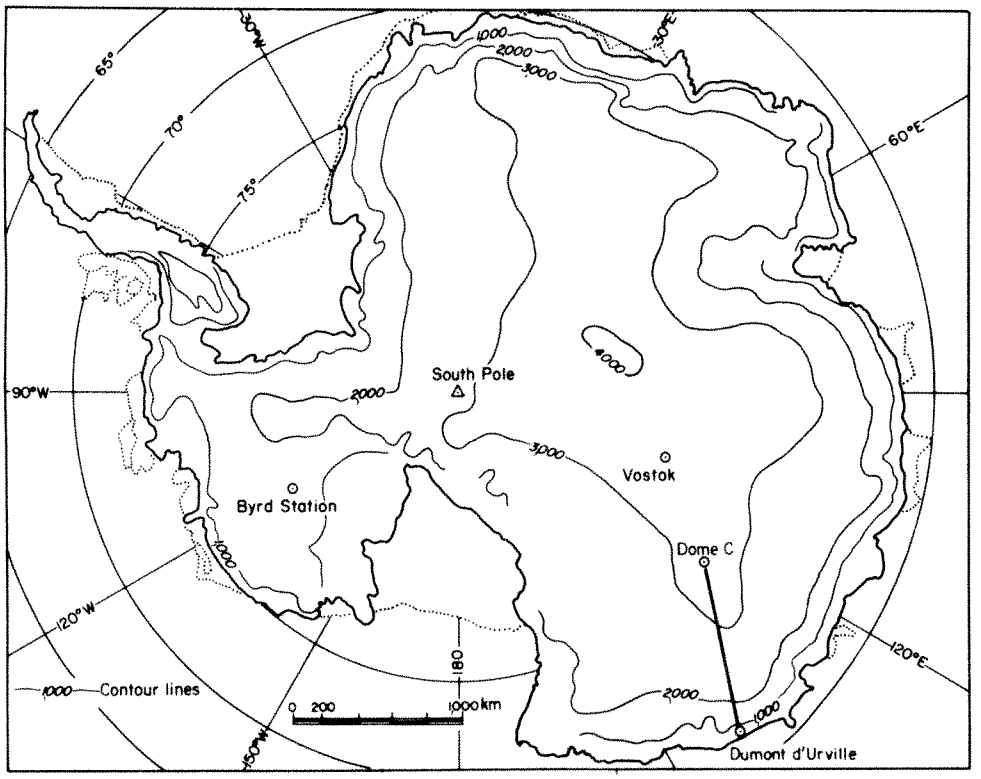


Fig. 1 Map of Antarctica showing the location of several stations (including the drilling sites) and of the traverse from Dumont d'Urville towards Dome C (dashed line). Recent data[32] indicate a dome-like shape around the Dome C site.

lower densities in the upper firn layers. Each increment samples a time interval a little longer than one century. A smooth curve was drawn from adjustment on the basis of a spline function of the order of 2 with a parameter of adjustment $\rho = 0.001$. The main features of the δ curve indicate relatively uniform conditions down to 381 m, with a mean value of −49.9‰ (standard deviation $\sigma = 0.46$) followed by a rapid decrease down to 510 m. From then and down to 687 m δ fluctuates around −55.3% ($\sigma = 0.37$); isotopic fluctuations and presumably those of climate are of similar magnitude in this depth interval to those of the upper 381 m, when mean values over the order of 100 yr are considered. The general trend is then towards less negative values down to the bottom (875 m, ice equivalent). Significant events are superimposed on these main features; some of these are indicated in Fig. 2.

Ice core chronology

Different methods may be used for dating deep ice cores[7,8] including measurements of radioactive decay, periodic changes of various parameters, and specific horizons dated from other sources. Some of these studies will be performed later, although they may not be successful in the low accumulation of the Dome C area. At present, a preliminary time scale can be calculated from the simplest ice flow model[9] which assumes a uniform vertical strain rate and a constant value of snow accumulation. Although this model has other limitations[10] it can be applied with reasonable confidence to ice dome areas when bedrock is flat and when considering the upper part of the ice sheet; both these conditions are fulfilled for the Dome C ice core. However, a large uncertainty results from the assumption of steady-state conditions over a time range which goes back to the last ice age. This is particularly true for the rate of snow accumulation which has so far been determined only over the past 25 yr from the radioactive layers formed from nuclear tests (0.037 m ice $yr^{-1} \pm 0.004$). The age of the ice (t yr) with depth can be calculated from

$$t = \frac{H}{\lambda} \ln \frac{H}{H - z} \qquad (1)$$

where λ is the accumulation rate, H the total ice thickness (3,400 m) and z the depth, all expressed in metres of ice equivalent. The ages so calculated are given in Fig. 2 (numbers in parentheses).

If the main isotopic features reflect climatic changes, we can try to get chronological information from climatic records obtained from other sources. Data from continental areas of the Southern Hemisphere are scarce but some reference data may be obtained from marine sediment cores which provide a climatic record of surface seawater temperature, assuming that typical climatic events are synchronous with those recorded in Antarctic snows. Previous results[11] from core MD 73025 (Indian Ocean; 43° 49′ S, 51° 19′ E) have been provided by J. C. Duplessy and G. Delibrias (personal communication). Eight ^{14}C ages were determined on the top 3.5 m of this core and the comparison of pelagic and benthic foraminifera isotopic profiles shows that some of the characteristic events recorded in the Dome C ice core are also apparent in this marine core. The selected climatic features can be seen in Fig. 2; depth in both cores with the three ^{14}C interpolated marine sediments ages are given in Table 1. According to Duplessy these climatic features can be recognised in several northern and southern ocean deep-sea cores having a high accumulation rate with roughly the same ^{14}C ages within experimental errors.

Table 1 Depth and interpolated marine sediment age of Dome C and MO 73025 cores

Dome C ice core Depth (m ice equivalent)	MD 73025 marine core Depth (m)	MD 73025 marine core ^{14}C (yr)
381	2.36 ± 0.02	10,550 ± 300
437	2.82 ± 0.02	12,600 ± 300
510	3.10 ± 0.05	15,500 ± 500

Snow accumulation rate changes

From these marine dates, we calculate by equation (1) a mean rate of snow accumulation in different ^{14}C time intervals. This gives 0.038 m yr^{-1} for the past 10,550 yr, 0.031 m yr^{-1} from 10,550 to 12,600 yr BP and 0.029 m yr^{-1} from 12,600 to 15,500 yr BP.

Although there are many uncertainties in such an approach and the accuracy of the accumulation figures may not be better than 10–15% (Table 1), the two last values are based on differences between ^{14}C ages, thus reducing problems of a possible time lag between marine and ice records and of errors of ^{14}C dating.

The rate of accumulation over the past 10,000 yr agrees very well with the value (0.037) measured over the past 25 yr. Since we know there are short-term changes in the accumulation rate this may seem to be a coincidence. However, the conclusion that present-day accumulation rates are typical of those for the past few thousand years has already been stated[12] for both the Greenland and Antarctic ice sheets from dating[7] and inferred from observed temperature–depth gradients[13]. Our figures suggest a decrease of the rate of snow accumulation associated with colder climatic conditions, the accumulation 14,000 yr ago being about 75% of the present value.

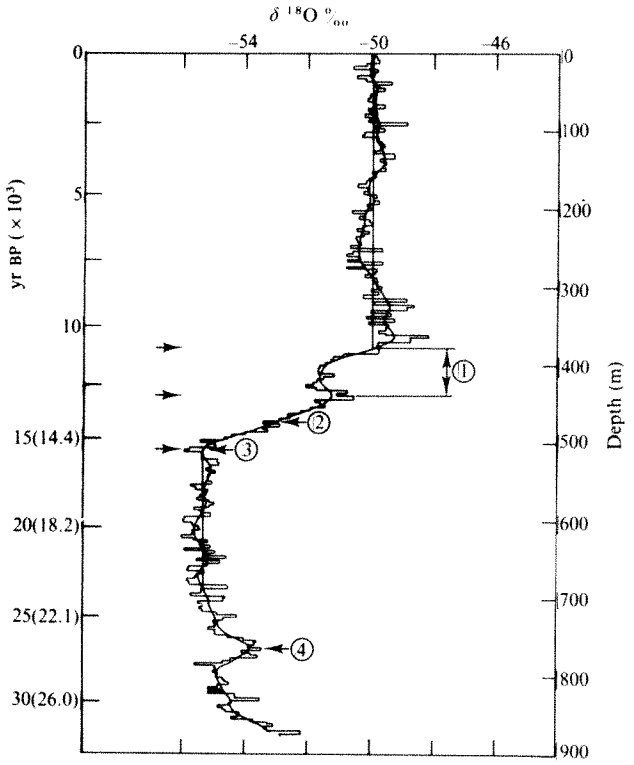


Fig. 2 δ record from the Dome C ice core plotted for about 4-m long increments and smoothed curve. The straight lines indicate mean values over the past 11,000 yr and during the late part of the last ice age. Significant isotopic events are numbered on the right hand side. On the left hand side arrows indicate reference levels corresponding to ^{14}C dates obtained from marine sediments. All depths are expressed in metres of ice equivalent. Ages are estimated from a simple ice flow model assuming a variable or constant (numbers in brackets) rate of accumulation.

Note that the mean annual accumulation in Antarctica is primarily governed by the amount of water vapour that can be carried in the atmosphere circulating above the surface inversion layer[12]. This is governed by the atmospheric temperature and by the saturation water vapour pressure over ice above the inversion. For condensation temperatures of around −40 °C in the Dome C area, a ratio of 0.75 for the vapour pressures corresponds to a condensation temperature change of about 3 °C.

The study of precipitation[14] at the South Pole gives a gradient of $d\delta^{18}O/dT_c = 1.4‰$ per °C, where T_c is the condensation temperature obtained from radiosonde measurements of cloud temperature. Analysis of snow samples collected between Dumont d'Urville and Dome C (Fig. 1) has provided an empirical relationship[15] between the mean surface temperature T_m and the isotopic deuterium content of $\delta D‰ = 6.04\ T_m$ (°C) −51 equivalent to $d\delta^{18}O/dT_m = 0.75‰$ per °C. In Antarctica mean temperatures above the atmospheric inversion layer (T_i) are similar to the condensation temperatures[12], while the inversion strength is linearly related[16] to mean surface temperature. A survey of existing data for eight stations with T_m values in the range from −10 to −56 °C gives $dT_i/dT_m = 0.67$ with a correlation coefficient of 0.99. Assuming $T_i = T_c$ leads us to interpret our traverse results of equivalent to $d\delta^{18}O/dT_c = 1.1‰$ per °C, a value which is in reasonable agreement with that obtained at the South Pole.

A change of 3 °C for the temperature of condensation previously deduced from accumulation data would lead to a change of 3.3‰ between ice recently deposited and that deposited 14,000 yr ago. The agreement with the value obtained from the Dome C record (3.1‰) is very satisfactory considering the large number of assumptions involved, which confirms conclusions about snow accumulation changes inferred from the Indian Ocean marine core data.

To obtain a continuous time scale we use relation (1) with a constant rate of accumulation (0.037 m ice yr^{-1}) down to a depth of 381 m, and then decreasing to 75% of this value down to 510 m; this last figure is used down to 687 m. Further down it does not make a significant difference whether or not we assume an increasing accumulation linked to the $\delta^{18}O‰$ change. The calculated time scale is given in Fig. 2. According to this model the age of the bottom of the 905 m deep ice core is about 32,000 yr.

Climatic interpretation

Two other ice cores with depths comparable or greater than that from Dome C have been recovered in Antarctica (Fig. 1). At Byrd Station (80° 01′ S, 119° 31′ W) the 2,160-m deep ice core covers a much longer time period and the isotopic change towards much more negative values is located between 1,000 and 1,400 m. The location and characteristics of the Vostok ice core are more comparable to those of Dome C one, the isotopic change at Vostok occurring between 250 and 375 m. Part of the published[2,17] isotope profiles plotted against ages are shown in Fig. 3.

Comparison of Figs 2 and 3 shows similar trends in the three δ profiles. Event 1 from Fig. 2 is well marked in all three locations and there is a parallelism between the Byrd and Dome C isotopic profiles down to event 3. Events 1, 2 and 3 are also apparent in the Vostok ice core when looking at a more detailed isotope profile[4], although there is a large scattering of the δ values probably due to discontinuous sampling technique. The less negative values of event 4 are also observed in the other ice cores.

Dating problems make precise age comparisons difficult, but it must be pointed out that the depths of the four events in the Vostok and Byrd ice cores relative to that of the Dome C record show a rather constant value. For the Vostok ice core where $\lambda = 0.024$ m ice yr^{-1} this value is similar to the ratio of the present rates of snow accumulation; this is not the case for the Byrd ice core, probably due to the very complicated regime in this area.

It appears then that the main characteristics of the δ profiles are similar for east Antarctica (Dome C, Vostok) and west Antarctica. Thus they probably reflect climatic events which affected both east and west Antarctica, rather than any ice sheet instability[18] because these two distinct areas have very different glaciological regimes. Furthermore, isotopic events observed in the Dome C ice core are also seen in the record of sea-surface temperature changes in marine sediments.

The ice core chronology from Dome C indicates a relatively cold period from 11,000 to 13,000 yr BP; this is shown also in the record of past temperature of the New Guinea Highland sum-

marised in ref. 19 from careful pollen analysis. The Dome C fluctuations observed during this period could be equivalent to the European Dryas–Bölling–Alleröid stages depicted in the Camp Century (Greenland) ice core[1] although dating uncertainties prevent any conclusion about their synchronism.

The last major glacial peak occurs around 16,000 yr BP, which agrees with the much less detailed pollen record from New Guinea[19] and the indication from various sources[20–22] that continental warming had probably begun in the Australian–New Guinea area by 16,000 ± 2,000 BP. This worldwide climatic feature has been found in many marine sediment cores both in the Northern and Southern Hemispheres[23,24] with an age of about 18,000 yr which may vary locally by several thousand years.

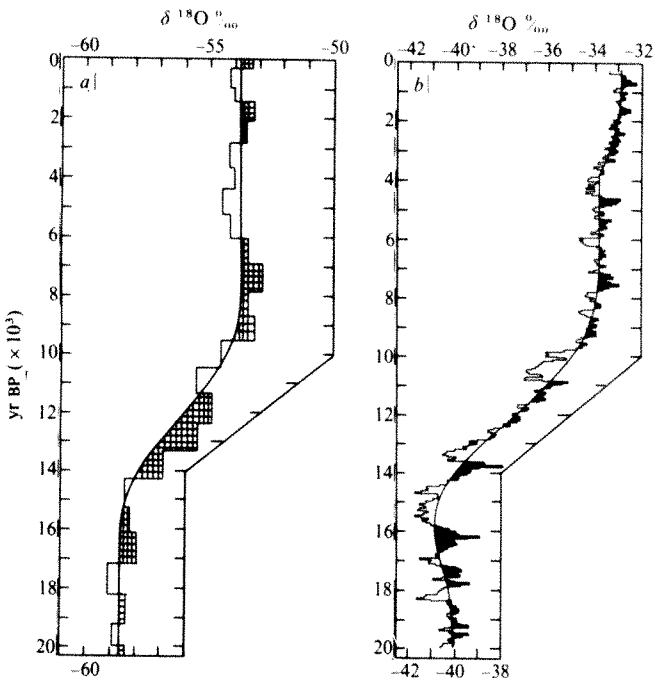


Fig. 3 The Vostok (*a*) and Byrd (*b*) δ records during the past 20,000 yr from ref. 2 (Byrd) and ref. 17 (Vostok). The δ values exceeding the smoothed curves are shown in black.

The warmer conditions shown on the Dome C δ record before 25,000 yr BP also agrees with conditions observed around this time in New Guinea by pollen analysis[22] and with North America glacial chronology[25].

The isotopic shift associated with the transition from the coldest part of the ice age towards mean climatic conditions from the last 10,000 yr is 5‰ for Vostok[4], 7‰ for Byrd[2] and 5.4‰ for Dome C. When values for Vostok and Byrd station are corrected[12] to allow for the origin further inland of the deeper ice and for a higher surface elevation of the ice sheet during the last ice age, the δ^{18}O‰ change associated with climatic changes would become 4.5 (Vostok) and 5.3 (Byrd). Although corrections for the same factors at Dome C could be made if measurements of total gas content[26] were available, severe cracking in the ice core from Dome C has made such a study impracticable. Since Dome C is at present a centre of outflow and if we assume that the form of the ice sheet has changed little over the past 10,000 yr, then the δ values in the upper 381 m of ice core should provide a useful climatic record over this period.

However, at the end of the last ice age, a lower sea level may have resulted in a slightly expanded ice sheet over east Antarctica, leading to greater ice thicknesses and increased surface elevations. These effects would be much greater in coastal areas, while in central east Antarctica estimates of the consequent surface rise are around 100 m (refs 4, 28). Such a rise, together with the present δ-elevation gradient observed in the Dome C area[15] would involve a correction of 1.5‰ to allow for changed elevation during the ice age. Thus it seems that the climatic change at the end of the last ice age was similar at Dome C, Vostok and Byrd, but further studies of ice thickness changes at Dome C are needed to confirm this conclusion.

The isotopic shift has still to be corrected for modifications of oceanic parameters during the last ice age. The most obvious is the change of mean isotopic value of oceanic waters. Due to the storage of water in δ-depleted ice sheets an increase of 1.6 δ^{18}O‰ with reference to the present conditions has been estimated[11]. This effect will probably be reflected in antarctic precipitation but from previous discussion it could be compensated by greater ice thickness during the last ice age.

The isotopic composition of ice cores, although principally governed by the temperature of condensation, can be affected by many other parameters[1]. However, it has been suggested[12] that the present empirical relationship between mean surface temperature and isotopic content may not have been too different in Antarctica during the last ice age. As previously mentioned a 0.75‰ per °C δ^{18}O change was obtained in this area for a large range of temperatures, which leads to a tentative estimation of a difference of about 7 °C for the surface temperature between the coldest part of the ice age and the present climate. From previous discussions the suggested changes for the temperatures of condensation and above the inversion layer are reduced by about half.

Although there are many assumptions in such interpretations, they agree with the results of atmospheric modelling of the ice age (18,000 yr BP) climate[29,30] which also indicate lower precipitation during cold conditions comparable with those described here. For the past 11,000 yr the climatic record inferred from the δ profiles (Fig. 2) shows that the warmest conditions occurred during the period 11,000–8,000 yr BP and the coldest conditions from about 8,000 to 4,000 yr ago. For the past 4,000 yr we observe a moderately warm period followed by a slight trend towards less positive δ values, with present surface conditions close to the mean value for Holocene.

Figure 3 shows that the Vostok δ profile indicates the same succession of climatic periods with a slightly different chronology. However, the Byrd record shows no apparent agreement with this sequence[2] due to the difficulty of interpreting its complicated glaciological regime.

The lack of comparative data from the Southern Hemisphere makes it difficult to estimate the spatial representativity of the Dome C Holocene record. During this period, the climatic evolution inferred from our δ profile gives a different picture from that obtained from New Guinea pollen analysis[19,20]. The warmest period (11,000–8,000 yr BP) that we observed at the end of the transition from the last ice age is much earlier than the usual estimates for the so-called hypsithermal in the Northern Hemisphere. This is, however, in excellent agreement with the fact that the warmest sea surface temperature since the last glacial age[31] in the Southern Hemisphere have a radiocarbon date of 9,400 yr (24).

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Shelf sea fronts' adjustments revealed by satellite IR imagery

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IR imagery from NOAA 5 has been used to study the movement of shelf sea fronts. After the elimination of tidal advection, the fronts show remarkable consistency of position and do not adjust to the strong semi-monthly variation in tidal stirring which indicates the operation of a feedback process in vertical mixing.

IR IMAGERY from NOAA 4/5 has provided convincing evidence of the role of tidal mixing in controlling the distribution of sea surface temperatures in the European shelf seas. In particular it has been shown that the positions of the surface manifestations of the shelf sea fronts are closely parallel to contours of the h/u^3 parameter[1,2], as previously predicted[3].

As the archive of imagery grows, it should be possible to use it to answer more detailed questions about the evolution of the fronts and the processes occurring on them. Indications have already been obtained from several images of the development of large scale (~25 km) instabilities in the along front flow which are clearly important as agencies of cross-frontal mixing[1,4].

In this article we attempt a more systematic analysis of two years data from NOAA 5 to investigate the extent to which the mean positions of the fronts adjust to variations in both the rate of tidal mixing and the surface heat flux.

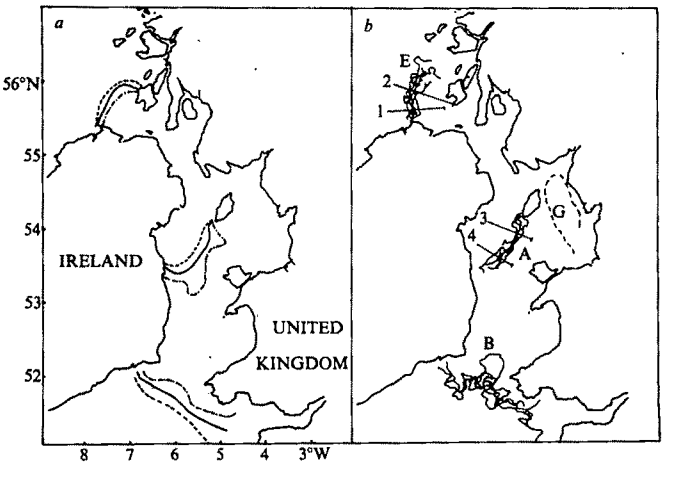


Fig. 1 *a*, Contours of $\log_{10} h/u_s^3$ -·-· 1.5; —— 1.9; – – – 2.2. h Is the depth (m) and u_s is the tidal stream amplitude at springs (m s^{-1}). The separation of the contours 1.5 and 2.2 indicates the range of hypothetical adjustment between neaps and springs. Contour shape based on Pingree and Griffiths[2]. *b*, Composite of all frontal positions observed in July 1977 from NOAA 5 imagery. Fronts are labelled as follows: A, Western Irish Sea (WIS); B, Celtic Sea; E, Islay; G, Liverpool Bay. Reference lines, along which displacements were measured, are numbered.

Equilibrium adjustment

We particularly wish to examine the possibility that the mean frontal positions vary during the semi-monthly range cycle of the tides. As the amplitude of the tidal streams increases by a factor ~1.8 from neaps to springs, the intensity of tidal dissipation, which is proportional to u^3, will rise by a factor ~5.8. The balance between surface heating and tidal and wind stirring at a front may be expressed in a constant efficiency model[1] as

$$R = 1 - a\left(\frac{\overline{u^3}}{Qh}\right) - b\left(\frac{\overline{w^3}}{Qh}\right) = 0$$

where h is the depth; u the tidal stream velocity; w the windspeed; Q the surface heating rate, and the parameters a and b, which contain the efficiencies of tide and wind mixing, may be assumed constant. The overbar denotes a time average.

If we further assume that Q and $\overline{w^3}$ are spatially uniform over a particular frontal region, the 'equilibrium positions' should be defined by

$$\chi = \frac{h}{\overline{u^3}} = \text{constant}$$

and, for slow changes in $\overline{u^3}$, the front would move to the appropriate contour of χ. This hypothetical adjustment, for a range change corresponding to the phase inequality cycle is shown in Fig. 1*a*. In the case of the Islay front, for example, the equilibrium displacement would be ~20 km. In other frontal

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Fig. 2 NOAA 5 IR image from 21 May 1978, at 09.30 GMT, showing Islay and WIS fronts.

regions this movement would be generally larger due to the weaker gradients in χ.

On the other hand, if the time scale for adjustment is long in relation to the fortnightly mixing cycle, adjustment may be substantially reduced. The mixing model of James[5], which incorporates a vertical transfer rate dependent on Richardson number, and hence stability, predicts negligible movement. Over longer periods we might also anticipate that the mean position of the front would adjust to changes in the heating rate Q which, at latitude 55° N, falls from a maximum of $\sim 140\ \mathrm{J\ m^{-2}\ s^{-1}}$ in mid-summer (day 172) to $\sim 30\ \mathrm{J\ m^{-2}\ s^{-1}}$ at 31 August (day 243) and reaches zero around mid September. Less regular adjustments due to variations in the level of wind mixing are also a possible source of variability in frontal positions.

Data acquisition and processing

The analogue data from the 10.5–12.5 μm band radiometer on NOAA 5 are received at the University of Dundee where they are converted to imagery with appropriate grey scales by a modified photo-facsimile machine. In the example shown in Fig. 2, where the darkness of the image increases in proportion to the radiation temperature, the positions of the Islay and western Irish Sea fronts are clearly visible. The 'front' is here taken to be the point of maximum surface gradient in sea surface temperature as judged from grey scale changes on the analogue images. Direct observations at sea indicate that it is close to, though not exactly coincident with, the transition to complete vertical mixing.

The photographs are corrected, by analogue techniques, for geometric distortion due to slant angle viewing of the Earth's surface from the spacecraft. As we are using the imagery to detect relatively small displacements of geographical position, it is important to quantify the extent of any residual distortion in the imagery. We have estimated this by determining, from a series of images, the relative positions of two headlands whose separation is known. The standard deviations of 17 measurements was $\sigma = 1.4$ km for a separation of 61 km, a value which is representative of the distance from coastal reference points at which fronts are located. With increasing distance from the coast the relative uncertainty of positions will be larger, while close to land it is limited by the spatial resolution of the scanner which is ~1 km.

For each cloud-free picture of a frontal region the displacement of the front along lines approximately perpendicular to the mean frontal direction was measured from a datum marker. Figure 1*b* shows the location of the reference lines together with a superposition of tracings of the frontal positions as seen on all the useful images during July 1977. The number of cloud-free views shown here is typical of the data yield for summer months. Other fronts, notably one evident in the original pictures in Liverpool Bay (Fig. 2) will be described elsewhere.

An indication of the variability of frontal positions may, of course, be obtained from composite diagrams like Fig. 1*b* but note that the apparent displacement here includes a contribution from tidal advection, by the stream component normal to the front, which may be as great as ±8 km. To remove this, we considered the attractive possibility of using the known times of sampling, which defines the relevant phase of the tide, together with the observed positions of the fronts to extract the tidal streams by regression analysis. This approach is feasible and an example is given below but there is an inherent danger. As the satellite is Sun-synchronous the sample time is constrained to essentially the same time each day. For the UK the morning pass occurs on average at ~10.00 GMT. Consequently the principal solar constituent S_2 in the tidal signal is sampled at an almost constant phase while M_2 is observed with a phase slowly changing at a rate of $24.38°\ \mathrm{d^{-1}}$. This generates an apparent displacement with a semi-monthly periodicity as can be seen in Fig.

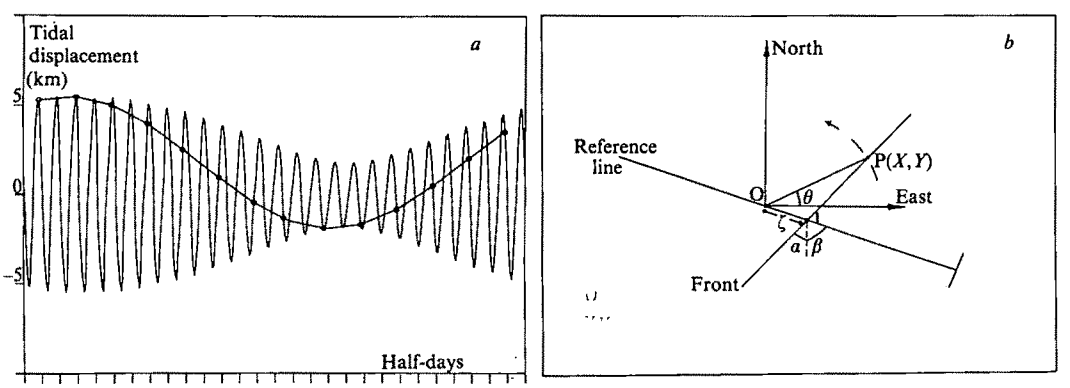


Fig. 3 *a*, Predicted tidal displacements for Islay line 2 based on current meter determination of M_2 and S_2. Circles indicate the effect of sampling daily at a fixed solar time of 10.00 GMT. The result is a signal of semi-monthly periodicity which may be ambiguous with springs–neaps adjustment. *b*, Displacement of the intersection point I of the front and reference line by an elliptical tidal excursion of a water particle P about its mean position 0.

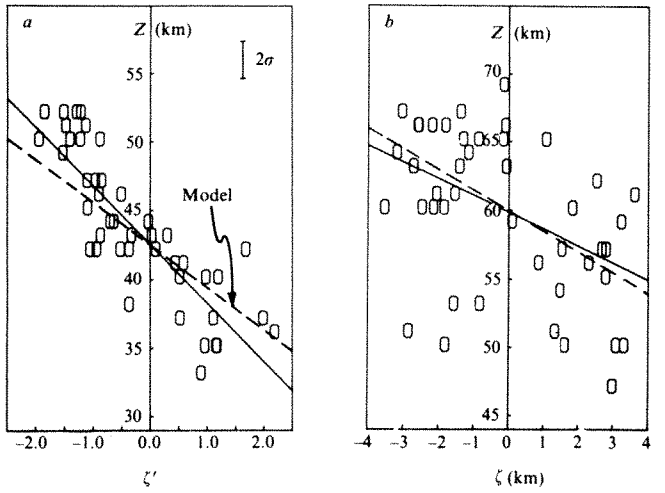


Fig. 4 Displacement Z versus a, predicted tidal displacement ζ' for Islay line 1, and b, predicted tidal displacement ζ for Islay line 2. The continuous lines are the results of a least squares fit. The dashed lines are based on a numerical model.

3a. This displacement will be indistinguishable from the neaps–springs mixing adjustment if the phases coincide. For a two component tide it can be shown that the phase difference of the range cycle and the apparent displacement is just

$$\Delta\nu = \phi_s - \omega_m \tau$$

where ϕ_s is the phase lead of the solar tide, ω_m is the frequency of the lunar tide and τ is the time before midday at which sampling takes place.

Ideally the ambiguity should be removed by doubling the sampling frequency using data from two satellites. In practice this is hardly necessary because of the spread in sampling time (±1.5 h) about the mean and the fact that the evening pass occurs on average 10 h (not 12 h) after the morning transit, an asymmetry which results from the inclination of the satellite orbit to the Earth's axis.

If an adequate sample of velocity data is available from a mooring near the front, prediction of tidal displacements X and Y of a water particle may be made by the usual harmonic method according to

$$X = \sum_1^N \frac{a_i}{\omega_i} \sin(\omega_i t + \kappa_i) \quad \text{and} \quad Y = \sum_1^N \frac{b_i}{\omega_i} \sin(\omega_i t + \lambda_i)$$

where the a_i, b_i, κ_i, λ_i are the amplitudes and phases determined by least squares harmonic analysis of the velocity data. The displacement of the front along the reference line, which is not the same as the displacement of a water particle, may then be found from

$$\zeta = \frac{(X^2+Y^2)^{1/2}\cos(\theta+\alpha)}{\sin(\alpha+\beta)} \quad \text{where} \quad \theta = \tan^{-1} Y/X$$

where the angles are defined in Fig. 3b. This approach assumes that the front is straight for a length large compared with the section of it which crosses the reference line during a tidal cycle. This requirement is least severe if we choose β so that $\alpha+\beta = \pi/2$, when the range of ζ has a minimum.

Where adequate tidal stream data is not available, we have compared the observed displacements to a non-dimensional tidal displacement defined by

$$\zeta' = F(t)\sin(\omega t + \phi)$$

where ω is the semi-diurnal tidal frequency and ϕ is a phase lead determined by reference to tidal atlas data. The slowly varying factor $F(t)$ is defined by

$$F(t) = \frac{\text{Elevation range at time } t}{\text{Elevation range at mean neaps}}$$

and found from elevation predictions at a convenient port. Comparison of ζ and ζ' in a case where suitable data were available showed acceptable agreement with an r.m.s. difference of 1.1 km.

Results

The Islay front: The tidal advection signal is illustrated in Fig. 4 where the observed displacements Z on lines 1 and 2 have been plotted against ζ' (Fig. 4a) and ζ (Fig. 4b). In the latter case, current meter data were available from a closely adjacent current meter station[6]. The slope of the regression line is 1.22 with a standard deviation of slope $\sigma_m = 0.44$, so that the observed displacements are consistent with the predictions although the scatter is considerable.

A better fit is apparent in Fig. 4a in which a regression line is compared with an estimate based on the numerical model of Pingree and Griffiths[2]. The difference in slope (1.1 km) is significant at the 1% level, but this may partly be due to the interpolation of values between model grid points in a region of high gradients. The observed slope difference corresponds to a change in position of only one grid interval of 9 km.

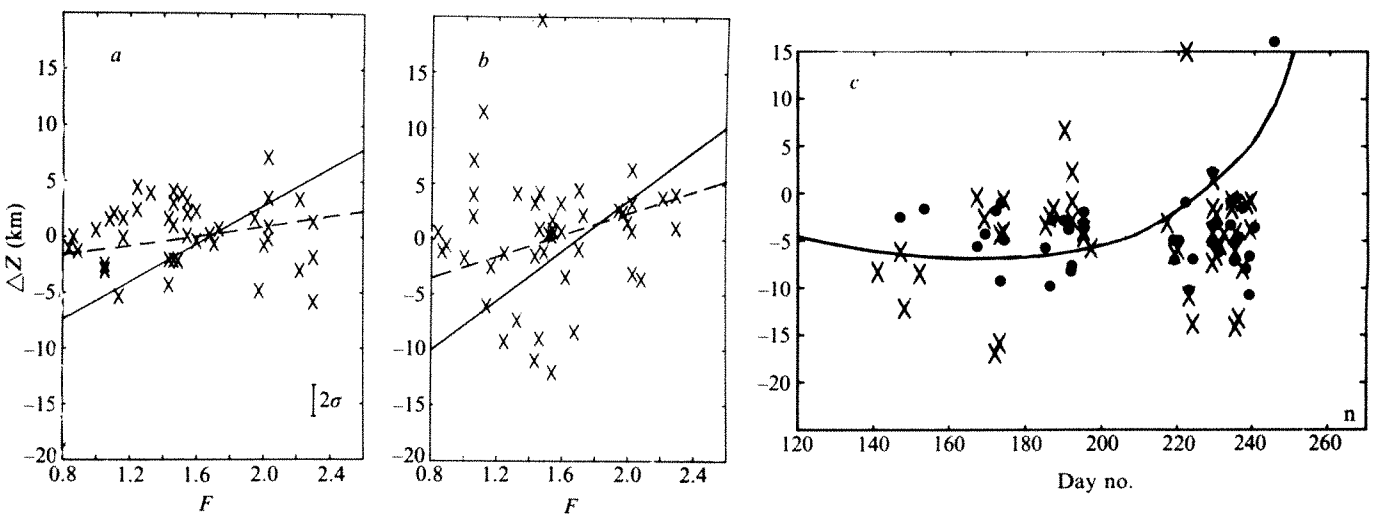


Fig. 5 a And b, residual displacement ΔZ against non-dimensional range index F for a Islay line 1; b Islay line 2. The extent of equilibrium adjustment is shown by the solid line. The dashed line has the largest slope consistent with the data points at 95% confidence ($F = 1$ at mean neaps). c, Residual displacement ΔZ versus day number n for the Islay front: ●, line 1; ×, line 2. The curve shows the form of the equilibrium adjustment to the seasonally variable heat flux.

An alternative explanation is the distortion of the Z–ζ relationship by curvature of the front, which we have neglected. When sufficient data are available, systematic departures from a straight line may be used to estimate frontal curvature and distinguish between fixed meanders and evolving eddies whose contributions should be random.

Subtracting the tidal advection signal, we obtain estimates of the residual displacement ΔZ which are plotted against tidal range in Fig. 5 along with a line which represents the extent of equilibrium adjustment. The data points are clearly inconsistent with such adjustment and the slope of the regression line through them is not significantly different from zero. The most probable adjustment between neaps ($F = 1$) and springs ($F = 2$), is determined from the regression as 0.25 km for line 1 and 1.2 km for line 2. Upper bounds are 1.9 and 4.8 km respectively at the 95% confidence level.

The marked difference in scatter between the two lines is apparently due to the front's tendency to form eddies which increases northwards. The lack of such instabilities on the Islay front near the Irish coast contrasts with that commonly observed on the shelf sea fronts, and may mean that observations of adjustment here are untypical of these features. Moreover the Islay front exhibits a strong salinity contract which distinguishes

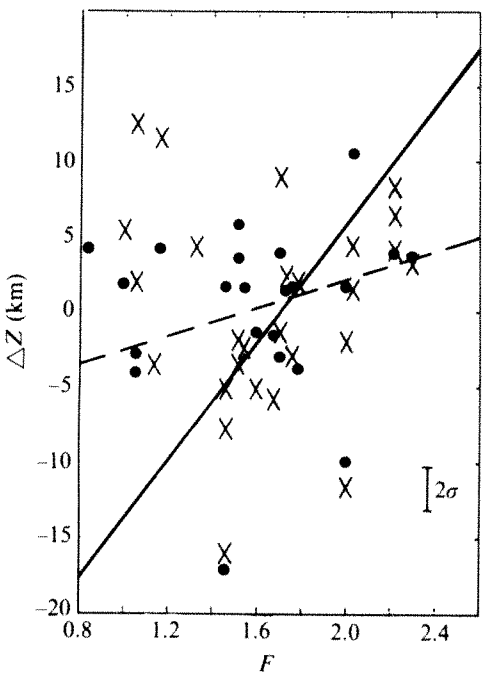


Fig. 6 Residual displacement ΔZ versus non-dimensional tidal range F for western Irish Sea lines 3 and 4 combined. (●, line 3; ×, line 4.) The extent of equilibrium adjustment is shown by the continuous line. The dashed line has the largest slope consistent with the data points at 95% confidence ($F = 1$ at mean neaps).

it from most other fronts and may contribute to anomalous behaviour. We therefore consider a second example.

The western Irish Sea: In this case density is essentially controlled by temperature. A second difference is that the tidal streams are almost parallel to the front so that the tidal advection signal is weak. After subtracting an estimate of ζ based on numerical model results[2], we have obtained the estimates of ΔZ for both lines 3 and 4 which are plotted against tidal range F in Fig. 6. As in the case of the Islay front, there is no evidence of semi-monthly adjustment. The most probable neaps to springs adjustment is 0.7 km with an upper bound, at 95% confidence, of 4.5 km.

The relatively large scatter is again probably due to frontal meanders and eddies (see Fig. 1*b*) which frequently occur on this front.

Seasonal and other adjustments

To test for frontal movement on a longer time scale, we have plotted ΔZ versus Julian day number n in Fig. 5*c*, together with an estimate of adjustment based on the mean nett heat flux through the sea surface. The scatter of points is such that a seasonal trend cannot be reliably identified with the present data, particularly as there are very few data points after $n = 240$ when the seasonal effect may become conspicuous. Moreover, because of the stored potential energy deficit, the retreat of the front may be slower than predicted by an equilibrium model especially as $Q \to 0$ at the autumnal equinox. Note also that the sampling scheme is biased, in the sense that images are most often available during anticyclonic weather conditions when surface heating will be above average. This may account for the large cluster of points with low values of ΔZ occurring between days 220 and 240. A large proportion of these points are from images during a period of exceptional fine weather in August 1976.

If the observed displacements from the mean position are not semi-monthly or seasonal in origin, to what, apart from frontal instabilities, could they be attributed? One possibility is that they represent movement induced by large scale advection. The residual velocity field in the Irish Sea, averaged, over periods of ~1 month, is small and not known with confidence, but over shorter time scales, residual flows of a few centimetres per second probably occur[7] and these could be responsible for fluctuations in the frontal position of the observed magnitude of $\simeq \pm 5$ km.

To assess the relevance of this hypothesis, we have examined the data for coherent adjustment on adjacent reference lines. No significant correlation is found for example, between ΔZ on lines 1 and 2 for the Islay front, implying that other movements with a length scale shorter than the line spacing of 30 km, are dominant.

Discussion

The results reported here indicate that any adjustment of the shelf sea fronts to the semi-monthly mixing cycle is minimal. There is also little support in the data for the notion of seasonal positional adjustment between June and the end of August. The shelf sea fronts therefore represent a set of almost fixed geographical boundaries throughout the summer season, moved significantly only by tidal advection.

There remains the possibility of regular adjustment on a very small spatial scale ~2 km, which cannot be resolved by the present approach, but which may be of considerable biological importance. It may be significant that the three regression coefficients of ΔZ versus F, although small, are all positive, which is the sense of the anticipated adjustment.

The remarkable consistency of frontal positions confirms the prediction of the James model and points to the operation of a feedback process in determining the rate of vertical mixing, that is, once stratification is established the efficiency of mixing falls, so that even when stirring increases at spring tides, the stratified structure survives. The notion of feedback is also consistent with the results of mixing tank experiments[8] which indicate that the flux Richardson number (efficiency) decreases with stability above a threshold stability level. An interesting experiment described by Loder (unpublished) suggests that direct laboratory study of this hysteresis effect may be profitable.

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The nitrogen budget of a salt marsh ecosystem

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Salt marshes reduce oxidised nitrogenous compounds to ammonium and particulate nitrogen and export these reduced forms to coastal waters. The internal demands exceed the net inputs of nitrogen by rain, groundwater flow and fixation, suggesting very active uptake, conversion, release and recycling of nitrogen within a marsh ecosystem. Nitrogen losses are mainly through tidal exchanges and denitrification, and these two outputs balance the gains. The chemical exchanges among uplands, marshes and coastal water are important in structuring these ecosystems.

NITROGEN is a key nutrient in coastal ecosystems[1]. In salt marshes, nitrogen has a critical role in determining the function and structure of the ecosystem. Salt marsh floras receiving increased amounts of nitrogen show increased rates of primary production[2-5], the abundance of certain species changes and the nitrogen content of the plants increases[6]. Provision of excess nitrogen raises the per cent nitrogen in tissues of marsh grasses from levels typical of C_4 plants to those of C_3 plants[7,8]. This is important because low nitrogen content is one reason why C_4 plants are relatively free from herbivores[9]. The dominant plants (*Spartina alterniflora*, *S. patens* and *Distichlis spicata*) in salt marshes of the Atlantic coast of the US are C_4 grasses, but where the supply of nitrogen is increased the number of marsh herbivores increases due to the lowered C/N ratios in the plant tissues[6].

Increases in nitrogen supply also lead to increases in the activity and standing crops of decomposer organisms and detritus-feeding invertebrates as the percent nitrogen of the litter helps to regulate decomposers (unpublished results). In general, the biomass of invertebrates in salt marsh plots receiving added nitrogen is larger than in untreated plots. We have also found that increased nitrogen supply changes the morphology of stands of *Spartina alterniflora* from a short to a tall form[10]. Concomitant with this is increased spacing among the taller plants leading to a more open habitat which in turn allows predatory fish and crabs a greater chance of finding prey on the marsh surface[11]. Increases in the supply of nitrogen therefore enhance primary production, decomposer activity, secondary production and changes in the physical structure of a marsh environment that facilitate predation by fish. Thus the rate of turnover of the entire system to a large extent depends on nitrogen supply.

A second important feature of nitrogen in salt marshes involves the export of nitrogenous materials and marsh-produced organic matter to coastal waters. The proposition that marshes provide food for coastal and estuarine fin and shell fish populations, including economically important species, has been the main argument for conservation of coastal marshes. With the recognition that nitrogen has a limiting role for coastal phytoplankton production[1], it can be appreciated that nitrogen exchange between coastal waters and marshes is significant for both these ecosystems.

We present here the first complete nitrogen budget for a marine ecosystem where each of the various inputs and outputs from a salt marsh has been accounted for. Great Sippewissett Marsh, our study marsh, is located on the western shore of Cape Cod, Massachusetts. Seawater from Buzzards Bay flows into Great Sippewissett Marsh through a single entrance (Sippewissett Creek) and floods the marsh twice daily with a maximum tidal excursion of about 1.6 m. The total area of the marsh is 483,800 m^2 and includes muddy (104,900 m^2) and sandy (61,700 m^2) creek bottoms, algal mats (13,000 m^2), low marsh dominated by short (122,500 m^2) or tall (91,100 m^2) *Spartina alterniflora* and high marsh (89,300 m^2) where the grasses *S. patens* and *D. spicata* are found. The marsh is surrounded by glacial moraine on the south, east and north and by sand dunes to the west. The relief and the ground water table slope generally to the west and towards the marsh and bay.

Inputs and outputs

Nitrogen enters Great Sippewissett Marsh through precipitation and flow of groundwater, the latter flowing into the marsh through numerous small underground springs. Other sources of nitrogen are fixation of atmospheric nitrogen by blue-green algae and bacteria in the sediments. Tidal flushing brings in and removes dissolved and suspended nitrogen from the marsh. The second major loss of nitrogen is through denitrification. Other minor fluxes include volatilisation of ammonia, removal of shellfish, loss to sediments and deposition of faeces by roosting birds.

Groundwater flow. Groundwater was sampled over 2 yr at seven small underground springs that flowed from the upland into the marsh[12]. The amounts of ammonium, nitrite and organic nitrogen were determined using an autoanalyser. The total flow of groundwater into the marsh lowered the salinity of the ebbing tidal water by about 2‰. This lowered salinity was used to calculate the volume of freshwater contributed by groundwater. Salinities were collected routinely during entire tidal cycles through the year. With these estimates of concentrations and volumes, the amount of each nutrient entering the marsh at different times of the year was calculated (Fig. 1). Nitrate was the major inorganic nitrogenous constituent of groundwater (averaging over 50 $\mu mol\ l^{-1}$), with substantial amounts of dissolved organic nitrogen (DON) (Table 1). There was a small reduction of nitrate flow during the autumn (Fig. 1). The amount

Cover photograph shows false colour IR aerial view of Great Sippewissett Marsh, Massachusetts. The entire system is drained by Sippewissett Creek. The dark blue tidal creeks have muddy bottoms while sandy bottoms show as light blue. Small pannes with no obvious drainage are scattered through the marsh. Algal mats occur on sand flats (top right). The sand surface is very unstable except where consolidated by the grey–blue mats of blue-green algae and purple sulphur bacteria. The largest area of the marsh is low marsh, covered by *Spartina alterniflora* (dark pink). The taller stands of *S. alterniflora*, showing as a thin red ribbon, can be seen on creek banks. Above the elevation of the low marsh, visible as areas of lighter pink, is high marsh where *S. patens* and *Distichlis spicata* grow. The marsh is surrounded by upland vegetation on the glacial hills.

of ammonium increased in summer, presumably due to the increased decay of litter prompted by higher temperatures at that time of year. The amounts of nitrite involved were minimal and were not included in Fig. 1. The total annual input of nitrogen to Great Sippewissett Marsh by groundwater was 6,120 kg yr^{-1}.

Precipitation. The amount of precipitation and the concentration of ammonium, nitrate, nitrite, DON and particulate nitrogen were measured in rain gauge collections at various sites over 2 yr (ref. 12). Nutrient inputs for the four seasons was calculated by multiplying the concentrations of nitrogenous nutrients by the amount of rain for each rainfall. The nutrient concentrations were typical of semirural areas[13–15] and there was little seasonal change. Nitrate and DON were the principal forms of nitrogen delivered by rain (Fig. 2 and Table 1). The concentration of all nutrients was higher in the groundwater than in precipitation[12] primarily due to evapotranspiration by terrestrial vegetation. There is little evidence of any increase in nitrogenous concentration in groundwater due to leaching from septic tanks upstream. The volume of rain that fell on the marsh was much less than that which reached the marsh as groundwater, so only 380 kg yr^{-1} of nitrogen reached the marsh through rainfall (Table 1).

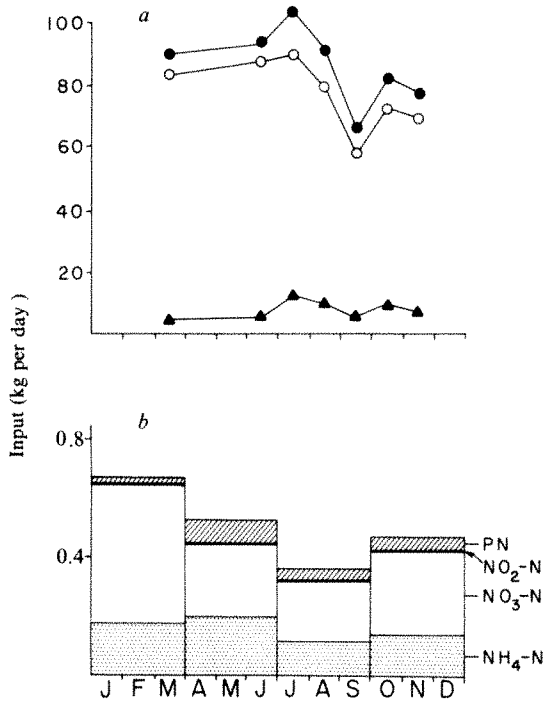


Fig. 1 Amounts of nitrogenous nutrients brought in by groundwater flow (*a*) and precipitation (*b*) into Great Sippewissett Marsh throughout the year. Values for precipitation are averaged for 3 months periods. Even though rain fell in episodic fashion, results are averaged on a per day basis over an entire 3-month period for ease of comparison with the other inputs and outputs. ●, Dissolved organic nitrogen; ○, NO_3—N; ▲, NH_4—N.

Nitrogen fixation by bacteria. Fixation of nitrogen by bacteria in the marsh is due to free-living bacteria in sediments lacking vegetation and bacteria closely associated with roots of marsh plants[16]. Rates of bacterial fixation in each of the marsh habitats were measured by incubating cores of sediment for 24 h in a gas mixture of 86% nitrogen, 10% acetylene, 4% oxygen. We then reflushed the headspace above the core with gas of a similar composition and measured ethylene production 24 h later by gas chromatography[17].

Low marsh was the major site of bacterial fixation due to the higher rates (100–500 ng N cm^{-2} h^{-1} during the warm part of the year) and larger area involved (Fig. 2). High marsh had lower rates (50–150 ng N cm^{-2} h^{-1}) and smaller area. Fixation peaked in the vegetated habitats during spring and early autumn, when roots were actively growing or senescence was taking place. Perhaps root exudates are most abundant at those times. Fixation rates in creek bottoms were considerably lower (maximum of 80 and 20 ng N cm^{-2} h^{-1} for muddy and sandy creeks) than in those recorded in sediments supporting plants and peak fixation occurred in midsummer (Fig. 2). Nitrogen fixation totalled 2,980 kg yr^{-1}.

Table 1 Nitrogen budget for Great Sippewissett Marsh (kg yr^{-1})

Processes	Input		Output	Net exchange
Precipitation		380		380
NO_3—N	110			
NO_2—N	0.4			
NH_4—N	70			
DON	190			
Particulate N	15			
Groundwater flow		6,120		6,120
NO_3—N	2,920			
NO_2—N	30			
NH_4—N	460			
DON	2,710			
N_2 fixation		3,280		3,280
Algal	297			
Rhizosphere bacteria	2,595			
Non-rhizosphere bacteria	384			
Tidal water exchange		26,200	31,600	−5,350
NO_3—N	390	1,210		
NO_2—N	150	170		
NH_4—N	2,620	3,540		
DON	16,300	18,500		
Particulate N	6,740	8,200		
Denitrification			4,120 (2,820)	−6,940
Sedimentation			1,295	−1,295
Volatilisation of NH_3			17	−17
Deposition of bird faeces		9		9
Shellfish harvest			9	−9
Totals		35,990	39,860	−3,870

Totals for each process are offset to the right. Losses from the marsh to Buzzards Bay are shown as negative numbers in the net exchange column. The amount of nitrogen added to the denitrification value is not equal to the amount of nitrogen fixed because net denitrification was not active in the blue green algal mats. Some of the values in this table and in the text may not agree with those of refs. 12, 16, 18, 20. The entries here were computed using corrected areas for the habitats and supersede earlier estimates.

Nitrogen fixation by algae. Algal fixation was measured for each habitat by an adaptation of the acetylene reduction technique[18]. Although pannes and algal mats had the highest fixation rates (up to 500–600 ng N cm^{-2} h^{-1}), the area of these habitats was so small that their contribution of N to the total marsh was small. Low marsh and creek bottoms showed lower rates[18] but their larger areas resulted in a greater contribution to the nitrogen economy of the marsh. The total nitrogen introduced into the marsh by algal fixation was 297 kg yr^{-1}, an order of magnitude smaller than that contributed by bacterial fixation.

Tidal exchange. The flow of seawater and nitrogenous nutrients through the mouth of Sippewissett Creek were measured during entire tidal cycles about monthly throughout the year. Water movement in and out of the marsh was measured with mechanical flow meters throughout each tidal cycle[12]. Tidal water was sampled hourly during each cycle and the concentrations of dissolved and particulate nutrients were determined. With these data and knowledge of the cross-sectional area and tidal height of Sippewissett Creek, water flux and nitrogenous nutrients entering and leaving the marsh during each hour of a tidal cycle were calculated. We summed over the tidal cycle to obtain net exchange (Fig. 3*a*).

Ammonium was the principal form of dissolved inorganic nitrogen (DIN), averaging about 1 μmol NH_4—N l^{-1} in winter and spring and 2–6 μmol NH_4—N l^{-1} in summer and autumn. For the first half of the year there were only trivial exchanges of

ammonium between the marsh and Buzzards Bay (Fig. 3*a*). From June to July there was a substantial import of ammonium into the marsh. This took place while the vegetation was actively growing and setting seed. The growth rate of *S. alterniflora* in Great Sippewissett Marsh is such that this species by itself consumes more than 39 kg per day of NH_4—N. During the same time of year about 9 kg per day of NO^-_3 and NH^+_4 nitrogen are brought into the marsh daily by groundwater (Fig. 3*b*). The tidal import of DIN into the marsh (Fig. 3*b*) was 8 kg per day, making a daily total of 17 kg DIN per day, a rate lower than the 39 kg per day used by plants during their period of active growth.

Towards the end of August there was a marked export of ammonium into Buzzards Bay (Fig. 3*a*), related to the maturity and senescence of marsh vegetation. The peak export of ammonium from the marsh was after the plants had matured and began flowering (Fig. 4); mature and senescent plants are most subject to leaching[19]. In a salt marsh with twice daily submergence in a good electrolyte, leaching should be important. Measurements of leaching of NH_4—N from marsh grasses show that such losses peak in August and that the standing crop of marsh grasses can release about 7 kg N per day through leaching (unpublished results).

In late summer the DIN conveyed into the marsh by groundwater (about 8 kg N per day), is no longer intercepted by the marsh ecosystem. Adding this amount of groundwater DIN to the 7 kg N per day leached from plants, a value (15 kg N per day) that is near the observed export of DIN (roughly 12 kg N per day) from the marsh to the Bay in late August and September is obtained (Fig. 3*b*). Perhaps additional ammonium is released by anaerobic respiration of plant exudates. In August the sediments become markedly more anoxic (B. Howes, personal communication). We are investigating the relationship between root activity and redox condition but as yet are unable to estimate the contribution of this mechanism to tidal export of DIN.

Nitrite is found in low concentration (up to 0.2 μg—at NO_2—N l^{-1}) in marsh water and it is difficult to know if the fluctuations seen in Fig. 3 (top) are of significance. Nitrate concentrations were higher (averaging up to 1 μmol l^{-1}), with net exports in autumn and winter (Fig. 3*a*).

Figure 3*b* shows the seasonal changes of total dissolved inorganic nitrogen. The marsh intercepts most of the groundwater DIN (mostly nitrate) during winter and spring. This may be due to the conversion of nitrate dissolved in the water into nitrogen gas by denitrifying organisms, described later. During midsummer the marsh imports DIN, as the grasses take up ammonium during maximum growth. With the onset of senescence, plant leaching and decay lead to export of DIN. The coming of winter slows all these processes. Throughout most of the year the marsh intercepts DIN (principally nitrate) that otherwise would flow into deeper waters.

Denitrification. In low and high marsh—habitats where plant roots consolidate sediments—rates of denitrification were measured using a bell jar method. Darkened glass bell jars were pushed about 20 cm into the sediment and the headspace was flushed with helium. Argon was injected into the headspace as an internal standard. After a 4-h incubation, samples of the gas in the headspace were removed and the ratio of nitrogen-to-argon was measured by gas chromatography.

The denitrification rates of the wet flocculent sediment of creek bottoms and pannes were assayed using a gas-partitioning technique. Samples of the sediment were placed in serum vials and flushed with helium. After incubation for 24 h the gas in the sediment was stripped onto the headspace by shaking. The gases were then sampled and analysed by gas chromatography[20].

The rates of denitrification were measured monthly at each of the habitats (Fig. 2). Due to the higher rates (maximum of 5 mg N m^{-2} h^{-1}) and large area of creek bottoms[20], this habitat was the site of over 50% of the net denitrification for the marsh. The low marsh had peak denitrification rates of 2–3 mg N m^{-2} h^{-1}, while the high marsh rates were only ~1 mg N m^{-2} h^{-1}. The rates in the pannes reached over 5 mg N m^{-2} h^{-1} but their small surface area did not make a large contribution to losses of N from the marsh as a whole. In the algal mats the high fixation rates were matched by high rates of denitrification[20]. In all habitats there was a strong seasonal pattern associated with the annual temperature cycle.

Both techniques used to measure denitrification yield results that represent the difference between true denitrification rates and simultaneous nitrogen fixation within the sediment. In Table 1 we added the measured amounts of nitrogen fixed to the amount of nitrogen lost by denitrification to obtain the total amount denitrified. This rate of total denitrification shows that low marsh and creek bottoms are the habitats where denitrifiers are most active and contributed the most to losses of N by denitrification (Fig. 2). The other habitats are of minor importance and the whole marsh exports about 6,940 kg N yr^{-1} as nitrogen gas.

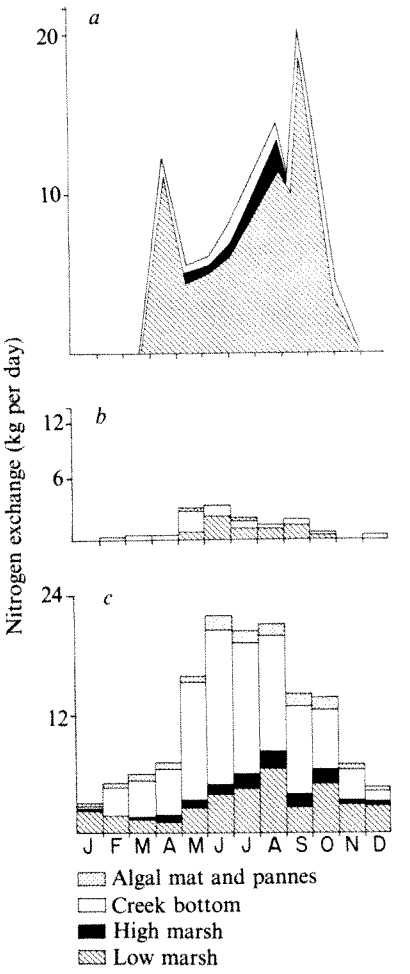


Fig. 2 Nitrogen exchanged by microbial processes in Great Sippewissett Marsh. Each major habitat is shown separately. Values are the products of rates × areas of each of the habitats. *a*, Bacterial N_2 fixation; *b*, algal N_2 fixation; *c*, denitrification.

Nitrate is not exported during the spring and summer. This is the time of year when rates of denitrification are highest, suggesting that denitrifiers are active enough to substantially alter the chemistry of tidal water.

Losses to sediments. The rate of accretion of sediments was obtained from measurements made for marshes near Great Sippewissett[21,22]. For well-established vegetated zones, we used a value of 0.15 cm yr^{-1} as the rate of sediment accumulation. In sediments of Great Sippewissett Marsh there is an average of

1.4% N and the specific gravity of 0.2 g dry weight cm^{-3}. We estimate that in the area stabilised by the growth of vegetation (302,900 m^2), 1295 kg N yr^{-1} is lost to the sediments. Although the loss of nitrogen to sediments is substantial relative to other inputs and outputs (Table 1), the amount of nitrogen sedimented is only about 1% of the estimated 116,800 kg N stored in the top 15 cm of marsh sediments. The bulk of the roots of marsh plants are found above a depth of 15 cm (ref. 23) and therefore any nitrogen found above 15 cm could be potentially recirculated by plant uptake. We are concerned here with losses that occur when nitrogen is left below a depth of 15–20 cm as the marsh peat accumulates.

Minor sources and losses. Our value for the amount of nitrogen lost from Great Sippewissett Marsh through volatilisation of NH_3 is based on rates measured in North Carolina[24]. At the range of *p*H values (5–8) measured in our marsh sediments, volatilisation would be small (Table 1).

Flocks of gulls use the marsh as a roost. As they feed largely elsewhere, we consider their defaecation to be an input of nitrogen. We calculated this small input (Table 1) by noting the average number of birds in the marsh, the average defaecations per unit time and the weight and per cent of nitrogen in the faeces.

There is active shellfishing in Great Sippewissett Marsh. The removal of nitrogen by this pathway was estimated by using the records kept by the shellfish warden and the per cent nitrogen in the shellfish meats. This is also a small figure (Table 1).

The balance sheets

Table 1 summarises annual nitrogen transport. The sum of the inputs is remarkably close to the sum of the outputs (only about 11% difference). This balance would be required for long-term persistence of an ecosystem in its present state.

Although adequate replication and estimates of variation for each process are available[12,16,18,20], as only one marsh was studied the variability for each entry in Table 1 cannot be easily estimated. There is the further problem that even very small errors in, for example, rates of flow of tidal water may be more important than larger errors in measurements of nutrient concentrations. This is inherent in the arithmetic used in making the estimates of Table 1. From our experience and by pooling estimates of variation from the data available[12,16,18,20], we believe that the coefficient of variation of each entry of Table 1 would be about 20% of the mean. Thus, the small 11% variation between inputs and outputs of Table 1 is fortuitous. This agreement suggests that major parts of the nitrogen budget have been accounted for.

Does the marsh have much flexibility to adjust to increased eutrophication of ground and tidal waters? Of the total nitrogen budget of Great Sippewissett Marsh, 91% of the inputs and 83% of the outputs were driven by physical forces which might indicate little room for adjustment. However, if the refractory (see below) dissolved organic nitrogen is not considered, less than 50% of the exchanges are purely physical and the possibility of considerable biological adjustment may be indicated. As dissolved ammonium increases, nitrogen fixation is sharply curtailed[25]. Fixation amounts to 9 or 20% of the input, again depending on whether or not the DON is considered an inert fraction of the nitrogen budget. In either case this biological feedback system can make only a relatively small change in the system's ability to handle increased nitrogen inputs. Perhaps increased rates of denitrification with increased supply of nitrogen can also serve as a feedback mechanism for adjusting nitrogen flow through the marsh. Our evidence is not conclusive[26], although others have shown increased denitrification after nitrate enrichment[27]. To be effective, increases would have to be large and our rates are already among the higher values reported[20]. Salt marsh sediments show very large rates of sulphate reduction[28]. As nitrate is energetically more favourable as an electron acceptor than sulphate, large increases in nitrate reduction are possible if new supplies of nitrate are made available.

Internal mechanisms, nevertheless, are unlikely to regulate the amount of nitrogen entering salt marshes. Eutrophication of entering fresh and tidal waters will probably result in an increase of the nitrogen pool within the marsh, especially in sediments and vegetation. This will to some extent enhance primary production in salt marshes[2–5]. Human contamination affects principally ground and tidal water, the two largest processes providing nitrogen to the marsh. Of the two, contamination of groundwater may be more important as flow is one way into the marsh and, at least in North America, disposal of sewerage wastes is conducted primarily by leaching wastewater through cesspools and septic tanks into the soil. This may result in enhanced concentrations of nutrients, particularly nitrate, in groundwater throughout the coastal zone.

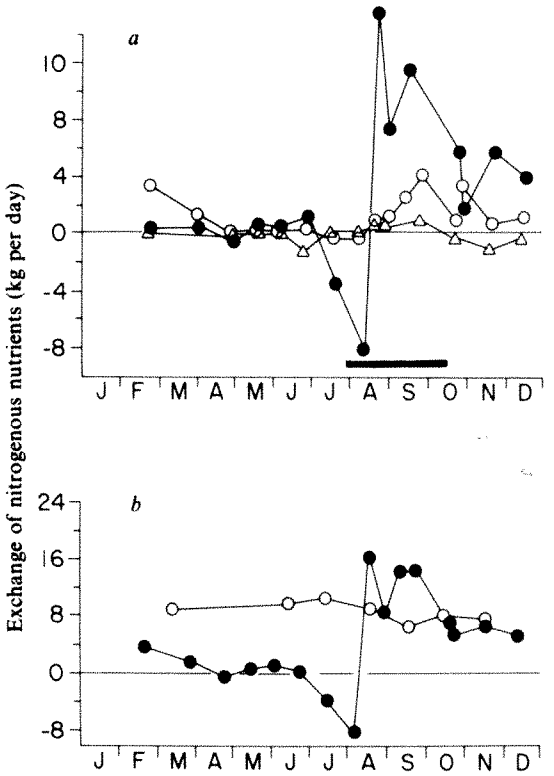


Fig. 3 Exchanges of nitrogenous nutrients between Great Sippewissett Marsh and Buzzards Bay through the year. In *a*, points are net exchanges for a given tidal cycle and recalculated in units of kg per day. ●, NH_4—N; ○, NO_3—N; △, NO_2—N. The solid bar shows the flowering season for *Spartina alterniflora*. *b*, Compares net tidal exchanges of dissolved inorganic nitrogen (●) with the dissolved inorganic nitrogen furnished by groundwater (○) flowing into the marsh. Values in both graphs above the 0 in the *y* intercept are net exports from Great Sippewissett Marsh, while negative values show an import into the marsh.

A better view of the action of a salt marsh ecosystem as a transformer of chemical forms of nitrogen can be obtained by rearranging the entries of the nitrogen budget. The annual exchanges of the major forms of nitrogen for Great Sippewissett Marsh are shown in Table 2. Sixty-four percent of the nitrogen entering the marsh as nitrate is intercepted by the marsh. The oxidised nitrogen imported into the marsh is reduced to various forms within the marsh. Ultimately most of the entering nitrogen is exported by tidal water in the form of particulate, ammonium and molecular nitrogen.

These exports are substantial. Export of dissolved inorganic nitrogen from the area of marshland around Buzzards Bay at a rate comparable to that found in Great Sippewissett Marsh in

Table 2 Annual exchanges of major forms of nitrogen for Great Sippewissett Marsh (kg yr^{-1})

Form of N	Input	Output	Net	% of input
NO_3—N	3,420	1,220	2,200	64
NH_4—N	3,150	3,550	−400	−13
DON	19,200	18,500	700	4
Particulate N	6,750	8,200	−1,460	−22
N_2	3,280	6,940	−3,660	−112
Totals	35,800	38,410	−2,610	−7

The exchanges of nitrite and the nitrogen lost to the sediments are not included.

August could entirely replace the nitrate content of Buzzards Bay in 100 days[12]. The amount of particulate organic matter exported yearly from the marsh is equivalent to about 40% of the net annual above ground production of the marsh[12]. As salt marshes are among the most productive ecosystems on a per unit area basis, these amounts exported are quantitatively significant and must be important to detritus feeders in coastal waters.

As annual denitrification rates exceed fixation, there are large losses of nitrogen from the marsh as N_2 gas. This is the second major pathway of nitrogen export.

The large amounts of dissolved organic nitrogen (Tables 1, 2) are mostly unaffected by passage through the marsh. This agrees with other results that suggest that the bulk of DON is refractory to microbial attack[29], although DON is the principal form of nitrogen in terms of total amounts.

Approximately 6,940 kg of nitrate nitrogen are required to supply the marsh denitrification annually. This is a larger amount than is available through the net import of nitrate (2,200 kg yr^{-1}, Table 2). This means that some nitrification of ammonium must take place within the sediments.

The amount of ammonium entering the marsh is composed of three sources; (1) net exchange by tides, groundwater and rain (1,450 kg N yr^{-1}); (2) the small amount of mineralisation of DON (700 kg N yr^{-1}); and (3) the amount fixed by microorganisms (3,280 kg N yr^{-1}). The total of these three sources is 5,430 kg N yr^{-1}. After allowing 1,460 kg N yr^{-1} for export of particulates and 1,295 kg N yr^{-1} for losses to sediments, only 2,675 kg N yr^{-1} are available for use within the marsh. Yet we estimate that growth of marsh plants must use about 8,800 kg N yr^{-1}. Thus, it is clear that very active uptake, release, conversion and recycling of nitrogen must take place within the marsh.

If the salt marsh were not present, Buzzards Bay would receive the nitrate from the groundwater and the phytoplankton would be the principal part of the Bay's ecosystem that would be affected. The marsh intercepts much of this nitrate and exports primarily particulate nitrogen to the sea. This export principally affects the animals that feed on particles, a very different component of the coastal food web. These interactions between marsh and bay maintain the structure of benthic and fish communities in the bay. Similarly, the flow of groundwater structures the marsh biota by providing dissolved inorganic nitrogen for plants and denitrifiers.

These results demonstrate that there are significant interactions among units of landscape, in our case nutrient exchanges among upland, marsh and coastal waters. In view of their importance it is obvious that these interactions among ecosystems have not received enough attention. Many aspects of the structure of an ecosystem, such as what plants, animals or microbes are present, and how many of each kind, are strongly affected by nutrients delivered from outside the particular ecosystem.

Conclusions

Our findings show that there are significant exchanges of nutrients between upland, marsh and coastal waters, and these interactions affect the structure and composition of these systems.

Groundwater often imports more N into salt marshes than does precipitation. Contamination of coastal zone groundwater by sewage is probably important to the marsh nitrogen economy. However, tidal water causes a net export of all nutrients studied, especially ammonium and particulate nitrogen. Quantitatively, tidal exchange is the most important nitrogen transport mechanism. Ammonium export seems to be linked to the activity of marsh grasses, and uptake and leaching are important mechanisms. Nitrogen fixation by bacteria is larger than that by blue-green algae and most bacterial fixation is associated with the roots of marsh grasses.

Dissolved organic nitrogen is the major form of N in the water but little of this is available to decomposers. Despite this rates of denitrification are high. Denitrification may be responsible for the interception by the marsh of the nitrate transported by the flow of groundwater. Annual denitrification for the entire marsh exceeds nitrogen fixed, causing a net export of N_2 to the atmosphere.

Loss of nitrogen to deeper sediments is significant, but this is small in proportion to the nitrogen pool found in the surface sediments where plant roots are found.

The amount of dissolved inorganic nitrogen required to satisfy growth of higher plants and supply the nitrate for denitrification is larger than imports of DIN, which suggests that nitrification, nitrogen fixation and active recycling of N are all important within the marsh. Nitrogen export by the marsh is also important for coastal waters and the transformation of oxidised imports to reduced exports is significant to the coastal ecosystems that receive marsh exports.

Altogether, the imports and exports of nitrogen are in balance. This, along with other evidence, suggests that an old salt marsh such as that studied is in a long-term steady state. Young marshes may be traps for sediment and particulate nutrients suspended in tidal water, but this becomes less marked as the marsh develops, with mature marshes exporting particulates.

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Phenotype stabilisation and integration of transferred material in chromosome-mediated gene transfer

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The human genes for thymidine kinase, galactokinase and procollagen type I, located on chromosome 17, have been transferred to cultured mouse cells. In unstable cell lines the transferred genetic material is shown to exist independently, whereas in stable cell lines the transferred material has become associated with chromosomes of the recipient cell. The size of the transferred genetic material exceeds 1% of the human haploid genome in some of the transformed cell lines. In addition, the data indicate a gene order of: centromere–galactokinase–(thymidine kinase, procollagen type I), on the long arm of human chromosome 17.

PURIFIED metaphase chromosomes have been used as vectors for the transfer of genetic information between cultured mammalian cells[1-7]. This process, referred to as chromosome-mediated gene transfer (CMGT), typically results in the transfer of subchromosomal amounts of genetic material[2-4]. Expression of the transferred genetic material (transgenome) can be either stable or unstable following return of gene transfer cell lines (transformants) to non-selective culture conditions[1,3,5-7] (for detailed reviews of the CMGT process see refs 8, 9).

To examine the nature of the CMGT process, we have transferred the human chromosome 17 gene thymidine kinase (TK, EC 2.7.1.75) to cultured mouse cells. In some instances co-transfer of the syntenic genes galactokinase[10] (GK, EC 2.7.1.6) and type I procollagen (PCI)[11] was detected. This three-gene segment has provided a means of defining the length of a transgenome and facilitated examination of the fate of the transgenome. In addition, we present data concerning the order of these three genes and assess the usefulness of CMGT as an intergenic mapping procedure. We have also observed donor chromosome fragments and co-transfer of asyntenic genes in some transformants.

Generation and characterisation of primary transformants

Human chromosomes isolated from HeLa S3 (TK^+) cells were applied to the mouse cell line LM(TK^-) which contains a non-reverting ($<10^{-9}$) mutant allele of TK. The CMGT procedure used[3] produced transformed colonies at an overall frequency of 2.3×10^{-7} per recipient cell (Table 1). In control experiments without donor chromosomes no colonies were observed.

Seven independent cell lines, designated TGT, were isolated from two transfer experiments. Two of the transformants, 2TGT2 and 2TGT4, were found to express the human form of GK (Table 2). Human PC I production was assayed by Ouchterlony double immunodiffusion using an antibody to the purified human protein[11]. Of the seven cell lines examined, only 2TGT4 expressed human PC I.

The stability of the transferred phenotype was assayed by cultivating the transformants in non-selective medium and periodically determining the proportion of cells able to form colonies in hypoxanthine, aminopterin and thymidine (HAT) selective medium (see Fig. 2 legend). Transformants TGT1, 2TGT1, 2TGT2 and TGT3 expressed the TK^+ phenotype stably, that is, >90% of the cells remained HAT resistant after 30 d in non-selective medium. Transformants 2TGT4 and 2TGT5 were unstable, losing the HAT-resistant phenotype with first order kinetics at a rate of 2.5% per cell generation. The remaining transformant, TGT2, seemed to be a mixture of stable and unstable cells.

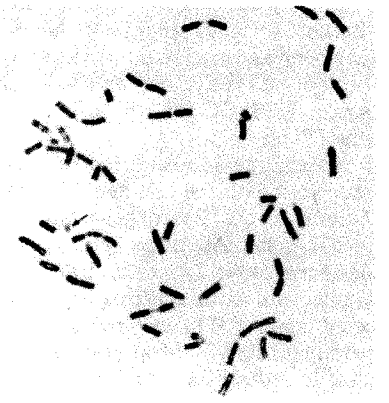


Fig. 1 Alkaline Giemsa[12] stained metaphase spread of transformant 2TGT4 containing an independent human chromosome fragment (arrow). Human chromosomal material appears lightly stained, whereas mouse chromosomes stain darkly, with the exception of their centromeric regions (lightly stained). Twenty metaphase spreads were observed and photographed for each cell line analysed.

Karyotypic analyses of the transformants were carried out using the alkaline Giemsa staining technique[12]. This method produces a differential staining of rodent and human chromosomes and is effective in detecting small amounts of human chromosomal material in a predominantly mouse background. Of the seven transformed cell lines, two possessed detectable human chromosome fragments. Transformant TGT2 contained a large human fragment that was translocated to a

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Table 1 Summary of TK transfer experiments

Expt	No. of LM(TK⁻) recipient cells	Cell equiv. of HeLa chromosomes	No. of flasks with colonies	Transfer frequency
1	2×10^7	3×10^7	2/20	1×10^{-7}
2	1×10^7	1×10^7	5/10	5×10^{-7}
3	2×10^7	0×10^7	0/20	0

Mitotic HeLa cells were obtained by treating cultures with 5 atm. nitrous oxide for 18 h. Chromosomes were isolated and applied to LM(TK⁻) cells according to the method of Willecke and Ruddle[3]. Following incubation, recipient cells were diluted with minimal essential medium (αMEM) plus 10% fetal calf serum, and 1×10^6 cells were plated per 75-cm² Corning culture flask. HAT selection was begun 24 h later[24]. Transformed colonies appeared after 2–3 weeks and were expanded for further analysis. All cell lines were negative for mycoplasma contamination by the culture method of Hayflick as modified by Barile *et al.*[25]. For the calculation of transfer frequency, each flask containing colonies was considered as one event, irrespective of the number of colonies present.

mouse chromosome. Transformant 2TGT4 contained an independent human fragment (Fig. 1). This fragment was similar in size to the long arm of human chromosome 17 and possessed a terminally constricted centromere-like region. G-banded chromosome preparations[13] revealed that this chromosome fragment possessed the banding pattern characteristic of the long arm of human chromosome 17. These observations of cytologically detectable chromosome fragments confirm previous work from our laboratory[14]. It now seems that there is a range of transgenome sizes, the largest exceeding 1% of the human haploid genome.

Co-transfer of asyntenic genes

The transformed cell lines were analysed for the expression of 20 additional human isozymes assigned to 16 different human chromosomes[15,16]. Five of the seven transformants were negative for all human isozymes examined. One cell line, TGT2, expressed the human form of adenylate kinase (AK1, EC 2.7.4.3), which has been assigned to human chromosome 9, and another transformant, 2TGT1, expressed human mannose phosphate isomerase (MPI, EC 5.3.1.8), a human chromosome 15 marker enzyme (Table 2).

Two types of experiments indicate that the asyntenic genes have become associated with the selected TK gene. First, nine independent subclones of TGT2 were generated under selective pressure for the TK gene. All these subclones continued to express the human form of AK1. Second, transformed cell lines which had co-transferred both syntenic and asyntenic genes were back-selected against TK by growth in medium with 5-bromodeoxyuridine (Table 2). Mass populations of surviving cells were analysed to reduce the effect of spurious dual segregation events. Back-selectants of transformants 2TGT2 and 2TGT4 lost the linked human chromosome 17 genes concomitant with TK. Similarly, back-selectants of 2TGT1 and TGT2 lost the natively asyntenic genes MPI and AK1. Karyotypic analysis of back-selectants derived from lines containing human chromosome fragments demonstrated loss of the fragments coincident with loss of the transferred markers.

There are at least two mechanisms which could result in the transfer and association of asyntenic genes with selected genes. The HeLa cell line used as the chromosome donor is typical of continuous tissue culture lines in possessing some chromosome rearrangements. Rearrangements involving chromosomes 17, 9 and 15 could have created artificial synteny groups in the donor cell line which were subsequently picked up in the transfer experiments. Alternatively, the CMGT process could cause co-transfer of genuinely asyntenic genes. Transfer of unlinked markers has been observed in bacterial transformation experiments (congression)[17]. Such results are due to the cell's ability to ingest and express more than one piece of DNA. Mammalian cells are capable of ingesting more than one chromosome in CMGT conditions[18]. Uptake of more than one chromosome followed by partial degradation and reconstruction could explain co-transfer of asyntenic genes.

In other CMGT experiments we have observed five additional transformants expressing normally asyntenic genes (our unpublished results with Miller and Church). The first mechanism proposed would require extensive rearrangement of the HeLa karyotype to explain these results. As the HeLa cell line is basically triploid with a minimal number of rearrangements[19], we favour the second hypothesis. A rigorous test of the hypothesis will require transfer experiments using a primary cell line with a normal diploid karyotype as the chromosome donor. Such experiments are in progress.

The unstable transgenome and its conversion to stability

Various experiments have indicated that the transgenome in stable transformants has become associated with chromosomes

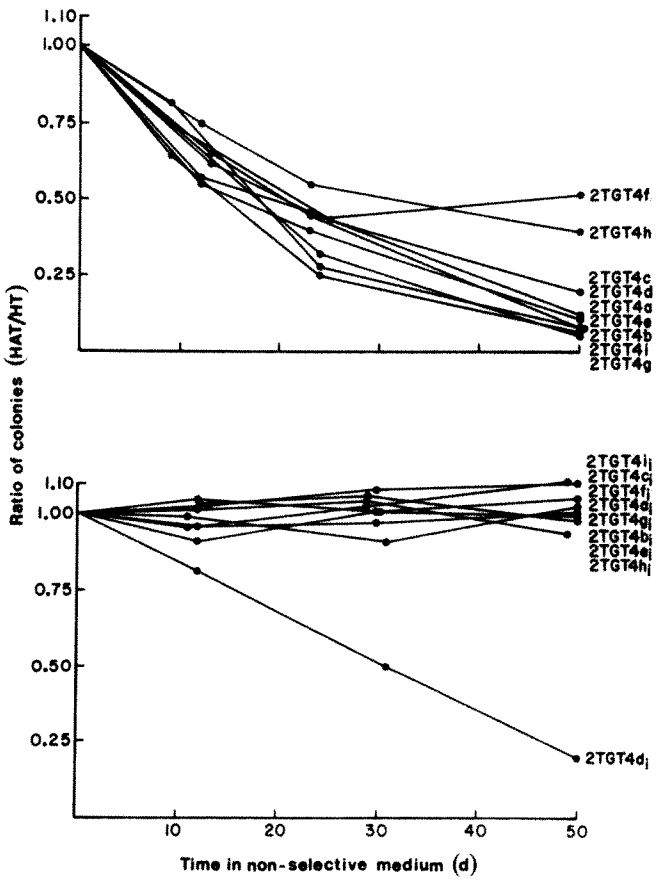


Fig. 2 Stability analyses of subclones of the 2TGT4 transformed cell line. *a*, Stability analyses of nine primary subclones, 2TGT4a–2TGT4i, of transformant 2TGT4. *b*, Second stability analyses of the nine subclones following their growth in non-selective medium for 75 d, and an additional period of growth with HAT-selective medium for 14 d. Eight of the nine subclones now stably express the transferred phenotype. Stability analyses were carried out by culturing transformed cell lines in non-selective medium for at least 30 d. At various times, 200 cells were plated in duplicate flasks with either HAT-selective medium or non-selective HT medium (medium supplemented with 0.1 mM hypoxanthine and 0.016 mM thymidine). After 9–10 d the resulting colonies were stained with 0.25% Wright's stain in methanol and counted. The number of colonies formed in HAT as against HT medium is taken as a measure of the fraction of cells retaining the transferred (TK⁺) phenotype.

of the recipient cell[20–23]. The precise nature of this association remains unclear. Even less is known about the state of the transgenome in unstable transformants. Prolonged culture in selective medium, however, often results in conversion from unstable to stable expression[5,7]. It has been postulated that stabilisation occurs infrequently as a unique event in a single cell. The resulting stable cell would thus acquire a growth advantage while in selective medium due to its ability to distribute the transgenome efficiently to daughter cells. Stable cells would then gradually overgrow the unstable cells, giving rise to a completely stable population.

Transformant 2TGT4, which expresses all three human chromosome 17 genes and contains a detectable human chromosome fragment, was chosen for investigating the nature of the unstable transgenome and its conversion to stability. Nine independent subclones (2TGT4a to 2TGT4i) of transformant 2TGT4 were derived and analysed for stability (Fig. 2). Six of the subclones were completely unstable, as more than 90% of the cells lost the HAT-resistance phenotype after 50 d of growth on non-selective medium. Subclones 2TGT4c, 2TGT4f and 2TGT4h did not meet this criterion for unstable expression and will be discussed separately. Each of the six unstable subclones continued to express human GK and PC I. Karyotypic analyses of each of these cell lines revealed a single human chromosome fragment indistinguishable from the fragment present in the 2TGT4 cell line. Thus, the unstable transgenome is an independent and constant entity, losing no genetic information and undergoing no gross structural changes.

Table 2 Summary of TK transformed cell lines

Cell line	TK	GK	PC I	AK1	MPI	Stability	Detectable human chromosomal material
Primary transformants:							
TGT1	+	−	−	−	−	S	−
TGT2	+	−	−	+	−	Mixed	+
2TGT1	+	−	−	−	+	S	−
2TGT2	+	+	−	−	−	S	−
2TGT3	+	−	−	−	−	S	−
2TGT4	+	+	+	−	−	U	+
2TGT5	+	−	−	−	−	U	−
Back-selected transformants:							
TGT2BS	−	ND	ND	−	−	ND	−
2TGT1BS	−	ND	ND	−	−	ND	ND
2TGT2BS	−	−	ND	ND	ND	ND	ND
2TGT4BS	−	−	−	ND	ND	ND	−

Human PC I expression was assayed by Ouchterlony double immunodiffusion using an antibody raised against purified human type I procollagen[11]. Human GK activity was detected by the starch gel electrophoretic method of Nichols *et al.*[26]. In addition to human AK1 and MPI, all transformants were assayed for the expression of 18 other human isozymes[15]. Back selection of primary transformed cell lines was carried out by growing cells in 30 $\mu g\ ml^{-1}$ of 5-bromodeoxyuridine for 24 h. Culture medium was then removed and replaced with phosphate-buffered saline and the cultures exposed to long-wave UV light for 30 min. The cells were returned to growth in culture medium with 5-bromodeoxyuridine, and mass populations of surviving cells were analysed as back-selectants. +, Expression of human phenotype; −, expression of human phenotype not detected; U, unstable expression of transferred phenotype; S, stable expression of transferred phenotype; ND, not determined.

To study the conversion to stability, we isolated stable derivatives of the 2TGT4 subclones. Each of the nine subclones was grown on non-selective medium for 75 d to remove most unstable HAT-resistant cells from the population, and then returned to HAT-selective medium for 14 d to select and fix any pre-existing, small subpopulation of stable cells. The cell populations carried through this regime (designated 2TGT4a_i to 2TGT4i_i) were again analysed for stability (Fig. 2). All the cell lines, with the exception of 2TGT4d_i, then expressed the transferred phenotype stably. Although all eight stable subclones continued to express human PC I, three of the cell lines no longer expressed human GK (Fig. 3). Karyotypic examination revealed that the human chromosome fragment had become associated with a mouse chromosome in all the stable subclones (Fig. 3). In each subclone the fragment was associated with a single mouse chromosome, but the mouse chromosome involved differed between cell lines. The rearrangements appeared either as centric fusions or terminal additions. When metaphase spreads were stained with the fluorescent dye, Hoechst 33258, each of the centric fusion type chromosomes had the brightly fluorescent centromeric region characteristic of mouse centromeric heterochromatin[13]. Neither the independent human fragment in transformant 2TGT4 nor any of its unstable subclones displayed centromeric fluorescence. These observations indicate that the mouse centromeric region has been retained during stabilisation.

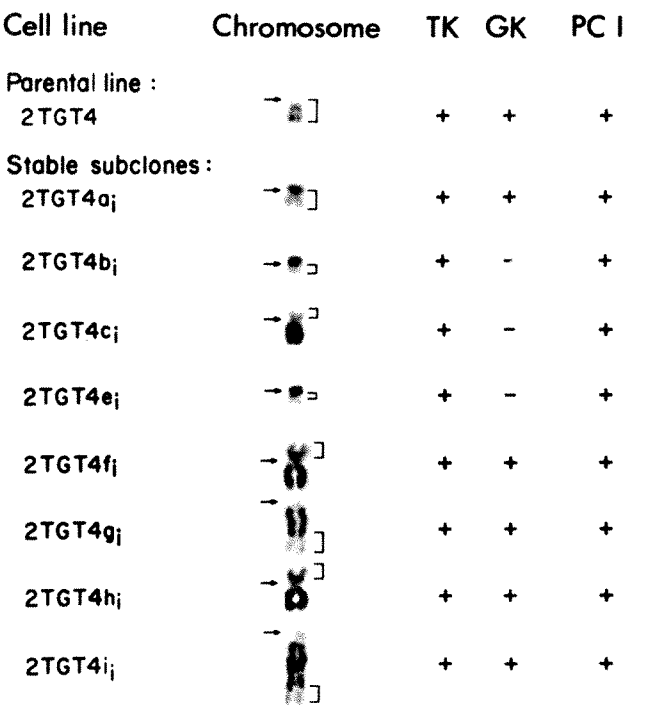


Fig. 3 Expression of human chromosome 17 genes and fate of the human chromosome fragment in the stabilised subclones of transformant 2TGT4. In each case the lightly stained human fragment, indicated by the bracket, has become associated with a darkly stained mouse chromosome. The human chromosome fragment has apparently been translocated to either a mouse chromosome's centromeric region (2TGT4a_i, 2TGT4b_i, 2TGT4c_i, 2TGT4e_i, 2TGT4f_i and 2TGT4h_i) or to the distal portion of a mouse chromosome (2TGT4g_i and 2TGT4i_i). Centromeric regions are identified by the arrows.

Finally, if stabilisation originates in a single cell which then gradually overgrows the population, it should be possible to find cell populations which transiently contain both stable and unstable cells. During a stability test a mixed population would be expected to display a plateau at the frequency of stable cells in the population. The cell lines 2TGT4c, 2TGT4f and 2TGT4h seem to be mixed populations, as they displayed plateaus at 16%, 40% and 50% HAT-resistant cells, respectively (Fig. 2). Karyotypic analysis of these subclones supported this hypothesis. Two types of human chromosome fragment were observed in each of these cell lines. One subset of cells contained the independent human fragment, and a second subset contained the human fragment translocated to a mouse chromosome. The frequency of cells containing the translocated chromosome was similar to the stability test plateau frequency for each cell line. Translocation chromosomes were found in

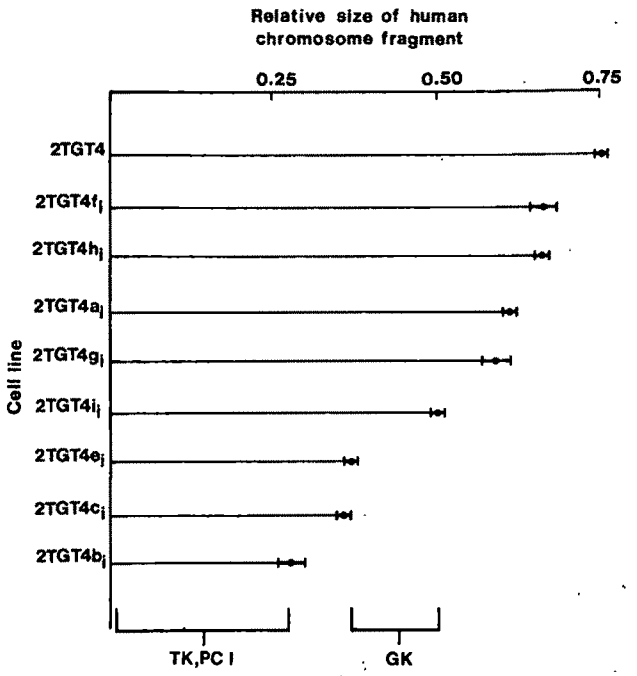


Fig. 4 Histogram displaying the sizes of the human fragments present in the transformed cell line 2TGT4 and its eight stabilised subclones. Relative size values were determined by measuring both the human chromosome fragment and the long arm of a mouse marker chromosome in each metaphase spread. By taking the ratio of the size of the human fragment to the size of the marker chromosome a relative size value was obtained which corrects for variable condensation between metaphase spreads. An average size value was obtained from measurement of at least five metaphase spreads for each cell line. The bars represent ±1 s.e.m. The regions containing the genes for TK, GK and PC I are indicated at the base of the histogram (see text).

10%, 25% and 35% of the cells of transformants 2TGT4c, 2TGT4f and 2TGT4h, respectively.

These experiments provide a visual record of the stabilisation process. The association of the transgenome with recipient cell chromosomes may be viewed as the acquisition of host centromeric activity, ensuring the orderly distribution of transferred genes at mitosis. Stabilisation does not involve the homologous genetic site in the recipient, as the transgenome is shown to associate with several recipient cell chromosomes. These results agree with those of previous studies[22,23]. The earlier studies, however, did not rule out the possibility of multiple sites of association within a single cell. We have shown here that each stable cell contains only one copy of the transgenome. Moreover, the fact that each stabilised subclone has only one type of chromosome associated with the transgenome, as well as the observation of mixed populations with regard to stability, argues that stabilisation originates in a single cell which then overgrows the population.

Genetic mapping by CMGT

Following stabilisation, three of the 2TGT4 subclones did not express human GK (Fig. 3). Moreover, the length of the human fragment decreased following translocation and was correlated with the number of human genes retained (Fig. 4): the largest fragments were in cell lines retaining all three human chromosome 17 genes, and the smallest were in cell lines retaining only TK and PC I. To examine the nature of the breakage during stabilisation, chromosome preparations were examined by G-banding[13]. All stable transformants, except 2TGT4g$_i$ and 2TGT4i$_i$ which could not be examined by G-banding due to the similarity of the translocation chromosome to other mouse chromosomes, retained the darkly staining terminal band (17q22–17q25) and various amounts of the remainder of the long arm of human chromosome 17. These results suggest that breaks have occurred near the centromere-like end of the fragment during stabilisation. If we assume that stabilisation involves a single break and fusion of the fragment, and not a complex rearrangement, a deletion map is generated. The map indicates a gene order of centromere–GK–(TK, PC I) (Fig. 4). The data do not allow unambiguous determination of the order of TK and PC I, but failure to segregate PC I suggests that it is distal to TK.

Deletion mapping following stabilisation of visible chromosome fragments provides a means of deriving qualitative intergenic mapping data. Alternatively, the co-transfer frequency of non-selected genes in primary transformants may be a source of quantitative intergenic mapping data[5,6,9,14]. Although the number of cell lines analysed here is too small to assess directly the usefulness of CMGT in this regard, two observations indicate that only primary unstable transformants should be used to determine a reproducible co-transfer frequency. First, stabilisation coincides with a decreased transgenome size. The co-transfer frequency of a given gene in a population of stable transformants will therefore be lower than in a population of unstable transformants. Second, the consistent loss of the centromere-like end of the chromosome fragment in the stable subclones of transformant 2TGT4 suggests transgenomes may undergo stabilisation in a polar fashion. Polar effects would differentially influence the co-transfer frequency of genes proximal and distal to the selected gene.

Additional transfer lines will have to be examined to determine how generally applicable these findings are. The ability to generate large numbers of transformed cell lines through recent modifications of the transfer technique[14] will aid in both confirming these results and providing quantitative mapping data. The further development of CMGT as a mapping procedure will provide an intermediate level of mapping resolution between restriction endonuclease analysis and somatic cell hybridisation.

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letters

IUE observations of an active region of HD206860

THE chromospheric Mg II *h* and *k* lines seem to have doubly reversed emission core in the spectra of the Sun and of solar and late type stars. In the solar spectrum the general appearance of Mg II *h* and *k* is very similar to the *H* and *K* of Ca II, but with larger intensity fluctuations. Current ideas suggest that the *h* and *k* lines are strongly sensitive to chromospheric parameters. In quiet areas of the Sun the Mg II emission shows asymmetric doubly reversed profile with a brighter blue peak, while in plages the emission is much stronger and nearly symmetrical[1]. The Mg II doublet seems to be a very useful way of investigating the presence of active regions on stars. We report here the results of our observations of the intensity of the Mg II *h* and *k* lines of the star HD206860 collected with the IUE at the Villafranca Satellite Tracking Station of the European Space Agency (ESA). The aim of the observations was to search for short-term variability of the chromospheric emission connected with the rotation period of the star.

HD206860 is a GOV star with prominent emission cores on the *H* and *K* lines of ionised calcium. Spectrophotometric observations by Wilson[2] show small amplitude but definite variations of the Ca II *H* and *K* emission components indicating activity in the chromosphere of HD206860. No clear long-term periodicity results from Wilson's observations which were carried out between 1966 and 1975. The data show an apparently random scatter much larger than the scatter of the standard stars. Such scatter may be due to fluctuations of the line intensity in a short time scale.

Since the stars with *H* and *K* chromospheric activity seem the most likely to show photospheric activity, HD206860 was included in a wide programme of UBV photoelectric observations of solar type and late-type stars, undertaken at the Catania Astrophysical Observatory to investigate light variations which might be ascribed to spots or more generally to active photospheric regions[3,4]. The analysis of the observations from 1970 to 1977 shows regular small amplitude variations in the light of HD206860. The amplitude in V light is 0.02 m and the period 24.9 d[4,5]. By the hypothesis that the light variations are due to spots on the star surface, the period of 24.9 d must be the rotation period of the star and this is an appropriate value for a GOV star like HD206860[5,6]. However, no correlation of the *H* and *K* line data from Wilson's observations with the 24.9 d period was found.

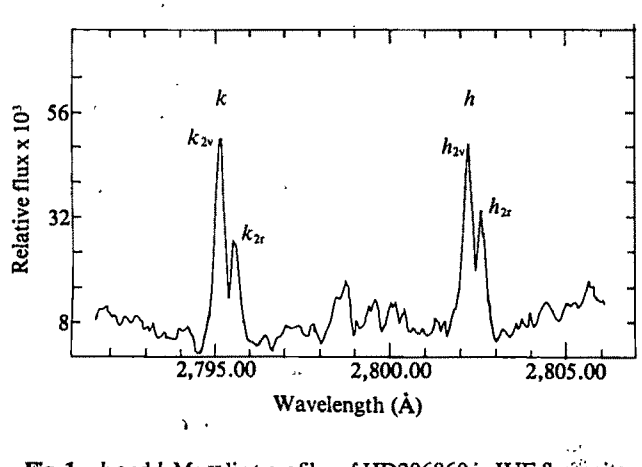


Fig. 1 *h* and *k* Mg II line profiles of HD206860 in IUE flux units. The blue emission component k_{2V} and the red emission component k_{2r} are outstanding.

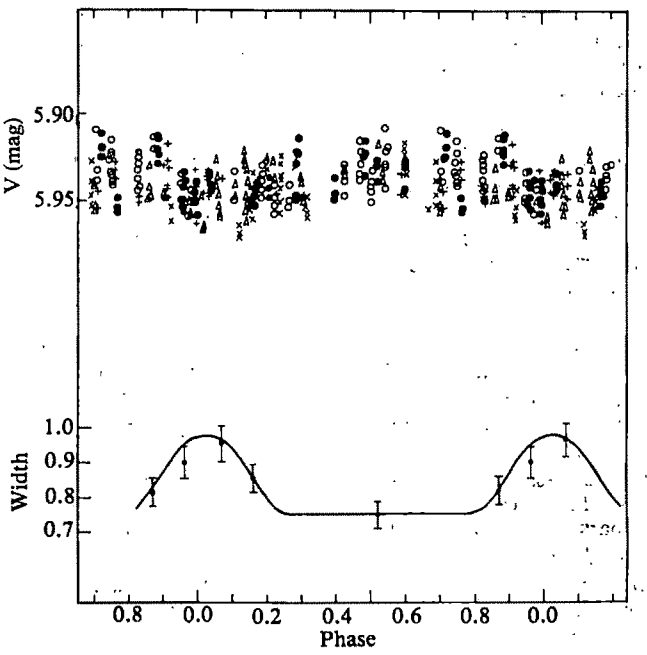


Fig. 2 Photometric observations of HD206860 in V light (different symbols refer to different years of observations) and equivalent width of the *k* emission component of the Mg II versus phases computed with the elements $(JD)_0 = 2440821.48$ and period of 24.9 d.

It is surprising that the Ca II *H* and *K* lines do not correlate with the 24.9 d, even if they show short term variability as deduced from the large random scatter in Wilson's observations. Different intrinsic and observational causes may contribute to the lack of the correlation: (1) the variations of the Ca II lines are less prominent than those of the Mg II ones; (2) Wilson's measurements[2] include photospheric contribution in the line which partially hides the variations of chromospheric origin.

To check these results repeated UV observations of HD206860 were made with the IUE. Five high-resolution spectra (~0.2 Å) in the long wavelength region (λ 1,900–3,200 Å) and two low resolution spectra (~7 Å) in the short wavelength region (λ 1,150–1,900 Å) were obtained from 18 November to 29 December 1978 at the Villafranca Observatory.

In the long wavelength region we concentrated our attention on the emission cores of the Mg II *h* (λ 2,803 Å) and *k* (λ 2,796 Å) resonance lines. A profile of the *k* line in IUE flux units is shown in Fig. 1, where the two emission peaks k_{2V} (the blueshifted component) and k_{2r} (the redshifted component) are evident.

Exposure times (Table 1) have been chosen to produce a well exposed spectrum at the k_2 peak, leading to a signal-to-noise

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Table 1 Observational data of HD206860 and measured quantities of the Mg II line referred to the line wing at λ 2,784.5 and λ 2,815

Date (1978)	Exposure time (min)	k_{2V}	k_{2V}/k_{2r}	$(W_k)_E$	φ
18 November	44	1.52	1.77	0.814	0.87
23 November	52	1.60	1.55	0.957	0.07
15 December	52	1.74	1.87	0.899	0.96
20 December	48	1.37	1.41	0.854	0.16
29 December	48	1.41	1.66	0.750	0.52

Equivalent widths of the emission $(W_k)_E$ are in Ångströms. The phases φ are computed with the elements $(JD)_0 = 2440821.48$ and period of 24.9 d.

ratio of about 20. Three or four pixels were satured at the k_2 peak on a few spectra. However, this has little influence on the evaluation of fluxes of the whole Mg II emission.

The output of the IUE software, that is the relative intensity spectrum uncorrected, for the wavelength dependence of the scientific instrument sensitivity has been used to study the change of the k lines of Mg II. To reduce the errors, mainly due to the background subtraction, relevant quantities of the emission profile have been referred to two points of the absorption wings at λ 2,784.5 Å and λ 2,815 Å. Since the line wings can be subject to changes due to the activity a check with the continuum at λ 2,685 Å and λ 2,950 Å was made. This check gave no evidence of such changes of the line wings of HD206860. Intensities at k_{2V} and k_{2r} emission peaks and the equivalent width have been measured on the IUE software plots.

Both the k_2 violet and red peaks were found to vary. The largest changes up to 25% were observed for the violet peak. Also the integrated emission component, that is the equivalent width, shows changes of 25%. Equivalent widths of the k line, measured in Angstrom and referred to the average value of the line wing at λ 2,784.5 Å and λ 2,815 Å, are given in Table 1. The estimated error on the equivalent width is ±5%. A plot of these values using the period and epoch of the photometric variation, given in Fig. 2, shows a fairly defined variation of the line intensity. Although we have only little data, the increase of intensity near the zero phase seems well defined.

A tentative interpretation of these line intensity changes may be given assuming that they result from a localised bright chromospheric active region like a plage. The continuous line in Fig. 2 represents the change of the k line intensity with a complete rotation of the star for an equatorial bright plage covering 10% of the stellar disk and 2.5 times brighter than the quiet chromosphere. A limb darkening coefficient equal to 0.5 was also assumed. The agreement with the observations seems fairly good.

Solar chromospheric faculae show an enhancement of the k (Mg II) line intensity[1] from two to three times the value of the quiet chromosphere, so the value 2.5 assumed in the case of the active region of the solar type star HD206860 seems to be reasonable.

No accurate value of the limb darkening for the solar k Mg II line is given in the literature but the value 0.12 derived from rocket observations[7] seems too small to fit the observed behaviour for the variations of HD206860.

The maximum intensity of the Mg II line seems to occur near the minimum of the V light (Fig. 2). The low accuracy of the photometric period and large time interval involved means that this could be only by chance. However, if this correspondence is real, HD206860 is a good example of a star with solar type activity, that is a photospheric dark spot underlying a bright chromospheric plage. So the variations of the intensity of the k line seem to confirm the rotation period of 24.9 d, first determined from the photoelectric observations.

Light variations attributed to spots are known for binary stars of the RS CVn or BY Dra class[8,9]. From radial velocity observations[10,11] HD206860 does not appear to be binary, it would therefore be the first single star with an active region detected from photometric observations and confirmed by the spectroscopic ones.

The two short-wavelength region spectra show spectral features indicating plasma temperatures hotter than 20,000 K. Important emission lines and temperatures of formation are: C II λ 1,335 (20,000 K), Si IV λ 1,394, 1,403 (80,000 K), He II λ 1,640 (80,000 K), C IV λ 1,549 (10^5 K). No clear changes are evident for these lines in the two spectra. Unfortunately, they were obtained at about the same phase on the Mg II variation, that is, during the increasing branch. It would be very interesting to see if any of these high temperature lines disappears or changes its intensity when the active region is not visible.

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Variations of the Sun's radius and temperature due to magnetic buoyancy

LIVINGSTON[1] has recently measured a decrease in the surface temperature of the Sun coincident with increased solar activity. He interpreted the temperature drop as implying a corresponding reduction in luminosity. I point out here that surface cooling could also be due to a radial expansion of the Sun, with no attendant reduction in luminosity. There is a plausible physical mechanism for such an expansion; namely, variations in magnetic buoyancy due to variations in the magnetic flux in the convection zone over the solar cycle.

The solar luminosity L may be expressed in terms of an effective surface temperature T as $L = 4\pi R^2 \sigma T^4$, where R is the solar radius. For constant R, a decrease in T implies a decrease in L. Alternatively, there can be a cooling (or heating) of the surface due to a radial expansion (or contraction) of the Sun with constant L. For small changes ΔT and ΔR with L constant,

$$\frac{\Delta R}{R} = -2\frac{\Delta T}{T} \qquad (1)$$

As $T \sim 6{,}000$ K, a drop in surface temperature of 1 K would correspond to a relative expansion $\Delta R/R \sim 3 \times 10^{-4}$.

The concept of magnetic buoyancy was introduced by Parker[2] and Jensen[3] and is an important ingredient in the solar dynamo. Consider an isolated magnetic flux tube of field strength B in the solar interior. The sum of the gas pressure p_i and the magnetic pressure $p_m = B^2/8\pi$ inside the tube must balance the external gas pressure p_e, so $p_i + p_m = p_e$, and $p_i < p_e$. If the flux tube is in

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thermal equilibrium with its surroundings ($T_i = T_e$), then the density inside the tube is lower than the density of the surroundings ($\rho_i < \rho_e$) and the flux tube is buoyant. Jensen[3] pointed out that, because of the density depression inside a magnetic flux tube, the presence of magnetic flux tubes in the solar interior will cause the volume of the Sun to increase; thus, we would expect the radius of the Sun to vary with the solar cycle, with maximum radius occurring near sunspot maximum. Jensen's suggestion, which has attracted little previous attention, is pursued here.

How much expansion and contraction of the Sun might we expect due to variations in magnetic buoyancy over the solar cycle? A precise estimate would require detailed knowledge of the magnetic field distribution in the convection zone over the solar cycle and a complete calculation of the effect of the magnetic field on the structure of the solar envelope. This is beyond present capabilities, so instead a crude calculation is used to get a very rough estimate. Consider a toroidal flux tube that circles the Sun at spherical radius R_1 and constant latitude ϕ. Let the tube have field strength B and cross-sectional radius a. The mass of gas within the tube is less than would occupy the same volume in the absence of the magnetic field. The difference in densities outside and inside the tube is given by

$$\Delta\rho = \rho_e - \rho_i = (p_e - p_i)/RT_1 = p_m/RT_1$$

where $T_1 = T_e = T_i$ is the temperature of the flux tube and its immediate surroundings. The total mass defect in the tube is equal to the volume of the tube times $\Delta\rho$, or

$$\Delta M = 2\pi R_1 \cos\phi\, \pi a^2 p_m/RT_1$$

Assuming that this mass ΔM is spread over a thin spherical shell of mean radius R_1 and thickness ΔR, then the mass in this shell is given by

$$\Delta M = 4\pi R_1^2 \Delta R \rho_e$$

Equating the two expressions for ΔM gives

$$\frac{\Delta R}{R} = \frac{\pi}{2}\cos\phi\left(\frac{R}{R_1}\right)\left(\frac{a}{R}\right)^2\frac{p_m}{p_e} \qquad (2)$$

This expression gives an estimate of the relative change in radius due to a single toroidal flux tube.

To obtain a numerical estimate we can, for example, use the results of the study by Weiss[4] of magnetic flux tubes in the solar convection zone, based on equipartition of magnetic energy and kinetic energy of convection. Weiss finds that a typical flux tube has a total flux of 4×10^{21} Mx. The radius and field strength of the flux tube vary slowly from 5,000 km and 5,700 G at the bottom of the convection zone to 14,000 km and 600 G at photosphere. The ratio p_m/p_e varies greatly, from 6×10^{-6} at the bottom of the convection zone to 2.8×10^{-1} at the photosphere. Thus, the dominant contribution to ΔR will come from flux tubes near the top of the convection zone. Using values given by Weiss for a and p_m/p_e in equation (2), for R_1/R in the range 0.98–1.0, we obtain values of $\Delta R/R$ in the range $5\times10^{-8}\cos\phi$ to $2\times10^{-4}\cos\phi$. For example, a single flux tube at a depth of 1,000 km at middle latitude gives $\Delta R/R \sim 2\times10^{-5}$. Now, to estimate the change in solar radius with the solar cycle, we need to estimate the difference in total magnetic flux in the upper convection zone between solar maximum and minimum. If we assume that the total magnetic flux emerging at the solar surface is a good indicator of the total flux in the uppermost layers of the convection zone, then observations suggest a difference in total flux of the order 10^{23} Mx between solar maximum and minimum. This is equivalent to some 25 or more of the individual flux tubes discussed above and could imply a relative change in radius $\Delta R/R \sim 5\times10^{-4}$ or more and a corresponding drop in surface temperature of ~1.5 K or more.

Magnetic field strengths in the convection zone may be higher than the equipartition limit used in the estimate above. Indeed, observations[5] have shown that almost all of the magnetic flux in the quiet photosphere is concentrated to strengths of 1,000–2,000 G, well above the equipartition limit, and the theory of Galloway, Proctor and Weiss[6] predicts that magnetic fields will be concentrated to 10 times the equipartition limit or more by the convection. Equation (2) can be rewritten as

$$\frac{\Delta R}{R} = \frac{FB\cos\phi}{16\pi R R_1 p_e}$$

where $F = \pi a^2 B$ is the total magnetic flux in the tube. For a tube of fixed total flux, the relative change in radius is proportional to the magnetic field strength. Thus, higher field strengths in the convection zone would increase the estimate of $\Delta R/R$ for a fixed difference in total flux over the solar cycle. With higher field strengths, flux tubes deeper in the convection zone would contribute significantly to $\Delta R/R$. Although there is uncertainty about magnetic fields in the convection zone, the estimates do suggest that the surface cooling of ~6 K observed by Livingston may be at least partly caused by expansion due to magnetic buoyancy.

The historical record of measurements of the solar radius gives no clear or consistent picture[7–9]. The currently accepted value[10] ($R = 959.63''$ at 1 AU) dates back to 1891 and is subject to correction for irradiation[11]. Variability of R with amplitudes ranging from ~0.05″ to ~2″ and periods of 7, 8, 11 and 22 yr has been reported. Giannuzzi[12], for example, found variations in R with amplitude 0.2–0.5″ and period 22 yr. Meyermann[13] reported variations with total amplitude ~0.15″ ($\Delta R/R \sim 1.5\times10^{-4}$) and period 11 yr with maximum radius occurring very near sunspot maximum; this is in good agreement with the theoretical argument above. Gething[8] finds variations in R with amplitude ~0.1–0.2″, but with no obvious correlation with the solar cycle. Improved measurements of the solar radius over a solar cycle are needed to test the ideas expressed here. Recent improvements[9,14] in the theoretical definition of the solar radius should give a considerable increase in accuracy. Changes in opacity during expansion may affect the visual change in solar radius. Note that the solar oblateness measured by Dicke and Goldenberg[15] ($\Delta R/R \sim 5\times10^{-5}$) and later disputed by Hill and Stebbins[16] is an order of magnitude smaller than the variation in mean radius estimated above. Even if the expansion due to magnetic buoyancy is too small to produce a measurable change in surface temperature, it may still be large enough to show up directly as a change in radius. Accurate monitoring of the solar radius over the solar cycle would give a measure of the variation of total magnetic flux in the convection zone and add to our understanding of the solar dynamo. Measurements of the solar radius from space are particularly desirable.

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Silicalite-2, a silica analogue of the aluminosilicate zeolite ZSM-11

THE structure of silicalite, a new polymorph of silica which was first prepared by Flanigen *et al.*[1], is so similar to that of the silica-rich aluminosilicate zeolite ZSM-5 (ref. 2) that we suggest that it should be regarded as the aluminium-free end member of this zeolite series. Therefore it should be possible to prepare silica analogues of other silica-rich aluminosilicate zeolites such as ZSM-11 (ref. 3), ZSM-12 (ref. 4), ZSM-21 (ref. 5) and ZSM-34 (ref. 6). We report here the preparation of what we believe is the analogue of ZSM-11, which we call silicalite-2, adopting the terminology silicalite-1 for the form prepared by Flanigen *et al.*[1]. The silicalite-1, ZSM-5, ZSM-11 and, by inference, silicalite-2 frameworks contain 4-, 5- and 6-membered rings linked to form a system of channels with 10-membered ring openings[1-3]. In silicalite-1 and ZSM-5 the channels are a combination of linear and zig-zag[1,2], while in ZSM-11[3] and silicalite-2 all the channels are linear. The lack of substitutional aluminium results in silicalites having no significant catalytic or cation exchange properties compared with the zeolites. However, silicalites are unusual in that they are the only known hydrophobic forms of silica and are capable of absorbing organic molecules up to ~6 Å kinetic diameter. Thermogravimetric studies of silicalites-1 and -2 containing absorbed long straight-chain hydrocarbon compounds (C_4–C_{16}) show that degassing occurs in two stages, which we consider to be due to self-blocking of the channels at intersection points.

The precursors of the silicalites are prepared by reacting tetraalkylammonium or tetraalkylphosphonium salts with a reactive form of silica at a high *p*H, at temperatures between 100 °C and 200 °C, in a closed system[4,5]. We have found that the silicalite-2 precursor can be prepared using tetra-*n* -butylammonium hydroxide only, although adding ammonium hydroxide or hydrazine hydrate as a source of extra hydroxyl ions increases the reaction rate considerably. A successful preparation was to mix 8.5 mol SiO_2 as silicic acid (74% SiO_2), 1.0 mol tetra-*n*-butylammonium hydroxide, 3.0 mol NH_4OH and 100 mol water in a steel bomb and heat at 170 °C for 3 days. The precursor crystals are ovate in shape, ~2–3 μm long and 1–1.5 μm diameter, similar to those reported for ZSM-11[3]. The silicalite-2 precursor will not form if Li, Na, K, Rb or Cs ions are present, in which case the silicalite-1 precursor is formed. The size of the tetraalkylammonium ion seems critical because replacement of the tetra-*n*-butylammonium hydroxide by other quaternary ammonium hydroxides (such as tetraethyl, tetrapropyl, triethylpropyl, and triethylbutyl hydroxides) results in amorphous products. Silicalite-2 precursor is stable at extended reaction times in the hydrothermal system. However, in similar conditions the silicalite-1 precursor converts to α-quartz, presumably due to the effect of alkali metal ions. The amount of Al present in silicalite-2 depends on the purity of the starting materials. We typically find <5 p.p.m. Al present. The precursor contains occluded tetraalkylammonium salts which, because of their size, can be removed only by thermal decomposition. We have observed, by means of thermal analysis and mass spectrometry, that the tetraalkylammonium ion decomposes at ~300 °C and is lost as the tertiary amine, alkene and water. This can be contrasted to the normal thermal decomposition at 200 °C of the same tetraalkylammonium salt in air.

Figure 1 shows the X-ray powder diffraction patterns we have obtained for silicalite-1 and silicalite-2, and for ZSM-5 and ZSM-11. Major differences between the patterns of silicalite-1 and silicalite-2 are that peaks at 9.06, 13.9, 15.5, 16.5, 20.8, 21.7, 22.1, 24.4, 26.6 and 27.0 degrees 2θ (CuKα radiation) are absent from the silicalite-2 pattern. Also, peaks at 8.8, 14.8, 17.6, 23.1, 23.9 and 29.9 degrees 2θ are singlets in the silicalite-2 pattern rather than doublets as in the silicalite-1 pattern. These differences are the same as those found between the diffraction patterns of orthorhombic ZSM-5 and tetragonal ZSM-11. Unit cell dimensions are given in Table 1 calculated on the assumption of tetragonal symmetry for silicalite-2, and show that the effect of the substituted aluminium in ZSM-5 and ZSM-11 is to increase the unit cell size over that of silicalite-1 and silicalite-2. The measured densities and refractive indices of silicalite-2 and its precursor are 1.82 and 1.98 g cm^{-3} and 1.41 and 1.48 respectively. Calculation of the unit cell volumes shows that silicalite-2 should be ~1% less dense than silicalite-1, whereas we find that silicalite-2 is ~3% more dense. The samples of silicalite-2 prepared here possibly contain a small percentage of another 'more dense' crystallographic form of silica.

Desorption or degassing of various organic molecules from silicalite-1 and silicalite-2 has been studied by thermogravimetric analysis. Rigid molecules, such as *p*-xylene, are lost in a single low-temperature step due to simple desorption. However, flexible molecules, such as *n*-alkanes or *n*-alcohols of chain length >C_5 are lost in two steps (Fig. 2). We believe this shows that self-blocking of the channel structure is occurring, probably by molecules bending through 90° into an intersecting channel,

Table 1 Lattice constants

	a	*b*	*c*
Silicalite-1	20.07 (1)	19.86 (1)	13.36 (1)
ZSM-5[2]	20.1	19.9	13.4
Silicalite-2	20.04 (1)	20.04 (1)	13.38 (1)
ZSM-11[3]	20.12	20.12	13.44

The figures in parentheses are 1 s.d.

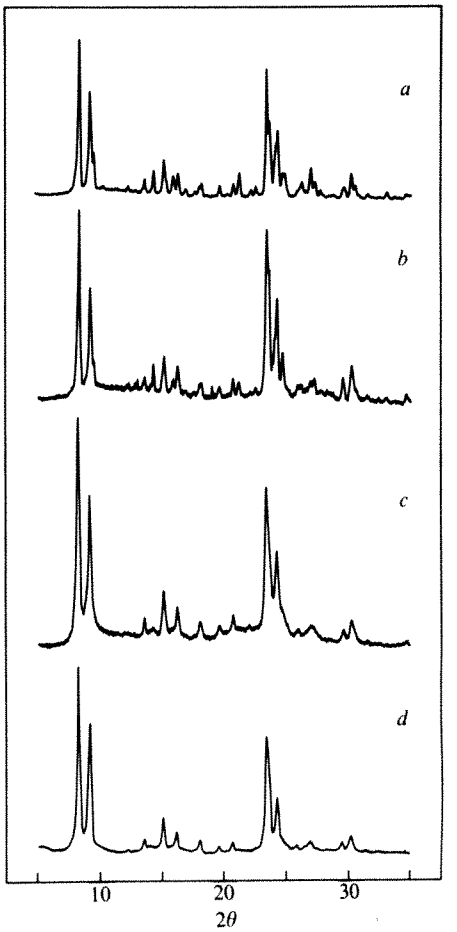


Fig. 1 X-ray diffraction patterns of silicalite-1 (*a*) and silicalite-2 (*c*) and the corresponding aluminosilicate zeolites ZSM-5 (*b*) and ZSM-11 (*d*).

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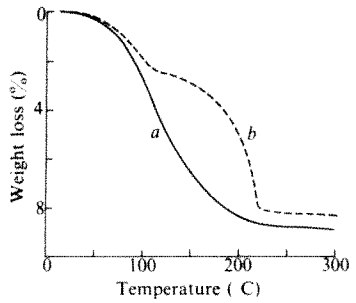


Fig. 2 Thermogravimetric curves showing the weight loss of: *a*, *p*-xylene; and *b* decane from silicalite-2 (heating rate = 2.5 °C min^{-1}, atmosphere is air).

and thus preventing egress of other molecules from both of the channels. A higher activation energy is required to overcome the awkward passage of these molecules, resulting in a higher temperature for weight loss when compared with desorption straight through the channel system. Initial results show that there is less self-blocking in silicalite-2 than in silicalite-1. Presumably the zig-zag channels in silicalite-1 are more prone to self-blocking since molecules are required to change direction even when continuing along the same channel, thus increasing the probability of bending into an intersecting channel.

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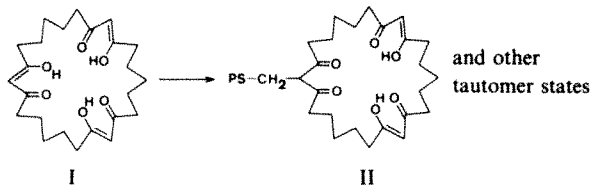
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Extraction of uranium from seawater by polymer-bound macrocyclic hexaketone

THE energy crisis means that a petroleum substitute is urgently needed and a promising candidate is nuclear energy. Uranium, however, like petroleum, is distributed in small limited areas. These circumstances could be dramatically improved if uranium in seawater were to be utilised as a resource. Although its concentration is extremely low (3.3 parts per 10^9), the total amount is huge, calculated at $4–4.5 \times 10^9$ tons. We have recently synthesised a macrocyclic hexaketone (I) which is a very strong host of uranyl (UO_2^{2+}) ion[1]. Its noteworthy characteristics are: (1) six oxygen atoms can be directed towards the inside of the ring, if necessary, to form hexadentate coordination in near coplanarity which corresponds to the crystalline structure of various uranyl salts[2,3]. (2) β-Diketone can easily be dissociated in seawater (pK_a is 8.95 for acetylacetone) to form a strong ligand, ketoenolate anion[4]. (3) The bound uranyl ion is readily liberated by the treatment with dilute aqueous acid. Here we report the successful extraction of uranyl ion directly from seawater using polymer-bound macrocyclic hexaketone.

The hexaketone (170 mg, 0.4 mmol) was treated with partially chloromethylated polystyrene (chloromethyl content: 18.8%, 82 mg, 0.14 mmol equiv. for CH_2Cl unit) in dimethylformamide (1 ml) in the presence of anhydrous potassium carbonate (138 mg, 1.0 mmol). An IR spectrum of the resulting polymer, after the usual purification, showed that the CH_2Cl absorption had almost disappeared and new absorptions at 1,720 and 1,600 cm^{-1} had appeared which correspond to keto and enol forms of hexaketone unit.



I II

Polymer-bound hexaketone (II) (98 mg, 0.1 mmol equiv. of (I)) dissolved in chloroform (30 ml) was stirred magnetically with 3.5 l of seawater sampled at 20 m off the coast of Ohara, Sotoboh, Chiba Prefecture, Japan (Fig. 2). After 18 h, a chloroform solution was separated and seawater was extracted with chloroform (5 ml × 2) to recover any dissolved polymer and/or polymer–uranyl complexes. From the combined chloroform solution, uranyl ion was liberated by the treatment with 1 M HCl solution (5 ml)[1]. The resulting acid solution was analysed by the arsenazo III method[5], showing that 0.84 p.p.m. of uranyl ion is present which corresponds to 36% of the UO_2^{2+} present in the original seawater. An independent analysis of UO_2^{2+} by atomic absorption spectrophotometry was also made to demonstrate the presence of approximately the same amount of uranyl ion. Repeated (three times) extraction and liberation of uranyl ion using the recovered hexaketone (II), accumulated 1.5 p.p.m. of UO_2^{2+} in 9.2 ml of acid solution (13.8 μg) (total seawater treated is 3 × 5 l). After these cycles, the same procedure using the hexaketone (II) recovered, was repeated to extract 11 μg of UO_2^{2+} from further 3 × 5 l of sea water. This demonstrated that the recovery and regeneration of hexaketone (II) was satisfactory. The extraction time was then reduced to 1 h and gave practically the same efficiency. These results clearly indicate that the polymer-bound hexaketone (II) can successfully extract uranyl ion directly from seawater and be liberated in concentrated form on treating with a dilute aqueous acid solution.

Attempts to extract uranium from seawater have so far concentrated on the method using the adsorbent hydrous titania sorber[6]. However, economical and environmental analyses are discouraging at present; the major problem is the large loss of titania sorber. It has been estimated that 68 pounds are needed to produce 1 pound of uranium[7]. The method described here avoids an appreciable loss of adsorbent by cross linking the supporting material via chemical bonding, and on further refinements it will give adsorbents specific to uranyl ion. The present procedure, however, is still not practical because the polystyrene backbone is too hydrophobic to achieve an efficient rapid contact with seawater if used in powdered form, although slow extraction does take place. In our preliminary experiments using powder of a partially sulphonated polystyrene, however,

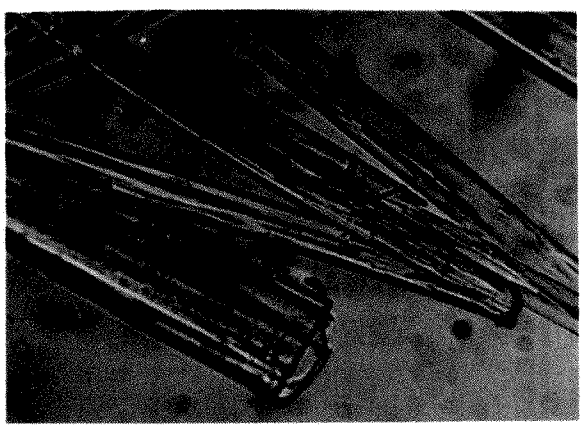


Fig. 1 Light microscopy of crystals of the hexaketone (I).

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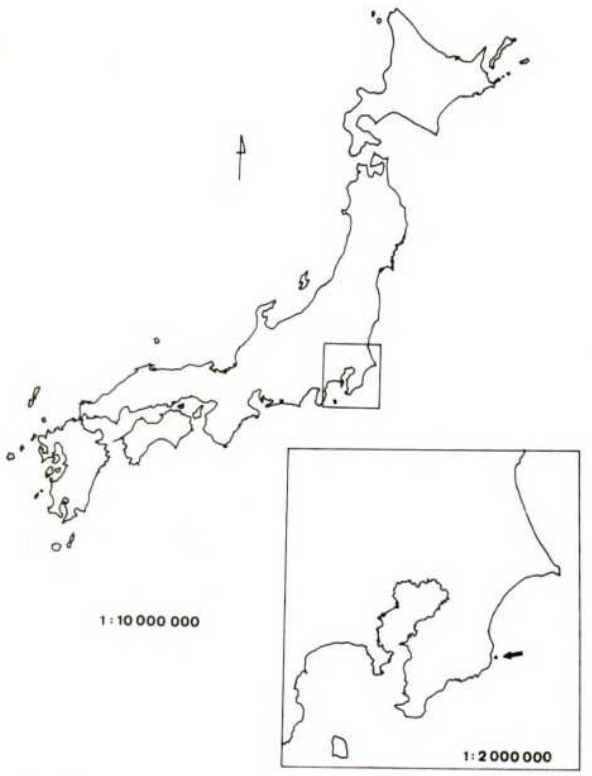


Fig. 2 Seawater was sampled at the spot asterisked along the Black Current.

uranyl ion was very effectively and rapidly extracted from seawater. The polystyrene was partially sulphonated by chlorosulphonic acid followed by the partial chloromethylation. Hexaketone was then attached to this polymer as described above. The details will be published elsewhere.

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Anodic coal reaction lowers energy consumption of metal electrowinning

THE metals which can be extracted in large amounts from aqueous solutions of their salts by electrowinning are Cr, Mn, Co, Ni, Cu, Zn, Ga, Cd, In and Tl. Gilroy[1] specifies an annual electrowinning production for 1971 of 2,400,000 tons of Zn and 540,000 tons of Ca, 53% and 10% respectively of total production at that time. We describe here a modification to the established electrowinning technique which lowers the energy consumption of the process.

Table 1 Current efficiency and electrical energy consumption during copper deposition in the conventional process

Reaction rate I (mA)	Total coulomb consumed It (C)	Theoretical Cu equivalent to the charge passed W_1 (g)	Measured amount of Cu deposited W_2 (g)	Current efficiency $(W_2/W_1) \times 100$ (%)	Electrical power consumed $E_1 = (I\int V\,dt)/W_2$ (kWh kg^{-1})
5.90	100.79	0.03319	0.03027	91.2	1.52
7.95	147.39	0.04853	0.04620	95.2	1.51
10.00	144.00	0.04741	0.04425	93.3	1.52
12.00	133.90	0.0442	1.04288	97.2	1.622

Electrolyte, 0.125 M $CuSO_4$ and 0.5 M H_2SO_4; Temperature, 60 °C.

The reactions for conventional electrowinning of a metal M are:

Anodic: $$X\mathrm{H_2O} \rightarrow \tfrac{1}{2}X\mathrm{O_2} + 2X\mathrm{H^+_{aq}} + 2X\mathrm{e^-} \quad (1)$$

Cathodic: $$\mathrm{M^{2x+}_{aq}} + 2X\mathrm{e^-} \rightarrow \mathrm{M} \quad (2)$$

The reversible thermodynamic potential, $E°$, of reaction (1) is 1.229 V. It and associated polarisation overpotentials, E_{op}, constitute a large portion of the total potential E_{tot} of the practical electrowinning cell:

$$E_{tot} = E° + E_{op} + E_m$$

where E_m is the half-cell potential for reducing the metal ion, reaction (2). Browall *et al.*[2] introduced the reducing agent, CO, at the anode to lower the cell potential for electrolytic production of hydrogen. Using coal as the reducing agent, we devised[3–5] a new electrochemical process which converts coal and water into two separate gaseous products—one at the anode, comprising essentially gaseous oxides of carbon, and the other at the cathode which is essentially pure hydrogen. We called this process electrochemical gasification of coal or, alternatively, coal-assisted water electrolysis. Coal contains a wide variety of organic molecules rich in carbon. Focusing only on the carbon of the coal the reactions of the electrochemical coal-gasification process, in simplified stoichiometry, would be:

Anode: $$\frac{X}{2}\mathrm{C(s)} + X\mathrm{H_2O(l)} \rightarrow \frac{X}{2}\mathrm{CO_2(g)} + 2X\mathrm{H^+} + 2X\mathrm{e^-} \quad (3)$$

Cathode: $$2X\mathrm{H^+} + 2X\mathrm{e^-} \rightarrow 2X\mathrm{H_2(g)} \quad (4)$$

The reversible thermodynamic potential, $E°$, of half-cell reaction (3) is only about 0.21 V. We have now extended electrochemical coal gasification to the electrowinning of metals by substituting the half-cell reaction (3) (which consumes coal) for the half-cell reaction (1) which usually takes place during conventional electrowinning of metals from aqueous solutions of

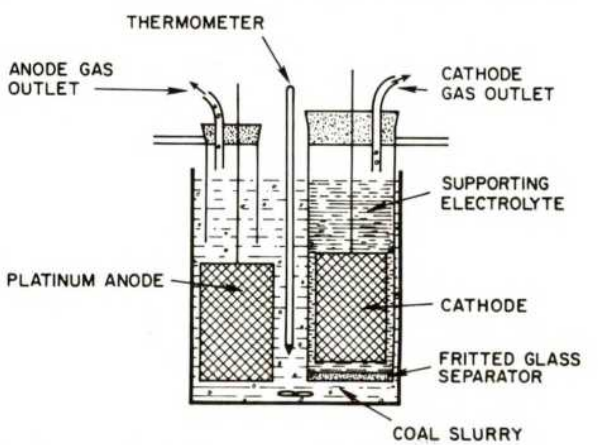


Fig. 1 Coal gasification cell: electrolyte volume, 80 cm^3; anode, 5.3 cm^2 (geometrical) platinum mesh, gauge 52; cathode, 8.4 cm^2 (geometrical) platinum mesh, gauge 52; stirring, magnetic, $\frac{7}{8}$-inch magnet).

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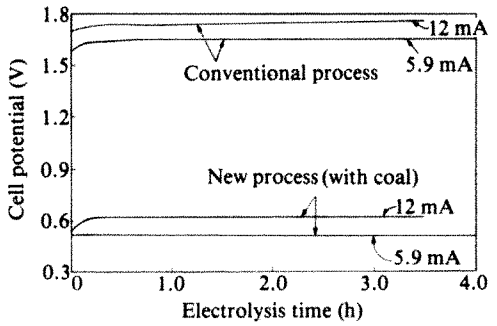


Fig. 2 Comparison of cell potentials of the copper electrowinning processes in galvanostatic conditions: Electrolyte, 0.125 M $CuSO_4$ and 0.5 M H_2SO_4; coal sample, N. Dakota lignite; slurry concentration, 0.15 g cm^{-3}; particle size, 74–88 μm; temperature, 60 °C.

their salts. By this coal-based innovation the total overall cell potential of the resulting metal electrowinning process is lowered by about 1.0 V with a corresponding significant reduction in the consumption of electrical energy. This new method of electrowinning from aqueous electrolytes is illustrated using preliminary experimental results for the coal-assisted copper electrowinning process.

Experiments were conducted in the simple cell shown in Fig. 1. The external e.m.f. was controlled by a potentiostat (PAR 371) and the electrodes (both anode and cathode) were Pt mesh, gauge 52 (0.004-inch diameter wire, Mathey Bishop Inc). Aqueous electrolytes were prepared with Baker analysed reagent grade sulphuric acid and Baker $CuSO_4 \cdot 5H_2O$ crystals. Coal slurries were prepared from pulverised North Dakota lignite coal; the ultimate analyses are described elsewhere[4]. Metal analysis was carried out on a Perkin-Elmer 460 atomic absorption spectrophotometer.

For comparison Cu was first deposited by conventional electrowinning without using coal. Accordingly, using the cell of Fig. 1, Cu was deposited on a platinum-mesh cathode from aqueous electrolyte (0.125 M $CuSO_4$ in 0.5 M H_2SO_4) at 60 °C, and at four different galvanostatic currents: 5.9, 7.95, 10 and 12 mA. The corresponding change in cell potential was recorded and typical cell potentials corresponding to the galvanostatic currents of 5.9 and 12 mA are plotted in Fig. 2. The conventional copper electrowinning reaction took place at corresponding cell potentials of about 1.65 and 1.73 V respectively. Figure 2 also shows the results obtained in parallel experiments, identical except that the coal was simultaneously oxidised at the anode according to equation (3) while Cu cations were reduced at the cathode according to equation (2). In these latter experiments, North Dakota lignite (NDL) coal slurry (0.15 g cm^{-3}) was introduced into the anode compartment of the cell and was anodically oxidised while copper deposited on the cathode in identical galvanostatic experimental conditions. As a result of the oxidation or gasification of the coal at the anode, the overall cell potential was lowered by ~1.1 V, compared to the conventional process. This is evident from Fig. 2. It is seen that the simultaneous anodic oxidation of coal lowers the potential by ~1.10 V, a value which is very close to the difference in half-cell potential between reaction (1) and (3).

Data on the current, electricity consumption and weight of Cu deposited are given in Table 1 for the conventional process, and in Table 2 for the new, coal-assisted process. Current efficiencies are also given computed from the ratio of the amount of Cu actually deposited to the equivalents of the charge passed during the experiment. The introduction of coal into the anode compartment during electrolysis evidently reduces current efficiency only slightly, at the worst about 2%. Comparing the right-hand columns of Tables 1 and 2 reveals that the simultaneous anodic oxidation of coal during Cu electrowinning reduces the electrical energy consumption by a factor of about two-thirds or more. Whereas the conventional process uses only electrical energy, our new process uses both electrical energy (E_2) and the chemical energy (E_3') of coal directly. For a reasonable comparison of the energy consumption of the two processes, the chemical energy of the coal consumed should be converted into equivalent electrical energy. By the stoichiometry of reaction (3), the mass of carbon consumed to produce 1 kg of copper is approximately:

$$\frac{12\ \text{kg C}}{\text{kg atom C}} \times \frac{1\ \text{kg atom C}}{2\ \text{kg atom Cu}} \times \frac{1}{63.54} \frac{\text{kg atom Cu}}{\text{kg Cu}} = 0.0944 \frac{\text{kg C}}{\text{kg Cu}}$$

Based on the enthalpy of combustion of carbon, this corresponds to a chemicothermal energy consumption of

$$E_3' = 0.0944 \frac{\text{kg C}}{\text{kg Cu}} \times \frac{94{,}052\ \text{kcal}}{12\ \text{kg C}} \times \frac{4180\ \text{J}}{\text{kcal}} \times \frac{1\ \text{kWs}}{10^3\ \text{J}} \times \frac{1\ \text{h}}{3{,}600\ \text{s}}$$

$$= 0.859 \frac{\text{kWh}}{\text{kg Cu}}$$

Assuming a practical efficiency of 35% for conversion of the combustion enthalpy of carbon (coal) into electrical energy, the electrical energy equivalent to the coal consumed is:

$$E_3 = (0.859)(0.35) = 0.300 \frac{\text{kWh}}{\text{kg Cu}}$$

Using these values of E_3' and E_3 with the values of E_1 and E_2 from Tables 1 and 2, the total energy requirements and also the net energy savings have been calculated for the new process and are reported in Table 3. Compared to the conventional process, the new coal-consuming process requires about 45% less net equivalent electrical energy for producing the same amount of copper.

A more telling comparison of these processes might be on the basis of relative energy costs based on assumed costs of US $0.02 per kWh for electricity and $35.00 per ton ($0.0514 per kg C) for coal assumed to be 75% carbon. Using mean values for E_1 and E_2 these costs are:

For the conventional process: Cost = ($0.02) · $\bar{E}_1$ = $0.031 per kg Cu.

For the new coal-assisted process: Cost = ($0.02) · $\bar{E}_2$ + (0.0944)($0.0514) = $0.0147 per kg Cu.

The savings in energy cost appears, therefore, to be ~50%.

In the experiments discussed so far, NDL coal which contains about 11.7% ash has been used as a reactant. The ash composition suggests the possibility of the presence of impurities such as Al, Fe, Ti, Ca, Mg, Na and K ions in the electrolytes. Consideration of the thermodynamic potential of the M–M^{x+} couple of these metals indicates that only iron might interfere with the copper deposition for which half-cell potentials have been reported[6] as follows:

$$Cu^{2+} + 2e^- = Cu \qquad E^\circ = 0.337\ \text{V}$$

$$Cu^{+} + e^- = Cu \qquad E^\circ = 0.521\ \text{V}$$

$$Cu^{2+} + e^- = Cu^{+} \qquad E^\circ = 0.153\ \text{V}$$

Table 2 Current efficiency and electrical energy consumption during copper deposition: new coal-assisted process

Reaction rate I (mA)	Total coulomb consumed It (C)	Theoretical Cu equivalent to the charge passed W_1 (g)	Measured amount of Cu deposited W_2 (g)	Current efficiency $(W_2/W_1) \times 100$ (%)	Electrical power consumed $E_2 = (I \int V\,dt)/W_2$ (kWh kg^{-1})
5.90	105.84	0.03485	0.031115	89.4	0.468
7.95	132.61	0.04366	0.04062	93.0	0.428
10.00	142.80	0.04702	0.04407	93.3	0.524
12.00	129.60	0.04267	0.04063	95.2	0.552

Electrolyte, 0.125 M $CuSO_4$ and 0.5 M H_2SO_4; Temperature, 60 °C; North Dakota lignite coal concentration 0.15 g cm^{-3}; particle size, 74–88 μm.

Table 3 Savings in energy consumption

Galvanostatic reaction rate I (mA)	Total energy consumption in the new process E_2+E_3 (kWh per kg Cu)	Net equivalent electrical energy consumed in this new process E_2+E_3 (kWh per kg Cu)	Net savings in equivalent electrical energy $E_1-E_2-E_3$ (kWh per kg Cu)	Net equivalent energy savings $\frac{(E_1-E_2-E_3)}{E_1}\times 100$ (%)
5.9	1.328	0.77	0.75	49.3
7.95	1.29	0.73	0.78	51.6
10.0	1.384	0.826	0.694	45.7
12.0	1.412	0.852	0.768	47.3

Reported[6] potentials of the different iron couples are:

$$Fe^{2+}+2e^-=Fe \qquad E^\circ=-0.440\ V$$
$$Fe^{3+}+e^-=Fe^{2+} \qquad E^\circ=0.771\ V$$
$$Fe^{3+}+3e^-=Fe \qquad E^\circ=-0.036\ V$$

The ash of NDL coal contains 12.5% Fe_2O_3 (other coals contain even less iron). Therefore iron is the only large-scale impurity which should be removed in a continuous process because reduction from the ferric to the ferrous state would reduce current efficiency for copper deposition and would increase the power consumption. Note that iron is generally also the only large-scale impurity present in copper ore itself and the technology for iron removal is well established, for example, by air oxidation of Fe^{2+} to Fe^{3+} which can easily be made to precipitate as the hydroxide. In some industrial processes ferric ion concentration is kept high to promote faster leaching of sulphide ores.

The purity of the electrodeposited copper was determined by dissolution in HNO_3 followed by atomic absorption spectrophotometry. In all experiments the purity of the deposited copper was >99%. The use of NDL or the presence of its ash in the electrolyte does not seem to have any significant effect on the copper purity. While our results, showing significant reduction in the energy requirement, pertain to one specific reaction system, similar observations are expected for other metal electrowinning reactions in aqueous electrolytes. Anode materials other than platinum have also been investigated. Graphite rods and graphite felt anode (Union Carbide Corp.) performed as well as platinum; copper plate cathodes have also been successfully tested.

Although, in an energy conscious environment this method looks promising, the practical applicability of the process depends on: (1) Achieving current densities sufficiently large to keep the capital cost economical; in our simple laboratory experiments no attempt has been made to increase current densities which were far lower than those used in industrial processes. (2) Almost total consumption of the carbon-value of the coal particles. (3) The influence of the particular coal and its ash content on electrowinning kinetics and on the properties and morphology of the deposited metal. (4) Preventing build-up in a continuous process of impurity cations which might compete with copper ions in electrolysis; such impurities would have to be removed continuously, for example by air oxidation of Fe^{2+} to precipitate ferric hydroxide.

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Impact of CO_2 on cooling of snow and water surfaces

THE levels of CO_2 in the atmosphere are being increased by the burning of fossil fuels and reduction of biomass[1–3]. It has been calculated that the increase in CO_2 levels should lead to global warming because of increased absorption by the atmosphere of terrestrial longwave radiation in the far IR (>5 μm)[4–7]. From model computations, CO_2 is expected to produce the largest climatic effect in high latitudes by reducing the size of ice and snow fields[4]. We present here computations of spectral radiative transfer and scattering within a snow pack and water. The results suggest that CO_2 significantly reduces the shortwave energy absorbed by the surface of snow and water. The energy deficit, when not compensated by downward atmospheric radiation, may delay the recrystallisation of snow and dissipation of pack-ice and result in a cooling rather than a warming effect.

We consider here the spectral absorption of CO_2 in the near IR portion (wavelength $0.75 \simeq 5.0$ μm) of incoming shortwave radiation with respect to the role of near IR in the heating of the uppermost (active) layer of snow and of water. Calculations were made to determine the impact of doubled CO_2 on the amount of short wave radiation absorbed in these layers in conditions typical of the start and the end of the local snow and ice season[8–10]. At this stage, other components of the heat balance such as transfer of sensible and latent heat, longwave atmospheric radiation, heat convection, and so on, were not considered.

To study the solar heating of snow, analytic expressions for the flux and divergence of flux (heat flux) were obtained by solving the radiative transfer equation in the modified two-stream approximation[11]. The scattering and absorption within the snow-cover by the randomly distributed ice grains were represented by a single scattering albedo and anisotropic phase function. Geometric optics calculations[11,12] were used to relate the scattering and absorption parameters to the grain size and density. The spectral flux, $F\lambda(z)$, and the negative of the derivative of spectral flux, $F^1\lambda(z)$, at a depth z within the snowpack were obtained from the equations:

$$F_\lambda(z)=[I_+(z)-I_-(z)]f_\lambda W_0$$
$$F^1{}_\lambda(z)=\sqrt{2}\gamma_e(1-\omega)[I_+(z)+I_-(z)]f_\lambda W_0$$

where

$$I_+(z)=c_1\left[1+\frac{(1-\omega)\sqrt{3}}{a}\right]\exp(-a\tau)+c_2\left[1-\frac{(1-2)\sqrt{3}}{a}\right]\exp(a\tau)$$

$$I_-(z)=c_1\left[1-\frac{(1-\omega)\sqrt{3}}{a}\right]\exp(-a\tau)+c_2\left[1+\frac{(1-\omega)\sqrt{3}}{a}\right]\exp(a\tau)$$

$$c_1=\frac{1}{\left[1+\frac{\sqrt{3}(1-\omega)}{a}\right]\left[1+\frac{\alpha(\alpha-R)}{R\alpha-1}\exp(-2a\tau_0)\right]}$$

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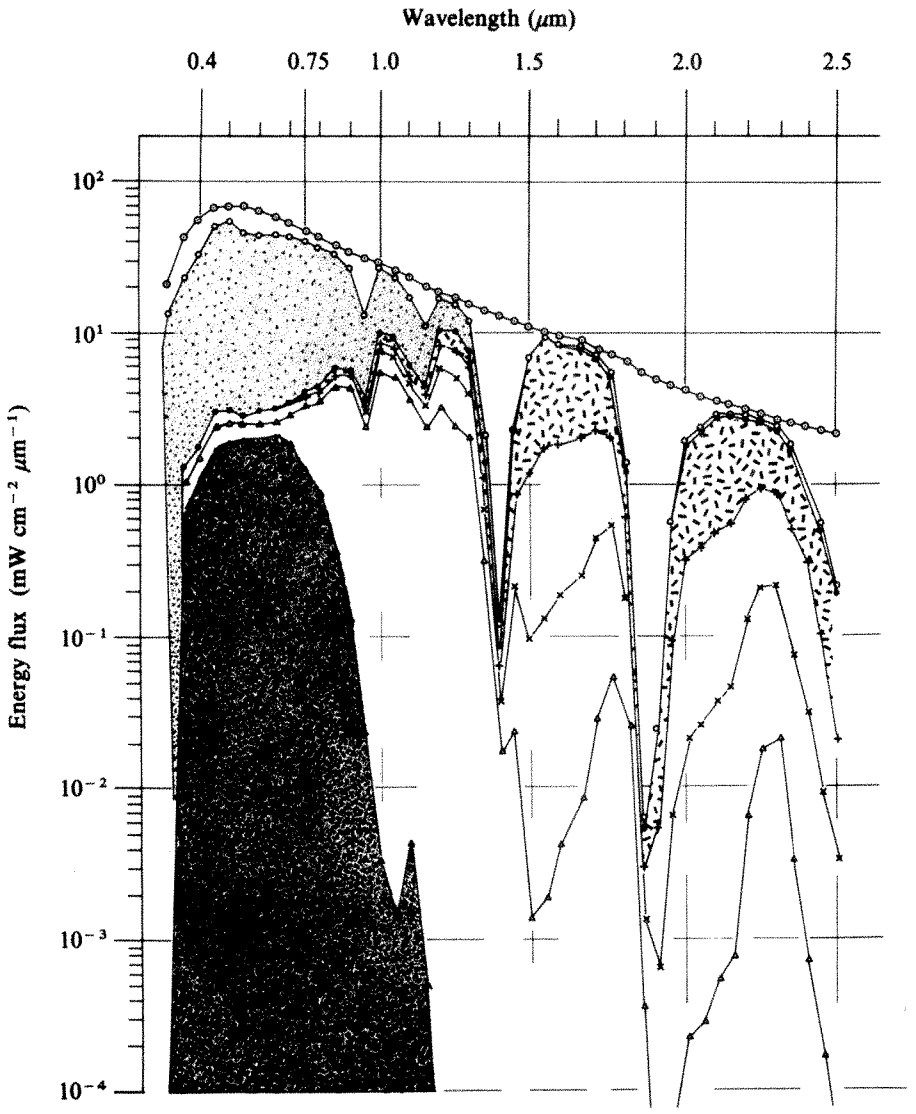


Fig. 1 Shortwave radiation flux in a snow pack in clear dry atmosphere at site A (see text), with 23° solar elevation and normal CO_2 level. Reflected radiation light stippled; energy absorbed in the uppermost 0.2 cm of a snowpack is marked with an intermediate tone; and energy penetrating into the underlying surface is heavy dark stippled. Almost all energy absorbed by snow is in the wavelength above 0.75 μm. ⊙ Top atmosphere; ○ surface; ● surface penetration; + 0.2 cm; × 0.5 cm; △ 1 cm; ⋀ 15 cm.

$$c_2 = \frac{\left(\frac{\alpha - R}{R\alpha - 1}\right)\exp(-2a\tau_0)}{\left[1+\frac{\sqrt{3}(1-\omega)}{a}\right]\left[1+\frac{\alpha(\alpha-R)}{R\alpha-1}\exp(-2a\tau_0)\right]}$$

where $I_+(z)$ is the intensity of downward oriented radiation; $I_-(z)$ the intensity of upward oriented radiation; f_λ the fraction of the solar flux incident on the snowpack within a narrow band centred around wavelength λ; W_0 the insolation to the top of the atmosphere; ν the radius of ice grains (cm); ρ the density of the snowpack (g cm^{-3}); h the thickness of the snowpack (cm); β the fraction of energy scattered in the backward hemisphere in single scattering (≃0.065); κ_λ the spectral absorption coefficient of ice (cm^{-1}); $C_{1,2}$ the integration constants; R the reflectance of the underlying snow surface (ice); $\tau = \gamma_e z$ the optical thickness; and $\tau_0 = \gamma_e h$ the total optical thickness of the pack.

Also

$$a = (3(1-\omega)(1-\omega+2\omega\beta))^{1/2}$$

$$\alpha = 1 - \frac{2(1-\omega)^{1/2}}{(1-\omega+2\omega\beta)^{1/2}+(1-\omega)^{1/2}} = \text{spectral reflectance}$$

$$\gamma_e = \left(\frac{3}{2\nu}\right)\frac{\rho}{0.917} = \text{extinction coefficient}$$

$$\omega = \tfrac{1}{2}+\tfrac{1}{2}\exp(-1.67\kappa_\lambda\nu) = \text{single scattering albedo}$$

In the absence of thermal conduction the rate of radiative heating was given by the equation:

$$H = \frac{1}{c\rho}\sum_\lambda F^1{}_\lambda(z)$$

where $c = 0.5$ is the specific heat of snow.

The values of the spectral reflectance, and the asymptotic flux extinction coefficient calculated from the model[13] are in good agreement with the observations[14]. We used the Delta–Edington approximation to calculate the radiative flux transfer and absorption in water[15]; the albedo was computed after Ivanoff[16]. Results agree with observations[17]. Atmospheric and radiative conditions approximately represent two sites. Site A is on land at latitude 43° N; water content in the atmospheric column is 0.9 g cm^{-2} and the Ångström turbidity factor 0.08. Noon solar elevation is 23°, as in the second half of December. Snow is underlain by soil. Site B is at latitude 78° N; atmospheric water content is 0.5 g cm^{-2} and the Ångström turbidity factor 0.02. Noon solar angle is 35°, as in early June when snow melt starts on top of the sea-ice.

In both cases the ozone path length is 0.35 cm, barometric pressure is 1,000 mbar, snow density is 0.22 g cm^{-3}, grain radius is 0.35 mm (that of moderately aged snow) and snow thickness is 15 and 30 cm. Normal concentration of CO_2 was conservatively taken as 315 p.p.m. although considerably higher winter values have been reported from the Northern Hemisphere in recent years[18]. Computations were performed for solar elevation changed in a daily cycle at hourly intervals. Flux was computed at a depth of 0, 0.2, 0.5, 1.0, 2.0, 5.0, 15.0 and 30.0 cm for snow

Table 1 Shortwave generated heating in snow and water

Medium	Case	Solar Time	Solar elevation (deg)	CO_2	Heating rate (°C min^{-1}) depth (cm) 0.0	0.2	0.5	1.0	5.0	15.0	100.0
Snow	A	9	11.46	Normal	1.7989	0.5441	0.1738	0.0806	0.0092	0.0002	
				Double	1.7570	0.5363	0.1731	0.0806	0.0092	0.0002	
				Diff. %	2.3292	1.4336	0.4028	0.0000	0.0000	0.0000	
		12	23.00	Normal	4.1561	1.2669	0.4111	0.1922	0.0226	0.0005	
				Double	4.0772	1.2523	0.4097	0.1922	0.0226	0.0005	
				Diff. %	1.8984	1.1524	0.3406	0.0000	0.0000	0.0000	
Snow	B	1	5.48	Normal	0.9277	0.2841	0.0925	0.0433	0.0050	0.0005	
				Double	0.9033	0.2795	0.0920	0.0433	0.0050	0.0005	
				Diff. %	2.6302	1.6192	0.5405	0.0000	0.0000	0.0000	
		7	23.16	Normal	4.6027	1.4188	0.4664	0.2180	0.0261	0.0026	
				Double	4.5121	1.4018	0.4647	0.2180	0.0261	0.0026	
				Diff. %	1.9684	1.1982	0.3645	0.0000	0.0000	0.0000	
		12	35.00	Normal	7.0564	2.1792	0.7167	0.3333	0.0399	0.0040	
				Double	6.9340	2.1561	0.7144	0.3333	0.0399	0.0040	
				Diff. %	1.7346	1.0600	0.3209	0.0000	0.0000	0.0000	
Water	A	12	23.00	Normal	1.1055	0.0984	0.0465	0.0288	0.0068	—	0.0004
				Double	1.0564	0.0981	0.0464	0.0288	0.0068	—	0.0004
				Diff. %	4.4414	0.2946	0.0646	0.0000	0.0000	—	0.0000

See text for atmospheric composition and snow properties.

and an additional 100 cm for water. In each case, parallel computation was done for a normal atmospheric content of CO_2, and then for the doubled level. The attenuation spectra of CO_2, water vapour, ozone and aerosols were taken from refs 19 and 20. CO_2 absorbs in the near IR bands 1.40–1.65 μm, 1.90–2.10 μm, and 2.80–2.90 μm.

Figure 1 shows the calculated spectral distribution of the shortwave radiation flux at different depth levels of the snowpack. The near IR is mostly absorbed in the upper 0.5 cm whereas the visible light is reflected or penetrates to the bottom of a pack. Similarly, in clear freshwater, the near IR is absorbed at the surface, whereas the visible radiation penetrates to deeper layers. Over 90% of the shortwave radiation absorbed in the uppermost 2 mm of snow and water is in the spectral range greater than 1 μm (Fig. 2).

Doubled CO_2 cuts the shortwave-generated heating rate at the snow surface on sites A and B by about 2% (1.7–2.6%, see Table 1) and that of the water surface on site A at noon by 4.4%. At a depth of 0.2 cm the difference is 1.0–1.6% in snow, but only 0.3% in water. The heat flux in snow and water layers below 1 cm is not affected.

Establishment of snow cover profoundly alters the radiative exchange taking place at the land surface because of the high visible albedo and high thermal emissivity of snow. Snow fields become areas of intensive radiative cooling where cold and dry polar atmospheric masses develop. A few days after a snowfall both the albedo and the surface emissivity of snow decreases. This is mainly due to the recrystallisation of snow in the active layer. The changes take place even in uninterrupted subfreezing weather[21–23]. The energy for snow metamorphism is provided largely from the absorbed near IR. As the shortwave transmissivity of ageing snow increases, the temperature at the base of the pack can rise to a sufficiently high level and initiate the bottom melt. Weaver *et al.*[24] described such internal melt of snow in subfreezing weather on top of the fast ice in the Western Davis Strait. Incipient meltwater puddles formed on the ice–snow interface. During 1971–73 the process began in early June and lasted 2–3 weeks, before the snow dissipated and open water pools formed.

It is logical to expect that the CO_2 related reduction of the near IR to the snow surface would delay the recrystallisation, maintain high albedo, and consequently delay the onset of subsurface melt.

Arctic sea-ice breaks under wind stress throughout the year, exposing leads of open water. In winter when the radiative input is low, and evaporative cooling strong, the water refreezes and the cracks are sealed. In spring and summer the exposed water does not refreeze, the ice floes disintegrate by wave action and are blown away into the open water where they melt. Thus the break-up starts when the absorbed radiation in the leads is sufficiently high to prevent refreezing. In clear cold weather, the

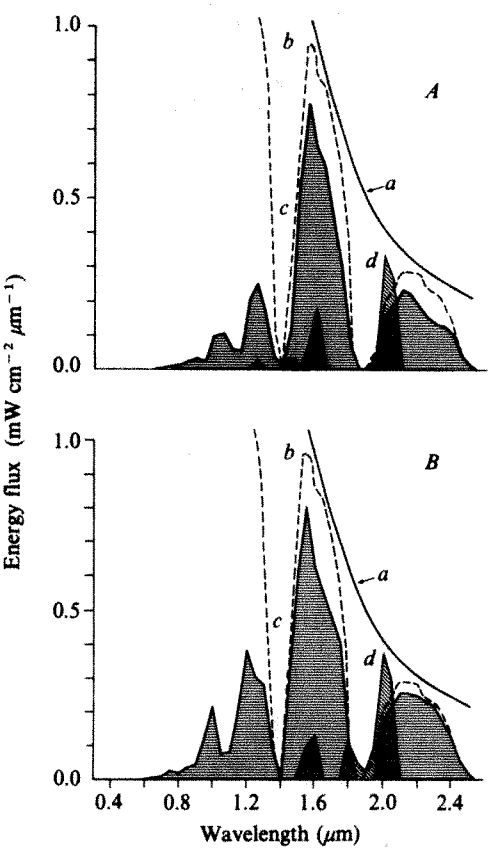


Fig. 2 Shortwave radiation absorbed in the uppermost 0.2 cm of snow (*A*) and clear fresh water (*B*) on site A at noon (see text). *a*, radiation at the top of the atmosphere; *b*, radiation reaching the surface; *c*, absorbed radiation; *d*, deficit due to CO_2 doubling, ×10.

increased CO_2 is likely to delay the onset of the break-up. It is also likely to facilitate an earlier freeze-up of shallow land based fresh water bodies. Apart from CO_2, some other trace gases such as NO_2 or NH_4, and the aerosols of volcanic or industrial origin also absorb in the critical bands and can have a similar effect.

We have to stress that several important radiative and micrometeorological processes were omitted in our model. For instance, the added heating of the atmosphere due to the presence of CO_2 was not calculated. The compensation cannot be complete in a dry subfreezing air of polar origin[27]. But in summer, when the air over the pack-ice in the central Arctic is relatively moist and thick clouds develop, less near IR reaches the ground, and CO_2 probably warms the system.

Obviously we are far from a comprehensive determination of the effect of CO_2 doubling on snow and ice fields. Our study demonstrates, nevertheless, that the final assessment of the CO_2 impact on global climate is too complicated to be reliably solved by simple annually-averaged general circulation models. Predictions of global warming derived from them should be considered highly tentative at best[27]. For reliable answers, improved models will have to be developed to handle in detail the different heating response of various surface types to changing spectral irradiance. Field observations will be needed to test the models.

The global impact of CO_2 increase remains uncertain, but it is possible that the related reduction of shortwave radiation absorbed in snow active layers and at the water surface may contribute to an extension of snow and ice seasons and to an intensified production of cold arctic air masses in winter, spring and autumn. Such a process would be marked by delayed snowmelt in spring, and early snow deposition in autumn, a development not inconsistent with the fluctuations of snow covers observed from satellites in the past 12 yr[25,26].

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An apparent periodicity in an index of volcanic activity

A STOCHASTIC SIMULATION of Lamb's dust veil index[1,2] (DVI) is used here to show that statistically significant peaks in the variance spectrum of geophysical or climatic data are not necessarily evidence of regular, or even quasi-regular, oscillations. The DVI is an annual measure of the amount of volcanic dust in the stratosphere, based on a variety of information, for the period 1500 to the present. Kelly[3] has noted the significant correlation of the DVI with various aspects of climatic change in the North Atlantic–European sector on time scales of 50 yr and more, and has shown that the variance spectrum of the DVI contains a statistically significant peak at 7–8 yr. This peak is also present in the spectra of certain climatic and atmospheric circulation parameters. Kelly[3] has suggested that this apparent periodicity in stratospheric dust content is more likely to be due to the chance occurrence of volcanic eruptions at 7-8 yr intervals during the limited period of record, rather than to a forced, regular cycle in volcanic activity. We now propose an alternate explanation: that the formulation of the DVI produces the spectral peak.

In his formulation of the DVI, Lamb assumes that, unless there is evidence to the contrary, after an explosive volcanic eruption the dust remains in the stratosphere in sufficient quantities to affect the radiation received at the Earth's surface, and hence climate, for four years, and that the dust amount decays linearly over that time. This assessment is largely based on the observed radiation deficits following the major eruptions of the past 100 yr and on the limited number of direct observations of the stratospheric dust load made in recent decades. Most studies of stratospheric dust loading due to volcanic activity assume an exponential, not linear, decay of the original dust injection with a relaxation time of about one year[4]. The linear decay approximates the period of most rapid fall-off of the exponential decay, integrated in yearly steps. Our results are not, however, valid for the case of an exponential decay, and the applicability of the general statistical point made here to the question of associations between volcanic activity and climate will be discussed elsewhere. The characteristic signature of a single eruption, as it appears in the DVI, is a decaying ramp function. The DVI record has an irregularity spaced saw-toothed and at times cyclical appearance due to the superposition of many such events (Fig. 1*a*).

Our hypothesis is that this formulation alone will produce a peak in the variance spectrum of the DVI at about 8 yr: twice the length of the ramp function. A stochastic simulation technique was used to test this hypothesis. Two parameters were allowed

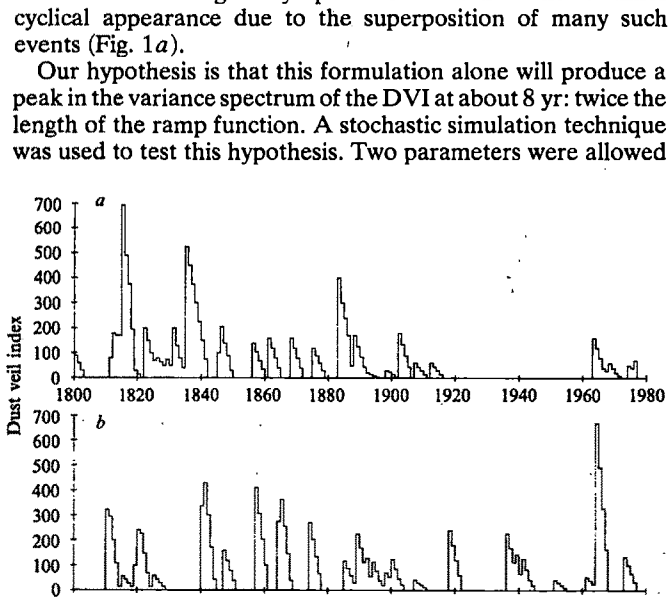


Fig. 1 *a*, Lamb's DVI: 1800–1976[1,2]. The isolated eruptions about 1860 show the ramp formulation clearly. *b*, Stochastic simulation of the DVI, with an eruption probability of 0.3 and ramp length of 4 yr.

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to vary in the stochastic simulations: the probability of an eruption within a particular year, and the ramp length. Simulated DVI records for a range of values of the two parameters were produced by a computer program using a random number generating routine. If the random number (in the range 0–1.0) assigned to a particular year was less than or equal to the eruption probability, an 'eruption' took place with a stratospheric dust injection given by a second exponentially-distributed random number. This distribution approximates to that of the actual DVI. The simulated dust-load was then linearly decayed over a period of years equal to the ramp length. A simulated DVI record is shown in Fig. 1*b*. In this case, the two variable parameters (the eruption probability and ramp length) have been set equal to those of the actual DVI. As it is a stochastic simulation the timing of the individual eruptions cannot be expected to be the same as in the actual DVI. In a series of experiments, the two parameters were varied through a realistic range of values. For each parameter pair, 1,000 records of length 117 yr were generated, the variance spectrum of each record was calculated, and a count was made of the number of peaks that were significant at the 5% level in each frequency band. The length of the simulated records used in the spectral analysis was 117 yr to facilitate comparison with the spectral analysis of the DVI given by Kelly[3]. The spectra were calculated using a fast Fourier algorithm, the spectral estimates were smoothed, and a white or red noise null continuum was assumed in a significance testing depending on whether or not the lag one autocorrelation coefficient departed significantly from zero.

Varying eruption probability over a wide range (0.1–0.9) produced little change in the resulting histograms (Fig. 2). This implies that a degree of superposition of the ramp functions does not obscure the spectral signal with a ramp of a few years' length. The following discussion is therefore restricted to the case of an eruption probability of 0.3 corresponding to that of the actual DVI. While this value is representative of the most reliable portion of the DVI record from 1800 to date, the eruption probability does in fact vary widely on the decadal and century-to-century time scale. The resulting histograms for ramp lengths of 4 and 6 yr are shown in Fig. 3. Clearly, as hypothesised, a spectral peak occurs at a period twice the length of the ramp function. Note that the odd harmonics of twice the length of the ramp function also appear in the histograms. As a control, we show the histogram resulting from an analysis of a set of simulations with zero ramp length (Fig. 3*c*). In this case, the total dust load was assigned to the year of the eruption and none to subsequent years. The histogram is smooth, as would be expected from a large sample of white noise spectra. This confirms that it is the ramp function alone which gives rise to the spectral signals seen in Figs 3*a* and 3*b*.

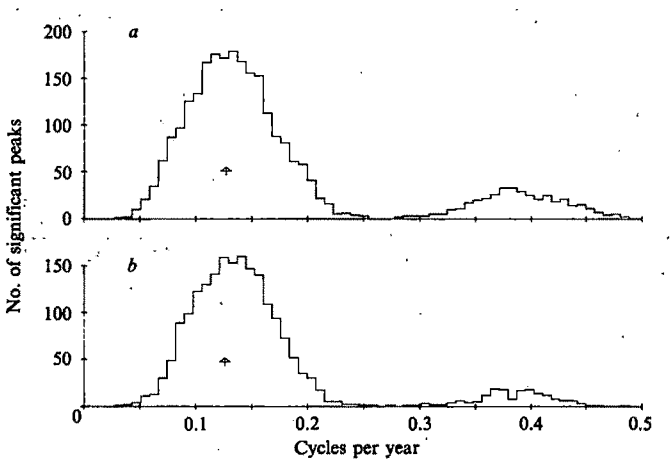


Fig. 2 Count of number of significant peaks per frequency band in the spectra of 1,000 simulations with ramp length of 4 yr and eruption probabilities of: *a*, 0·9; *b*, 0.1. Arrows indicate hypothesised location of spectral peaks.

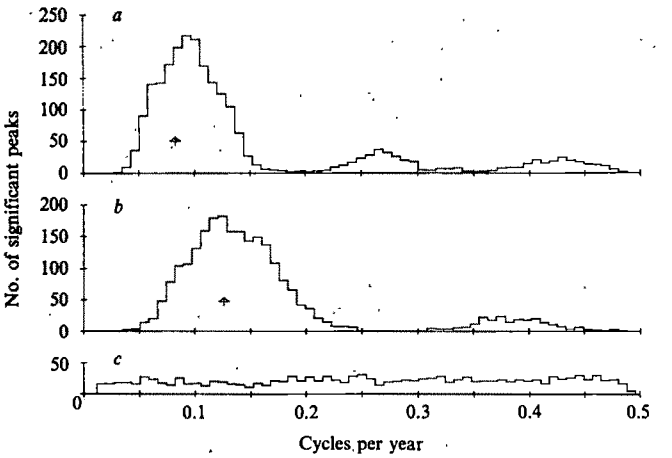


Fig. 3 Count of number of significant peaks per frequency band in the spectra of 1,000 simulations with an eruption probability of 0.3 and ramp lengths of: *a*, 6 yr; *b*, 4 yr; *c*, 0 yr. Arrows indicate hypothesised location of spectral peaks.

Two conclusions can be drawn from this analysis. First, the 7–8 yr peak in the variance spectrum of the DVI is not necessarily evidence of a regular, predictable cycle in volcanic activity. It arises from the formulation of the DVI and indicates the existence of ramp functions of length 4 yr in the data. A 7–8 yr cycle will not necessarily appear in the DVI when filtered, as the DVI is a summation of such ramp functions with random phase. This does not, however, detract from the value of the 7–8 yr spectral peak as a statistical characteristic of the DVI. It is the interpretation of this signal that is under discussion, not its reality, and it can be validly used for comparison with climatic data. The question of whether or not the linear decay is a realistic approximation to the fall-out of stratospheric dust and/or the climatic response to volcanic dust-veils, does, however, warrant further investigation. Second, we note that if there is a mechanism within the climate system which has the decay characteristics of a ramp function, for example, ocean–atmosphere interaction, then this could give rise to a spectral peak which may be misinterpreted as evidence of a regular climatic cycle.

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The Tweeddale Granite—a newly discovered batholith in the Southern Uplands

ABOUT 2,500 gravity stations have been established in South-east Scotland by members of the Department of Geophysics, University of Edinburgh (Fig. 1). We report here a negative long-wavelength gravity anomaly over the Southern Uplands, running SW to NE parallel to the Caledonian trend. The gravity anomaly is interpreted as being due to an underlying granite

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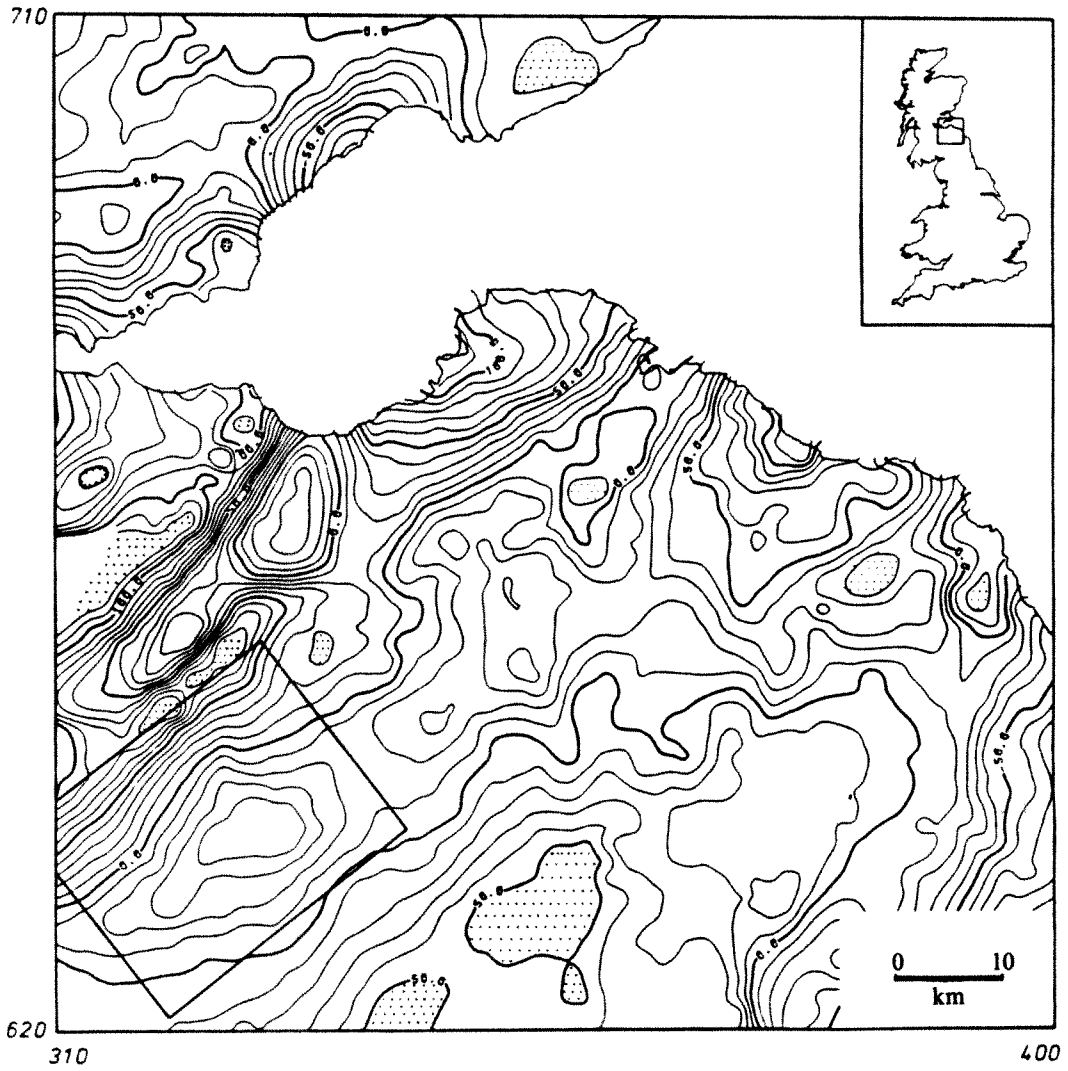


Fig. 1 Bouguer anomaly map of SE Scotland, outlining the area covered by Fig. 2, the residual anomaly map. Gravity data on both figures are referred to ISGN(1971)–GRS(1967). Contour interval 10 gu (= 1 mgal = 10^{-5} m s^{-2}, Stippled closures are high.

batholith, modelled in detail in the district of Tweeddale, where our station density averages 0.5 km^{-2}. Elsewhere in the Southern Uplands, the average coverage is 0.3 km^{-2}.

In Tweeddale the surface geological exposure to the south-east of the Southern Uplands Fault is relatively uniform. The SW–NE trending belt of Ordovician shales and graywackes is followed to the south by similar Silurian sediments as far as the Northumberland–Solway basin. Within the Lower Palaeozoic there are occasional small lenses of black shale, sometimes in the Northern Belt with radiolarian chert and pillow lavas. The whole sequence is interpreted as slices of oceanic sediment emplaced against a continental margin during the closure of the Iapetus Ocean[1]. Most of the small igneous features occur as felsite and porphyrite veins, with some relatively larger exposures, notably the porphyrites at Priesthope Hill (NT3540) and at Juniper Craigs (NT2536). Although most are now only evident as boulders in the drift, some small exposures of granite have been reported[2] and are shown on the Geological Survey map.

Because of the uniformity of the surface geology, the regional Bouguer anomaly field was estimated directly from the LISPB model[3,4], using the Nafe–Drake curve[5] to translate seismic velocity into density. The LISPB model was assumed to be two-dimensional, striking parallel to the Caledonian trend. The resulting residual Bouguer anomaly map is shown in Fig. 2, superimposed on a geological sketch map.

Our laboratory measurements on hand samples of Lower Palaeozoic sediments from many localities in the Southern Uplands fail to show sufficient systematic variation in density to cause the gravity low. Moreover, a two-dimensional extension of Nettleston's method[6], fitting a second degree surface separately to each 10 km National Grid square, also gave only a small density variation: over nine squares, the computed Bouguer density was $2{,}711 \pm 26$ kg m^{-3}, compared with an average from hand specimens of $2{,}708 \pm 21$ kg m^{-3}. The latter was calculated from 46 samples collected from eight locations. If we attempt to model the gravity low by a variation in sediment thickness, quite excessive values (>20 km) are required underlying the centre of the negative anomaly for any reasonable basement density; but it is here that the shortest wavelength features of the anomaly field occur, rather than at the margins. Evidence supporting a granite batholith is discussed below.

The centre of the main gravity low in Fig. 2 is near the outcrop of the numerous minor acid intrusives mapped in the Tweeddale area by the Geological Survey. This, in itself, suggests that the granites may be part of a more significant structure than is indicated in the field. To the SW along the Caledonian trend there are major granite exposures in South-west Scotland and Ireland, where detailed studies have already been carried out[7–11]. Recent regional interpretation of Bouguer anomalies in South-west Scotland[11] suggests that the granite plutons are connected at depth as a single massive batholith extending along the Caledonian trend. The existence of a granite batholith, both in South-west Scotland and along strike in South-east Scotland, is consistent with plate tectonic models for the evolution of the British Caledonides[12,13]. The Tweeddale area has been persistently elevated, at least since Upper Silurian times when it formed part of an emergent land mass called Cockburnland[1,14]. The anticipated mineralisation is only sporadic in the Southern Uplands and is not strongly developed in Tweeddale: historically, lead ore was worked at a few localities near the Tweed Valley[15,16], most extensively at the abandoned Grieston Mine (NT3136) but also at Dalwick (NT1734) and Dumbetha

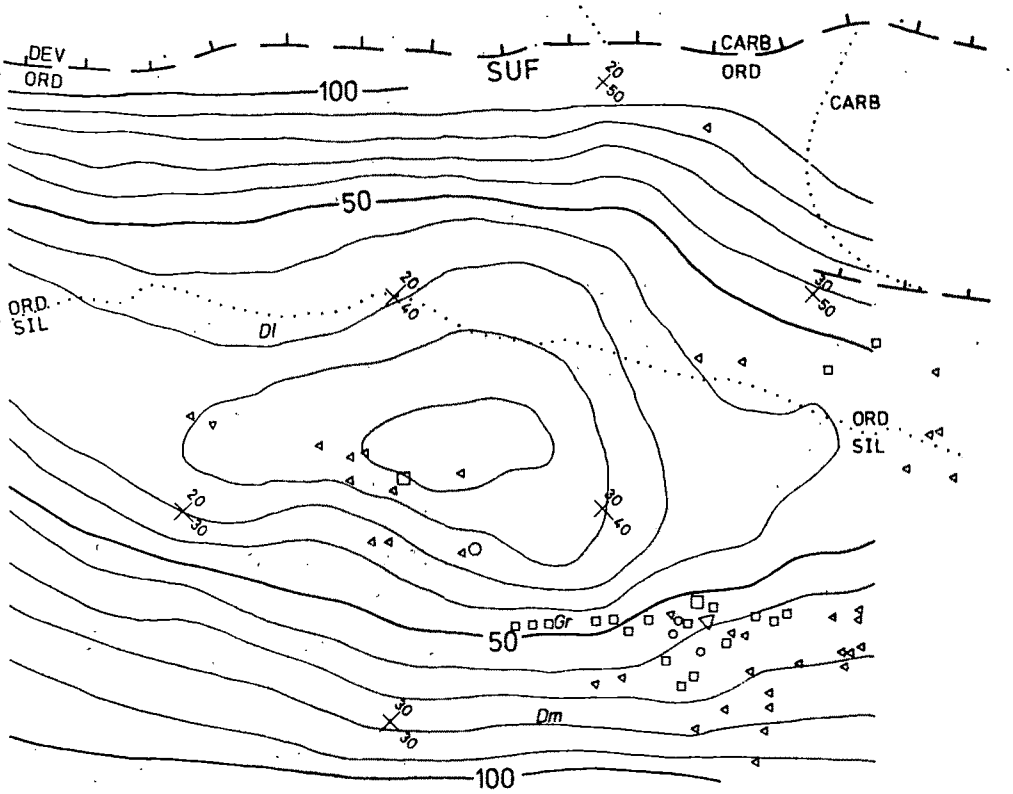


Fig. 2 Residual Bouguer anomaly map of the Tweeddale area, superimposed on a geological sketch map. Contour interval 10 gu. Intersections show the corners of 10 km National Grid squares. ORD, Ordovician; SIL, Silurian; DEV, Devonian; CARB, Carboniferous; SUF, Southern Uplands Fault; Dl, Dalwick; Gr, Griestón; Dm, Dumbetha; △, felsite; □, porphyrite; ○, granite.

(NT3433). Geochemical analyses of organic material in the Moffat Shales[17] do indicate sharply increased thermal alteration towards the north, where we model the granite intrusion.

Residual gravity anomalies, interpolated onto a regular grid, were inverted by a three-dimensional modelling program, which adjusts the topography on a single interface of contrasting density. A unique model inversion exists if the density contrast and the depth to the interface at one point are known and the depth is single-valued everywhere.

A density contrast of $-70\ kg\ m^{-3}$ between Ordovician and Silurian sediments and the granitic rock was assigned (2,720–2,650 $kg\ m^{-3}$). Published densities of exposed granitic rocks in South-east Scotland[2] vary between 2,630 and 2,800 $kg\ m^{-3}$ and do little to constrain the interpretation. From a few massive boulders on Kailzie Hill (NT2836) and Kernie Law (NT3439), we found a saturated density of 2,628 (2,644) ± 9 $kg\ m^{-3}$ (11 samples), with 2,663 (2,674) ± 27 $kg\ m^{-3}$ (42 samples) for nearby porphyrites and felsites (grain densities in brackets). In South-west Scotland, the Fleet granite has been modelled[10] with a density of 2,630 $kg\ m^{-3}$, while the Griffel granodirite was interpreted[8] with a gradational density between 2,640 and 2,710 $kg\ m^{-3}$.

The depth-scale is more uncertain: a three-point depth rule[18] suggested that the interface is less than 2.6 km deep near the centre of the anomaly, while the maximum amplitude/maximum gradient ratio put the centre of mass at < 6.5 km. Figure 3 shows a typical three dimensional model inversion, here with a central point fixed at 2 km depth. The shape is typical for a batholith with outward sloping sides extending to a depth of 10–12 km and three small bosses coming near to the surface close to the porphyrite outcrops. This model is consistent with the barycentre estimate as well as seismic and magnetotelluric models showing a boundary at about 12 km depth.

Ingham (personal communication) finds a highly resistive layer ($10^4\ \Omega m$) overlying a good conductor ($10^2\ \Omega m$) at a depth of 10–12 km, from a one-dimensional inversion of magnetotelluric data from sites at Earlyburn (NT2249), Peebles (NT2740), Yarrow (NT2924) and Borthwick Brae (NT3616). The high overlying resistivity ($10^4\ \Omega m$) is indicative of a low-porosity igneous or metamorphic rock and so is also consistent with our granite hypothesis. Jacob[19] interpreted seismic arrivals at the Eskdalemuir array in terms of a 6.44 $km\ s^{-1}$ refractor at a depth of 12 km, a model which is not inconsistent with the later LISPB result[3], where this mid-crustal refractor was only poorly constrained. Although the LISPB profile actually crosses the Tweeddale area, it should not be expected to show the lateral

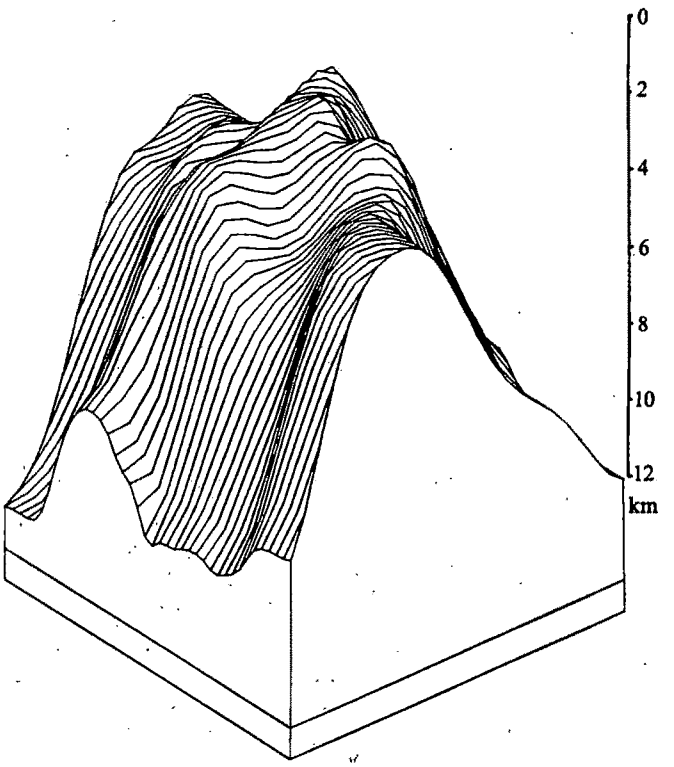


Fig. 3 Three-dimensional model of the upper surface of the Tweeddale Granite, viewed from the west and covering the same area as the residual anomaly map in Fig. 2. The computation includes infinite horizontal extensions from all edges of the model, the beginnings of which in the SW–NE direction are included in the drawing.

extent of the granite batholith: the 5.84 ± 0.02 km s^{-1} velocity observed for the supposedly Lower Palaeozoic sediments does not contrast with values found generally for granites[20,21], although the S-wave arrivals do give an unusually low Poisson's ratio[22], consistent with quartz enrichment.

Figure 1 suggests that the negative gravity anomaly, and therefore the granite batholith, extends northeastwards from the Tweeddale area towards the Berwickshire coast, although the pattern is confused by the Devonian sedimentary basins in Lauderdale and near Oldhamstocks (NT7471) and Eyemouth (NT9464). From the aeromagnetic map[23], and more clearly from filtered versions of it[24,25], there is reasonable long-wavelength continuity of a weak magnetic high, flanked to the north by a corresponding low, from the Criffel-Fleet-Doon plutons in SW Scotland to the Tweeddale area and thence northeastwards towards the coast. The 'granites' of Berwickshire and E. Lothian[2,26] show close petrological affinities to those in South-west Scotland. Only the Priestlaw (NT6562) and Lammerlaw (NT5163) exposures show localised, stocklike, positive magnetic anomalies, although all appear to be related to the same weak regional magnetic high traceable along the whole NE–SW length of the Southern Uplands. It seems reasonable to postulate that the whole Southern Uplands are underlain by the batholith, which is responsible for their longstanding and continued relatively high elevation and stability.

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Lichenometry and earthquake age determination in central Asia

THE age of rock surfaces can be determined from the size of lichens growing on them[1]. We present here measurements of the species *Lecidea lactea* growing on rocks from the avalanches which occurred after the 1907 (Karatag) and the 1949 (Khait) earthquakes in Tadjikistan, Middle Asia. We show that their forerunning strong earthquakes occurred more than 100–130 yr previously.

The method of using lichens to date rocks is based on two assumptions: first, that the yearly rate of lichen growth is constant (on average within a given area), and second, that lichens settle on a newly exposed rock surface during the first year of exposure. We used lichens of species *Lecidea lactea* Ach. growing approximately in circles, which are convenient shapes for measurements. Each sample included, wherever possible, 100 measurements of the lichen diameter on compact groups of homogeneous rock surfaces.

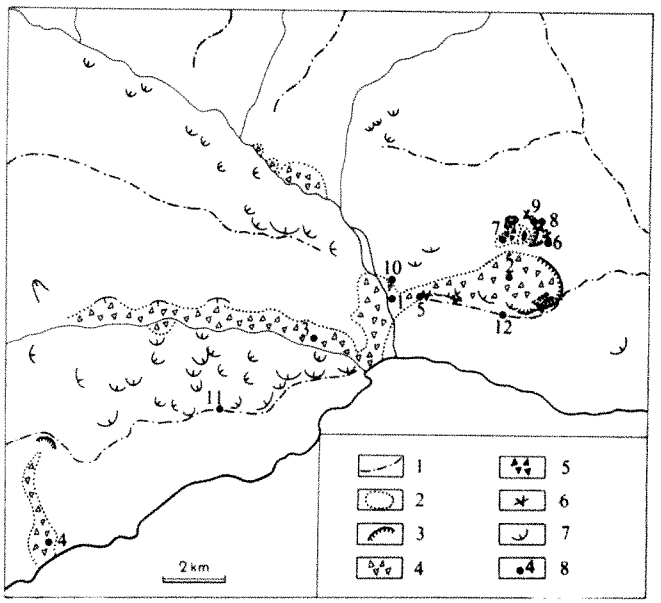


Fig. 1 Scheme of seismic dislocations and sites of lichenometric measurements in the epicentral area of the 1949 Khait earthquake. 1, Watersheds; 2, contours of landslides; 3, fracture walls of rock landslides; 4, landslides of 1949; 5, ancient landslides; 6, seismoravines in basement rock; 7, earth avalanches and slips of (mainly loess) rock; 8, sites of lichenometric measurements.

The investigation was in two stages: (1) the average growth rate of lichens on geologic objects of known age was determined, and (2) the age of undated objects was determined from measurements of the lichen diameter using the average growth rate values obtained.

The area of the 1949 Khait earthquake lies on the right bank of the Surkhob river in the outskirts of the village of Khait. The earthquake ($M = 7.4 \pm 0.1$) had caused a large landslide and an avalanche (length 10 km, height above 50 m) of mud and stone, as well as producing seismic ravines in hard rock[2–4]. To determine the average growth rate of lichens in this area, its dependence on exposure conditions and altitude (the ecology conditions) had to be estimated first. Two series of measurements were taken of boulders and debris in the upper and lower portions of a landslide[2,3] in the epicentral area of the 1949 Khait earthquake. The diameter distribution curves of lichens inhabiting the southern and northern surfaces of the boulders and debris were practically identical. Thus, the rate of growth can be taken to be independent of exposure conditions, but is highly dependent on altitude (Table 1) and this should be included in the calculations.

Table 1 shows that a number of statistical parameters of the rate of growth have been used: the arithmetic mean of diameter values (X_{mean}), the diameter of the most frequently occurring

Table 1 Basic statistical characteristics of average yearly growth (in mm) of lichens of species *Lecidea lactea*

Statistical characteristics of growth from diameter measurements	Khait area (mm yr^{-1}) Site 1 Altitude (m) 1,600	Khait area (mm yr^{-1}) Site 2 Altitude (m) 2,000	Karatag area (mm yr^{-1}) Site 1 Altitude (m) 1,700
Arithmetic mean, X_{mean}	0.54	0.47	0.38
Most frequent, X^*	0.48	0.40	0.38
Largest, C^*_{max}	1.20	0.94	—
Maximum, X_{max}	1.30	1.00	0.75
Yearly change of variance	0.14	0.13	0.092

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Table 2 Lichenometric characteristics and age of various objects on the epicentral zone of the 1949 Khait earthquake

Group	Measurement sites	Description of object	Altitude (m)	Sample size	Statistical characteristics of lichen diameter (mm above) and age determinations (yr below) X_{mean}	X^x	σ	X^x_{max}	X_{max}	Average age and confidence interval (yr)	Time of event (yr)
(1)	3	Boulders in landslide on right bank of Yagman river 2.5 km west of village of Khait	1,600	100	13.1 24	13.5 28	3.8 27	30.5 25	30.5 23	25±1	1950±55
(2)	4	Boulders in landslide on right bank of Surkhob river around village of Mirtob	1,500	100	15.5 27	11.5 23	6.4 44	36.5 28	36.5 27	30±7	1940–50
	5	Ravine walls in western part of latitudinal watershed 0.5 km NE of village of Khait	1,650–1,750	97	15.8 30	14.5 31	4.5 33	31.5 27	32.5 26	29±3	1940–50
	6	Renewed ravine walls in northern part of meridional watershed 5 km NE of village of Khait	3,000	16	13.9 27	13.5 36	2.3 19	— —	15.8 —	27±7	1940–60
	7	Ancient landslide 4 km NE of village of Khait	2,200	42	75.0 160	88.0 220	17.5 140	— —	— —	170±70	1740–1880
(3)	8	Walls of ancient ravine on meridional watershed 5 km NE of village of Khait	2,800	10	99.5 200	75.0 190	24.0 190	132.5 —	132.5 —	190±10	1780–1800
	9	Walls of another ancient ravine, same location	2,800–3,000	22	71.1 150	73.0 180	20.0 160	112.5 —	113.0 —	160±20	1800–40
(4)	10	Ancient exposed surface near the foot of a slope down to Yarkhych river, 0.7 km NE of village of Khait	1,600	62	102.8 190	94.0 195	26.0 185	170.0 140	176.9 130	170±30	1780–1840
	11	Rock exposures at the ridge of a range 6 km WSW of village of Khait	2,400–2,450	50	92.1 195	82.5 210	25.0 220	160.0 170	163.9 165	190±20	1770–1810
	12	Rock exposures at a latitudinal ridge 3 km east of village of Khait	2,500	37	107.4 230	105.0 206	25.0 200	195.0 210	197.5 200	220±30	1730–90

Table 3 Lichenometric characteristics and age of various objects on the epicentral zone of the 1907 Karatag earthquake

Group	Measurement sites	Description of object	Altitude (m)	Sample size	Statistical characteristics of age determinations (yr) from X_{mean}	X^x	X^x_{max}	X_{max}	Average age and confidence interval (yr)	Time of event (yr)
	2	Boulders in a small landslide on right bank of Karatag valley 1 km east of village of Chuyanchi	1,700	28	62	49	89	51	63±22	1890–1930
(1)	3	Walls of a seismoravine on watershed near village of Chuyanchi	1,800	6	77	—	71	49	65±25	1890–1940
	4	Walls of an adjacent ravine same location	1,800	20	69	69	61	60	65±6	1910–20
(2)	5	Boulders in a fresh landslide on left bank of Karatag river 2.5 km NNE of northern edge of village of Karatag	1,150	63	32	29	28	27	29±3	1945–50
	6	Ravine on a slope of Karatag valley same location	1,200	10	28	24	34	21	27±7	1940–60
	7	Boulders in an ancient landslide on left bank of Karatag river 4 km NE of northern edge of village of Karatag	1,700	73	105	84	174	130	123±43	1810–1900
(3)	8	Boulders in an ancient landslide on right bank of Karatag river above village of Naalbek	1,600	47	238	236	182	173	212±41	1720–1810
	9	Ancient seismoravine on left bank of Karatag river 3 km NNE of northern edge of village of Karatag	1,200–1,300	38	203	203	149	157	178±34	1770–1830

The average age has been calculated for a reference height of 1,700 m.

objects (X^x), the mean diameter of the largest objects (X^x_{max}), the maximum diameter (X_{max}). We have also estimated the variance (mean squared deviation) (σ). We believe that such an approach greatly improves the accuracy attainable using the maximum diameter measurements alone[1,5] and confirms the desirability of combining several characteristics of growth rate[6].

Before lichenometry could be applied to objects of unknown age, they had to be classified into several groups according to outward appearance and geomorphologic criteria: (1) boulders in landslides probably dating from the 1949 earthquake (from indirect indications see refs 2, 3, and outward appearance); (2) walls in ravines that were either produced or renewed by the earthquake (our unpublished data); (3) ancient ravines and a landslide that were probably produced by a large earthquake

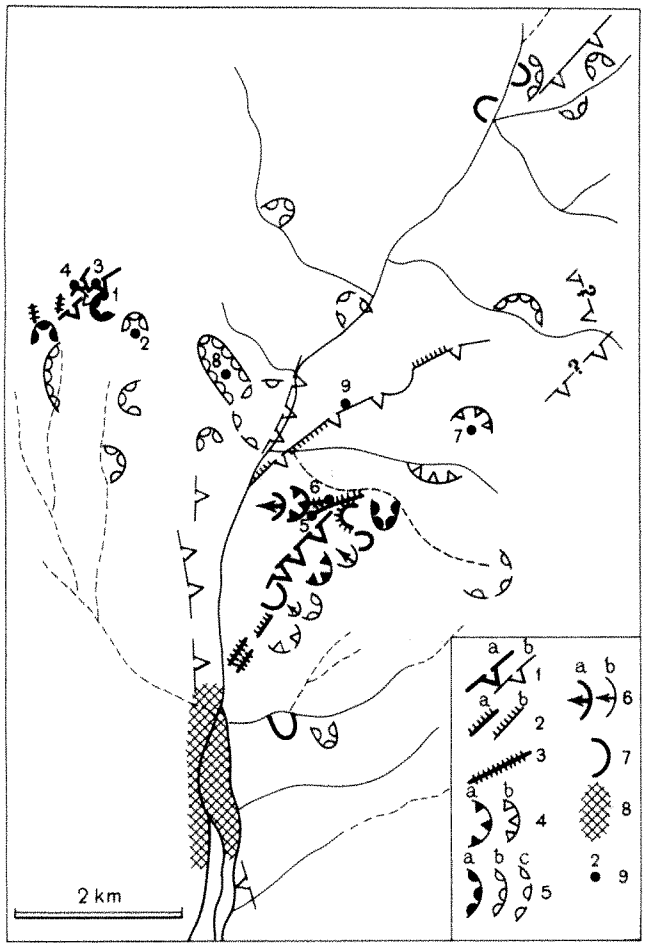


Fig. 2 Scheme of seismic dislocations and sites of lichenometric measurements in the epicentral zone of the 1907 Karatag earthquake. 1, Seismoravines; 2, terraces, escarpements; 3, cracks in loose sediments; 4, fracture walls of rock landslides; 5, landslides; 6, earthavalanches and slips of loose rock; 8, village; 9, sites of lichenometric measurement. Age by geomophologic criteria: a, fresh; b, somewhat earlier (late Holocene); c, ancient (pre-Holocene).

before 1949 (our unpublished data); (4) hard rock surfaces that are certainly older than the 1949 or any previous earthquake, or which were renewed during it.

Two or three objects from each group have been measured. Table 2 shows that recent landslides in the Yagman river valley and a part of the Surkhob valley around the village of Mirtob were actually formed during the 1949 earthquake; the latitudinal ravines in the watershed to the east of Khait were indeed formed during the 1949 earthquake, whereas the meridional ravines are much older, and were only renewed in 1949; the ancient landslide and ravines in the upper Daraihaus river are approximately of the same age, so that they can be considered as paragenetic formations of an earlier large seismic event (or several consecutive events); the earthquake-generated surfaces are younger than the other hard rock surfaces, that is the age of the early seismic dislocations in our study is not limited by the extreme size of lichens in the present conditions.

Thus, we may conclude that the epicentral area of the 1949 Khait earthquake had been struck by a similar sized earthquake a long time previously.

The 1907 Karatag earthquake area ($M = 7.4 \pm 0.1$) faces the southern foot of the Hissar range 50 km west of the town of Dushanbe. The objects studied by lichenometry in the epicentral area of the Karatag earthquake can also be classified into a number of groups: in addition to the wall of a joint that certainly results from the Chuyanchi earthquake of 21 October 1907 (ref. 7), and which is used here to determine the average growth rate of lichens in this part of the area (see Table 1), there are the following: (1) ravine walls and landslide boulders which we tentatively ascribe from their positions and outward appearance to the 21 October 1907 earthquake; (2) boulders in the landslide and walls in the ravine, supposedly a result of the 8 October 1907 earthquake; (3) boulders in ancient landslides and the walls of an ancient ravine, here associated with an earthquake(s) before 1907.

The results of lichenometric measurements in the Karatag area support the idea[8] that the landslide and ravines near the village of Chuyanchi (group (1)) are associated with the Chuyanchi earthquake of 21 October 1907. The landslides and ravines on the left bank (group (2)), which we had supposed to originate from the Karatag earthquake of 8 October 1907 have turned out to be much younger (29 ± 3 yr and 27 ± 7 yr from the age of the lichens. We consider, however, that this age only reflects a renewal of the surface rather than formation of the objects themselves.

The group (3) formations ascribed to an earlier earthquake some hundreds of years before 1907 (refs 8, 9), have an age of 200 yr according to lichenometry. A landslide on the left bank first investigated here (measurement site 7 in Table 3) can be tentatively associated with the large 1863 earthquake west of the Hissar range from some age determinations from the lichens on the boulders (123 ± 43 yr).

The two largest earthquakes that we know to have occurred in the Hissar–Kokshaal fault zone in Tajikistan cannot be considered to be random events as regards their place and time of occurrence. The investigation area has been struck with large ($6 < M \leq 7.5$) earthquakes during the past few hundred years with an interval of recurrence of at least 100–130 yr, but more likely 200–250 or more years.

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Do circadian oscillators ever stop in constant light?

A TWO-DIMENSIONAL limit cycle model (Fig. 1) predicts the behaviour in darkness of both the *Drosophila pseudoobscura* circadian eclosion rhythm[1,2] and the mosquito *Culex pipiens quinquefasciatus* Say (*C. p. fatigans* Wied.) flight activity rhythm[3,4]. This model has been extended to explain features of rhythms in very dim light, but in constant 'bright' light it has been suggested that the dynamics are fundamentally different, with the system having a globally stable equilibrium point and hence no stable oscillation[1]. We propose here a simplifying modification: for each light intensity the dynamics are topologically equivalent, and thus for each there is a limit cycle, its position and amplitude being continuous functions of intensity. This interpretation avoids the awkward discontinuity between 'light' and 'dark' dynamics and emphasises the possibility of time-keeping at all light intensities. Furthermore, it provides a simple explanation for novel features of the *Culex* rhythm described here and previously[3], and allows us to treat some

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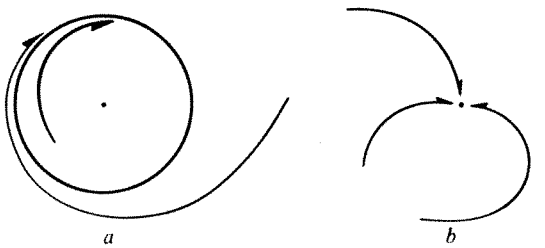


Fig. 1 *a*, Outline of the two-dimensional limit cycle model. The two variables are left unspecified, the oscillator is merely assumed to be attracted in darkness to a closed cycle, here represented by the bold circle. This cycle, the limit cycle, is assumed to be stable. If the oscillator is displaced from the cycle (typically by exposure to light), it will return as indicated by the two sample trajectories. There must be at least one point, the critical point, within the region bounded by the cycle, from which this return is indeterminate. *b*, A globally stable equilibrium point. All trajectories are attracted to a single point and thus such a system will always eventually reach a fixed non-oscillatory state.

important differences between *Culex* and *Drosophila* as merely quantitative. Finally, it suggests a new general strategy for the analysis of circadian rhythms and allows extensions of established theory that explain previously paradoxical results obtained for the two species (E.L.P. in preparation).

Both the *Drosophila* and *Culex* rhythms persist in continuous darkness, following, according to most models, a limit cycle in the two-dimensional state space. Phase is assigned to all limit cycle states relative to an arbitrary reference point. States out of the cycle can only be defined operationally: for example, that state realised after a phase 0 population is given a 1 s, 1 lx perturbation. However, states can be compared with the following protocol: transfer samples to darkness and let the original states be equivalent if the settled rhythms in darkness are in phase. The state space is thereby partitioned into equivalence classes or isochrons[2] (Fig. 2). Each isochron must include a unique limit cycle state and is labelled accordingly (isochron θ contains the state with phase θ). In practice, a state's isochron is determined by an assay experiment entailing transfer to darkness and comparison with a sample having known phase. In fact, not quite all states belong to an isochron, from some the return of the rhythm is unpredictable or not apparent. Such states comprise the phaseless set, which must include at least one point in the region bounded by the limit cycle.

The simplest possible topology is illustrated by

$$\dot{x} = y + \varepsilon x(a^2 - x^2 - y^2)$$
$$\dot{y} = -x + \varepsilon y(a^2 - x^2 - y^2) \qquad (1)$$

which has a limit cycle of radius a, centred on the phaseless critical point at the origin and straight, equally spaced radial isochrons. Each trajectory moves with constant angular velocity about the origin, independently of a and ε.

Our working hypothesis is that in darkness the circadian dynamical system has qualitatively the same form as equation (1). Note that in such cases, the isochrons divide state space much as longitudinal lines divide the Northern Hemisphere (Fig. 2), both systems yield incomplete yet useful information about location. The release–assay experiment uses this information to map a trajectory's route through state space: a population is synchronised at a chosen state, the release point, then released to free-run in constant test conditions—light of fixed intensity. The population follows the trajectory dictated by the dynamics at that light intensity. By periodically removing an aliquot and assaying the phase in constant darkness, the population's progress across the isochron map can be monitored. A plot of isochron against time gives an assay curve.

While this formal presentation and interpretation of release–assay experiments is new, the experimental design is not. Pittendrigh's *Drosophila* experiment[5] is one archetype (Fig. 2*a*). The dynamics in bright light apparently force trajectories to a point on one isochron, which, for this reason, we have defined to be isochron 0.

With these two concepts—a limit cycle in darkness and a globally stable equilibrium point in light—one can explain most light pulse phase resetting phenomena in *Drosophila*[2] and *Culex*. Yet, because the trajectory patterns in the two cases are topologically different (one with a limit cycle, one without), somewhere on the continuum of light intensity a change of form must occur. We prefer to eliminate the discontinuity, proposing instead that the trajectory patterns for all light intensities are topologically equivalent and, in particular, that a limit cycle always exists. For example,

$$\dot{x} = (y - b) + \varepsilon x(a^2 - x^2 - (y - b)^2)$$
$$\dot{y} = -x + \varepsilon(y - b)(a^2 - x^2 - (y - b)^2) \qquad (2)$$

where $a = \exp(-kI)$ and $b = y_\infty(1 - \exp(-kI))$ is a family of systems parametrically defined by light intensity, I. Each member of equation (2) is just a coordinate shift of a corresponding member of equation (1). Hence for each light intensity there is a limit cycle, radius a, centred on a single critical point $(0, b)$, the angular velocity of every trajectory around the critical point for that intensity is constant. As intensity tends to infinity, the corresponding system tends

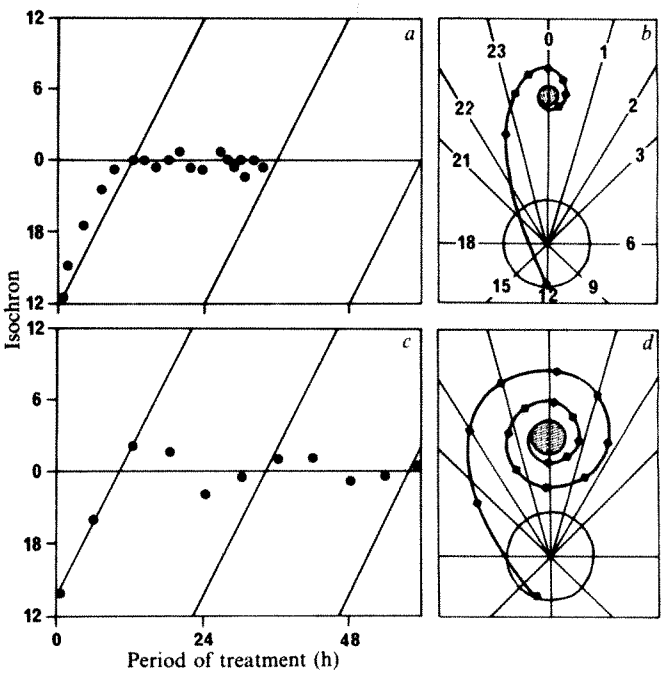


Fig. 2 *a*, *c*, Assay curves. The set of diagonals comprise the assay curve which would necessarily be obtained if the treatment condition were darkness and correspond to a population continuously following the dark limit cycle. *b*, *d*, Corresponding route maps. The route map is an interpretation of the associated assay curve. Each includes the dark limit cycle (unshaded circle), radial isochrons and the limit cycle assumed to exist in the given test conditions (shaded circle). Superimposed is the trajectory of the sample, inferred from the assay curve, assuming the dynamics are approximately those of equation (2). The predicted position of the sample every 3 h is indicated on the trajectory. *a*, *Drosophila pseudoobscura* assay curve. Data are redrawn from Fig. 7 of ref. 5. The release point was 'light on' in LD 12 : 12 (12 h light, 12 h dark), the test condition was 1,080 lx. Isochron 0 to which the assay curve seems to tend corresponds to Pittendrigh's ct: 12. *b*, *Drosophila pseudoobscura* route map. The proposed limit cycle is so small that any oscillation would be masked by the large experimental uncertainty. *c*, *Culex pipiens quinquefasciatus* assay curve redrawn from Fig. 2 of ref. 3. The release point was 'light on' in LD 12 : 12, the test condition was 50 lx. Each point is a mean of 24 mosquitoes with s.e. < 15 min. (The error bars in ref. 3 Fig. 2 are standard deviations.) *d*, *Culex pipiens quinquefasciatus* route map. The assay curve moves rather more quickly to its first maximum (12 h) than predicted, assuming constant angular motion around the 50 lx critical point.

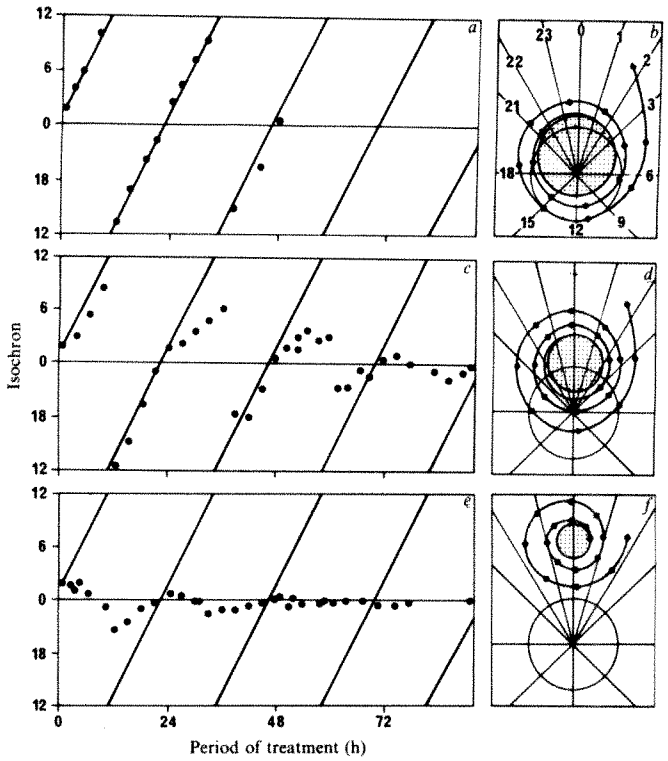


Fig. 3 The general organisation is as in Fig. 2. 'Light off' in LD 12:12 (180 lx) was chosen as the release point. (We have noted no difference between 50 and 180 lx.) Activity was monitored acoustically[4] and each point is the mean for 24 mosquitoes. *a*, *b*, 0.05 lx. The population follows the dark assay curve for the period examined. We infer that the 0.05 lx limit cycle is rather close in amplitude and position to the dark limit cycle. *c*, *d*, 0.5 lx. During the first 24 h the assay curve is near the dark assay curve. Correspondingly, the first cycle of the route map trajectory curls around the singularity cutting all isochrons. Over the second 24 h the assay curve jumps from isochron 6 to isochron 18, a result predicted if the trajectory cuts across the singularity at this time. Subsequent turns of spiral exclude the singularity, thereby failing to cut most isochrons. This is reflected by the reduced excursion of the assay curve in later cycles. *e*, *f*, 8 lx. The data points of Fig. 2*c* fall almost perfectly on those of *e* (after a 12-h time shift) indicating that the dynamic systems corresponding to 8 and 50 lx probably differ very little. The limit cycle, if it exists at this intensity, is too small to detect above experimental error, although the transient spiral is readily apparent.

smoothly toward the limiting case—the system with a stable focus at $(0, y_\infty)$.

This scheme is compatible with the *Drosophila* result, assuming that the amplitude of the limit cycle is sufficiently reduced for the oscillation to be experimentally irresolvable (Fig. 2*b*). However, it was the comparable experiment for *Culex*[3] (Fig. 2*c*) which yielded tangible evidence for the model. A spiral trajectory asymptotically approaching a limit cycle (Fig. 2*d*) is the only plausible explanation for the form of the assay curve, and the persistence of a circadian activity rhythm in the test conditions[3].

We propose that the organisation of these two dipteran circadian systems is qualitatively similar, discrepancies reflecting differences in treatment intensity and light sensitivity in the two cases. In support of this, note that a circadian eclosion rhythm continues in *Drosophila* in very dim light (0.05 lx white light[6] or 0.01 erg cm^{-2} blue light[2]), although an assay curve or its equivalent has never been presented.

The set of dim light assay curves for *Culex* suggests the smooth transitional sequence between darkness and light demanded by the model (Fig. 3). The curve for 0.5 lx (Fig. 3*c*) is of critical significance. There is, between 36 and 39 h, a phase jump of about 12 h, a feature compatible with the model only if the trajectory passes near the singularity of the isochron map during that time. Winfree[2] reports that for *Drosophila* the region around the singularity is a phaseless zone from which a return to rhythmicity is not observed in the dark. Results suggesting a similar phenomenon for *Culex* were seen in the 36 and 39 h assays. Depressed arrhythmic activity was recorded in these two samples, assumed to be lingering near the singularity. Although to some extent the result of population incoherence, the effect is obvious in individual activity records. This damping effect, like that reported for *Drosophila*, is reversed by a period of bright light, as the model requires.

We first proposed that the systems had straight isochrons merely to make diagrams easy to draw and predictions correspondingly clear. Even so, in addition to the phenomena considered here, other features including phase resetting, steps up and down in light intensity and entrainment can be explained without seriously weakening this simplifying assumption. Our philosophy is thus to maintain this general framework until an obvious inconsistency appears.

The existence in *Culex* of activity rhythms in light suggests an obvious test of the model's consistency. Choosing any intensity one can select a phase marker, partition the state space into a new set of isochrons corresponding to that intensity, and conduct release–assay experiments using this new isochron map as a reference frame. Test conditions can be any light intensity including darkness. The form of these new assay curves can be predicted from that of the old curves assuming constant angular motion. Such experiments are in progress.

We have considered here the dynamics of a single oscillator, although it is almost certain that circadian rhythms reflect the interaction between coupled oscillators. Nevertheless, as Pavlidis[7] argues, preserving this duality in the description of circadian rhythms is useful and leads to no important contradiction.

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Relative 'trophic' influences of excitatory and inhibitory innervation of locust skeletal muscle fibres

DENERVATION of locust skeletal muscle, where L-glutamate is thought to be the excitatory neurotransmitter[1], induces a pattern of physiological and pharmacological changes similar to that following denervation of vertebrate skeletal muscle[2,3]. The degeneration of nerve terminals on locust muscle which follows axotomy[4] is accompanied by a significant increase in the sensitivity of the extrajunctional membrane to L-glutamate[5–8], as well as a fall in resting membrane potential[8,9] and input conductance[8] of the denervated fibres. Locust skeletal muscle fibres are multiterminally and sometimes polyneuronally innervated. In addition to excitatory inputs some fibres receive endings from inhibitory axons in which transmission is probably mediated by γ-aminobutyric acid (GABA)[10]. This dual nervous control at the

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peripheral level allows examination of the separate 'trophic' influences of excitatory and inhibitory inputs to a muscle fibre. In this report we demonstrate that in at least one locust muscle, the metathoracic retractor unguis (RU) of *Schistocerca gregaria* which contains fibres receiving both excitatory and inhibitory inputs, denervation-induced changes can occur in the presence of functionally intact inhibitory innervation.

The femoral portion of the RU muscle consists of two bundles of long (1 cm) fibres, called 'red' and 'white', usually containing eight and nine fibres, respectively[4,11]. Each bundle of fibres is innervated by one of a pair of large ('fast') excitatory motor axons which run in a branch (B_2) (ref. 4) of metathoracic nerve 5 (Fig. 1). Previous studies have suggested that the innervation of the RU is restricted to excitatory neurones; however, antidromic stimulation of $5B_2$ (distal to the point where it sends a process to the RU muscle to avoid stimulation of the excitatory inputs to this muscle) as well as metathoracic nerves 3 and 4 (see Fig. 1) usually evoked either a small depolarisation or hyperpolarisation in the red but not in the white fibres (Fig. 1) (C. Walther, personal communication). The properties of this response were identical to those of inhibitory postsynaptic potentials (i.p.s.ps) found in other locust muscles[10]: for example, it was reversibly abolished in Cl-free saline, and it had a reversal potential within a few millivolts of the resting potential (−50 to −60 mV in 10 mM K, locust saline[10]) (Fig. 1). Although i.p.s.ps could sometimes be seen in red fibres only when the membrane potential was displaced by ±5 mV, they were never recorded from white fibres even when the membrane potential was altered by ±15 mV.

Topically applied GABA (10^{-4}–10^{-3} M) caused ~50% reduction in the input resistance of the red fibres and also abolished the i.p.s.ps. Picrotoxin (5×10^{-4} to 10^{-3} M) reversibly abolished the i.p.s.ps and blocked the change in input resistance caused by GABA. Responses to GABA ejected iontophoretically from one barrel of a double-barrelled micropipette could be obtained from those regions in the clefts between fibres where a large, fast-rising depolarisation to glutamate was obtained from the second barrel, but not from extrajunctional areas where the glutamate sensitivity was very low. The reversal potential of the GABA response was similar to that of the i.p.s.ps. Bath application of GABA caused a significant change in input resistance of some of the white fibres, suggesting that perhaps these fibres receive an inhibitory input, although i.p.s.ps were not recorded from them.

In part of this study the RU muscle was denervated by sectioning metathoracic nerve 5 using an aseptic procedure[9]. It is possible that, like the RU excitatory axons, the inhibitory innervation to this muscle runs in metathoracic nerve 5, although it probably supplies other muscle fibres in the leg in addition to those of the RU. The RU on one side of each animal was denervated; the contralateral muscle was left unoperated to serve as a control. Operated animals were maintained in separate containers at 30 °C and fed fresh grass daily. Severing nerve 5 interrupts both the excitatory and inhibitory inputs to the RU simultaneously, and it is not feasible to section selectively excitatory and inhibitory axons. To selectively remove the excitatory inputs to the muscle, we have developed a procedure which leaves the inhibitory innervation of the RU muscle intact while deleting the excitatory input to this muscle. The procedure involved surgically excising that part of the metathoracic ganglion which contains the cell bodies of the RU excitatory motoneurones (Fig. 1*a*), but metathoracic nerve 5 was not sectioned. Animals operated in this way survived well for up to 4 weeks, which was the longest period studied.

Excitatory motor nerve terminal degeneration was complete 2–3 d after section of nerve 5, as judged by the absence of spontaneous miniature excitatory postsynaptic potentials and lack of response of the RU muscle to stimulation of the distal stump of nerve 5 (ref. 12). The sensitivity of the extrajunctional muscle membrane to L-glutamate applied iontophoretically through high-resistance (100–200 MΩ, 0.1 M Na-glutamate, *p*H 8) micropipettes was examined at various times up to 36 d after section of nerve 5 (and sometimes also nerve 3). The extrajunctional glutamate sensitivity increased significantly compared to control levels 4–6 d after nerve section, and reached a peak after 12–15 d, when the extrajunctional glutamate sensitivity was about 30 times greater than that of control muscles. The increase in extrajunctional sensitivity was accompanied by a decline in membrane potential and an increase in the input resistance of the muscle fibres[13]. These changes were similar in the red and white fibres. Animals with operated ganglia were examined 14 or more days post-operatively. By this time excitatory innervation had degenerated and the extrajunctional membrane of the RU muscle exhibited a high

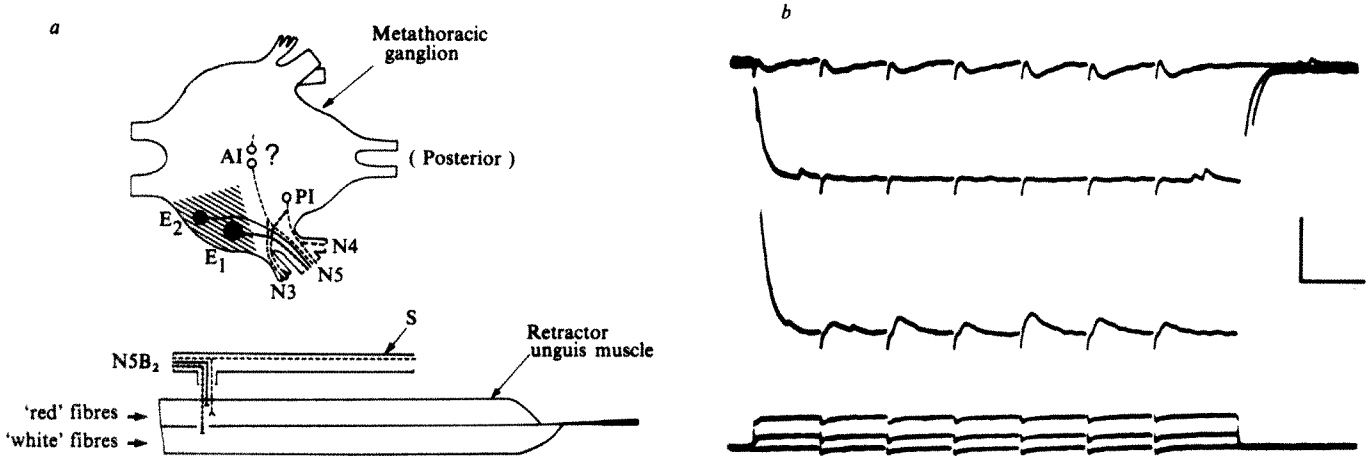


Fig. 1 *a*, Schematic diagram of the innervation of the femoral portion of the metathoracic retractor unguis (RU) muscle of *Schistocerca gregaria*. Each of the bundles of fibres ('red' and 'white') are innervated by one or two excitatory motoneurones in the metathoracic ganglion (E_1, E_2). The red fibres are also innervated by one or more inhibitory motoneurones, possibly branches of the anterior (AI) and posterior (PI) inhibitors which innervate the flexor tibiae (FT) muscle. The approximate positions of the cell bodies of E_1, E_2, AI and PI are shown (see refs 15 and 16 for details). Axons of both excitatory motoneurones run in metathoracic nerve 5, while axons of inhibitory motoneurones run in nerves 3, 4 and 5. The exact location of the inhibitory axons is not yet clear, and they are represented schematically. In some experiments the shaded part of the metathoracic ganglion was excised to delete selectively the excitatory innervation of the RU muscle. *b*, Intracellularly recorded inhibitory postsynaptic potentials (i.p.s.ps), evoked in a red fibre of the RU muscle by antidromic stimulation of branch B_2 of nerve 5 (S, in *a*). This nerve branch also innervates the FT muscle of the metathoracic leg. In this muscle fibre the i.p.s.ps were hyperpolarising at the resting potential, −54 mV. The membrane potential (upper traces) was altered by current injected through a second intracellular microelectrode (lower traces). Note that the i.p.s.ps disappeared when the membrane potential was hyperpolarised to −57 mV. Further hyperpolarisation reversed the polarity of the i.p.s.ps. Membrane potential, 2 mV; current, 20 nA; time, 100 ms. I.p.s.ps could also be recorded in RU fibres by antidromic stimulation of the inhibitory motoneurones through the axons which run in metathoracic nerves 3 and 4.

sensitivity to L-glutamate, similar to that of nerve-sectioned animals.

An example of the increased extrajunctional glutamate sensitivity which occurred on red fibres of denervated RU muscle is shown in Fig. 2. The excitatory innervation of the fibre had degenerated, but i.p.s.ps similar to those recorded from control fibres were recorded from the denervated fibre (Fig. 2). Qualitatively similar results were obtained from red fibres of muscles up to 18 d after nerve section, indicating that up to this time the inhibitory endings on these fibres were still capable of storing and releasing transmitter despite the fact that the axons had been sectioned. Similar results were obtained from animals with an operated ganglion, but in these cases not only could i.p.s.ps be recorded in red fibres on antidromic stimulation of $N5B_2$, but also during 'spontaneous' and 'reflex' activity of the inhibitory innervation and by stimulation of the inhibitory neurones through metathoracic nerves 3 and 4, indicating that the inhibitory inputs to the RU muscle were intact despite the disappearance of the excitatory innervation.

After periods of more than 21 d after section of nerve 5, i.p.s.ps were absent from all red fibres examined, suggesting that the inhibitory terminals had degenerated, although we have no structural evidence to support this conclusion at present. Electron microscope studies[4] of the branches of nerve trunk $5B_2$ which innervate the RU reveal that the excitatory axons are about 8–10 μm in diameter, whereas other axons in the branch, one or more of which are presumably inhibitory, are about 1 μm in diameter. The disappearance of the excitatory innervation 2–3 weeks before the disappearance of the inhibitory is probably due to more rapid degeneration of the large excitatory axons compared with the small inhibitory axons[4].

These experiments demonstrate that in red fibres of locust RU muscles, changes in properties resulting from degeneration of excitatory innervation, for example increased extrajunctional L-glutamate sensitivity, decline in membrane potential and increase in membrane resistance, can occur in the presence of a functionally intact inhibitory innervation. It is possible that trophic influences exerted over the red RU fibres by the inhibitory innervation may be relatively weak and nonspecific, as the inhibitory neurone which innervates the RU muscle probably innervates fibres of other muscles in the leg (for example flexor tibiae). In the case of the nerve section experiments any trophic influences exerted by the inhibitory innervation may be rapidly lost after section of nerve 5. The fact that mechanisms responsible for the production and release of inhibitory transmitter apparently remain intact up to about 3 weeks after nerve section gives little information about trophic factors associated with the inhibitory innervation which may be independent of the inhibitory transmitter. For example, trophic materials could be synthesised in the inhibitory neurone(s) and transported to the junctions by a fast component of axonal flow[14]. Following nerve section stores of such materials in the distal stump of the inhibitory axons would be rapidly depleted, although the nerve terminals could remain functional in a synaptic transmission sense for some time. However, these objections should not apply to the ganglion operation experiments, where the inhibitory innervation presumably remained intact.

One major difference between the excitatory and inhibitory inputs to the RU muscle lies in the ionic currents gated by the respective neurotransmitters. Whereas i.p.s.ps are generated exclusively by an increase in membrane permeability to Cl, the excitatory transmitter (probably L-glutamate) mediates an inward current of Na and Ca ions[17]. In view of the recent findings of Betz and Changeux[18] which suggest that Ca ions, together with cyclic nucleotides, may be important trophic factors in controlling the density of extrajunctional acetycholine receptors on vertebrate muscle, it is tempting to speculate that the changes in RU muscle properties that result from deletion of the excitatory inputs to the muscle arise indirectly from elimination of the influx of Ca ions into the muscle fibres which accompanies excitatory activity at the nerve–muscle junctions. The Ca ion influx would also be absent even if the inhibitory innervation to

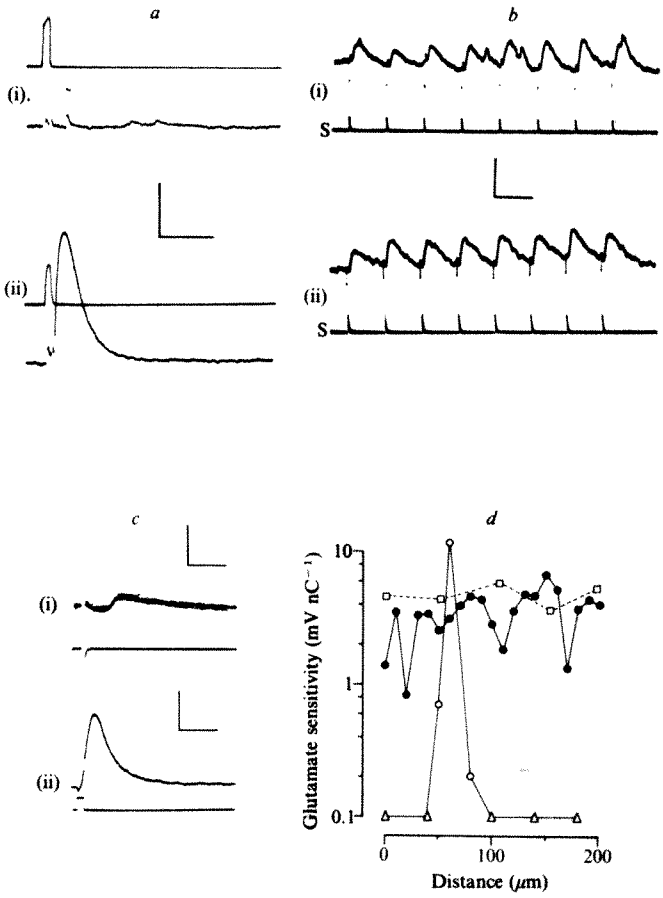


Fig. 2 *a*, Effect of denervation on the L-glutamate sensitivity of RU muscle fibres. (i) A dose of 2 nC (upper trace) applied to the extrajunctional membrane of an innervated control fibre failed to elicit a detectable response (lower trace). (Small responses were elicited with much higher doses of glutamate.) Note miniature excitatory postsynaptic potentials (min. e.p.s.ps). Resting potential of the fibre was −53 mV. (ii) A similar dose of glutamate applied to the extrajunctional membrane of a 'red' fibre of the muscle 16 d after nerve section evoked a large depolarisation. Note absence of min. e.p.s.ps. Resting potential of this fibre was −44 mV. Vertical and horizontal calibration bars, 2 mV, 100 ms, respectively. *b*, (i) I.p.s.ps recorded at the resting potential from the same control fibre as in (*a*). (ii). I.p.s.ps very similar to those recorded from the control fibre were recorded from the denervated fibre on stimulation of nerve $5B_2$ (see Fig. 1). Calibration bars, 0.5 mV, 100 ms, respectively. *c*, (i) Superimposed i.p.s.ps recorded from an RU muscle fibre of an animal 18 d after surgical deletion of the RU excitatory motoneurones. Resting potential of the fibre was −46 mV. These i.p.s.ps were recorded on stimulation of nerve $5B_2$, but similar responses were obtained on stimulation of metathoracic nerves 3 and 4. Calibration bars, 1 mV, 20 ms, respectively. (ii) Response to a 1-nC dose of glutamate (bottom trace) applied to the extrajunctional membrane of the same muscle fibre. Note that the excitatory innervation of the muscle had degenerated, that is no min. e.p.s.ps were observed on this or any other record from this fibre and e.p.s.ps. could not be evoked from the RU muscle fibres during stimulation of metathoracic nerve 5. Calibration bars, 2 mV, 200 ms, respectively. *d*, Comparison of the distribution of glutamate sensitivity of an innervated (Δ, ○) and a denervated (●) RU muscle fibre. The glutamate electrode was moved in 10–20-μm steps along the surface of the muscle fibre and the sensitivity to glutamate at each point measured in terms of the peak depolarisation produced by a 2-nC dose of glutamate. The sensitivity of the innervated fibre was less than 0.1 mV nC^{-1} (Δ) at most points on the fibre; the localised area of high sensitivity (○) corresponds to a neuromuscular junction. By contrast, extrajunctional membrane of the denervated fibre (16 d after section of nerve 5) had a large and nearly uniformly distributed glutamate sensitivity although not as high as that of former junctional sites (not shown). (□), Illustrates the extrajunctional glutamate sensitivity of an RU muscle fibre 16 d after deletion of the excitatory motoneurones.

the muscle remained intact, as Ca ions do not contribute to the inhibitory currents.

Further studies are in progress to evaluate the effect of selective deletion of inhibitory innervation on the properties of RU muscle.

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Differential labelling of retinal neurones by ^{3}H-2-deoxyglucose

THE delineation of structural and functional interconnections in the vertebrate retina has long been a central problem in vision research. The Golgi technique[1,2], combined with ultrastructural studies[3,4] and post-recording injection of intracellular stains[5,6], has revealed the morphological and physiological properties of many of the individual cell types of the retina. Recently, light and electron microscope autoradiography with putative neurotransmitters has tentatively identified certain retinal neurones with specific transmitters[7,8]. However, very few techniques exist which can reveal the interconnections of a 'pathway' of retinal neurones in a functionally dependent manner. Radioactive 2-deoxyglucose (2-DG) is commonly used as a tracer in the central nervous system, where biochemical and autoradiographic studies have shown it to be a good indicator of local functional activity[9–11]. Transport and phosphorylation of 2-DG are identical to those of glucose, but no further metabolism beyond 2-DG-6-PO_4 occurs[12]. Most published studies using 2-DG as a tracer have used the ^{14}C-labelled form and frozen thick-sectioned tissue, a combination in which the resolution of morphological structure is unacceptable for studying neuronal connections in the retina. In the studies reported here, we have used tritium-labelled 2-DG in combination with plastic embedding to produce stimulus-dependent labelling at the cellular level in the isolated goldfish retina; our results suggest that these modifications of the '2-deoxyglucose technique' are useful for studying the physiology and functional connections of retinal neurones.

We chose the retina of the goldfish (*Carassius auratus*) for these studies because of its well characterised morphology and physiology[1,3–6]. After overnight dark-adaptation, goldfish eyecups were removed using an IR image converter. Small pieces of isolated retinas were removed from the eyecups under the same illumination as was used during incubation. For dark incubation, only IR illumination was used. Retinas were incubated in 0.2–0.5 ml of teleost Ringer's solution containing 100 mM NaCl, 3.5 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM NaH_2PO_4, 30 mM $NaHCO_3$, 1.4 mM $CaCl_2$ and 2 mM glucose, *p*H 7.3, which had been presaturated with 95% O_2/5% CO_2. 2-Deoxy-D-[1-^{3}H]glucose (Amersham, TRK 383) was present at 1 mCi ml^{-1}.

Incubations were carried out in small vials sealed with cover slips and centred under the photostimulator beam. During incubation, retinas were maintained in the dark or stimulated with flashing broad-band white light or narrow-band red light of equal quanta. Stimulation was provided by a 6-V tungsten source with a maximum output of 300 μW cm^{-2}. The source gave a 22-mm diameter beam of collimated light. Neutral density filters were used to attenuate the light to 2×10^{12} quanta s^{-1} cm^{-2}, calculated at 550 nm (average 400–700 nm) for broad-band white light and isolated at 700 nm with an interference filter having a 13-nm half-peak bandwidth. This stimulus was delivered as a 1-Hz, 50% duty cycle square wave through the use of a rotating disk. After 20 min, an equal volume of double-strength fixative was added to the incubation mixture and stimulation continued for an additional 15 min. The pieces were

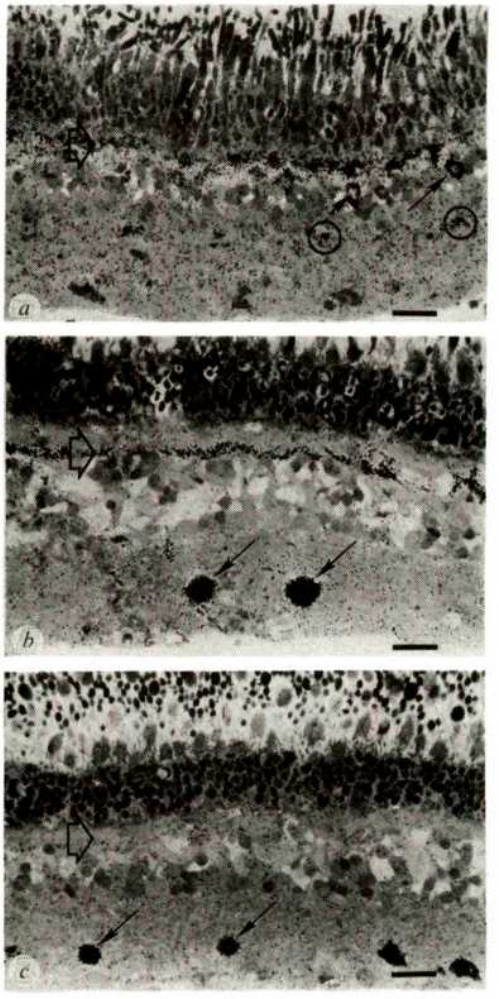


Fig. 1 Light microscope autoradiographs of isolated goldfish retinas incubated for 20 min in teleost Ringer's solution containing 1 mCi ml^{-1} ^{3}H-2-DG. *a*, Incubated in darkness after IR dissection. Label appears predominantly in photoreceptors, H1 horizontal cells (open arrow) and presumptive type *a* (centre-hyperpolarising) bipolar cells (closed arrows). The heavily labelled areas just beneath these cells, in sublamina *a* of the inner plexiform layer, are probably their axon terminals (open circles). *b*, Incubated under flashing narrow-band red light (700 nm) at 2×10^{12} quanta s^{-1} cm^{-2}. Label appears in H2 and/or H3 horizontal cells (open arrow) and type *b* (centre-depolarising) bipolar cell axon terminals (closed arrows). *c*, Incubated under flashing broad-band white light at 2×10^{12} quanta s^{-1} cm^{-2}. Very little label is seen in photoreceptors and horizontal cell layers (open arrow); only type *b* bipolar cell axon terminals are heavily labelled (closed arrows). Scale bars, 20 μm.

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transferred to fresh 3% glutaraldehyde for 1–2 h, then rinsed twice for 10 min in 0.1 M phosphate buffer, *p*H 7.3, post-fixed for 1 h in buffered 1% OsO_4, rinsed twice for 10 min in buffer, dehydrated in graded ethanol and acetone, then embedded in Epon–araldite and polymerised for 48 h at 65 °C. Longitudinal and tangential sections of 1 μm were transferred to glass slides, coated with Kodak NTB2 emulsion and exposed at 4 °C for 2–3 weeks. After exposure, the slides were developed in Dektol for 2 min at 17 °C, washed, then stained with 1% toluidine blue. Similar retinas which were not embedded in plastic, but were extracted and analysed by paper chromatography[13], revealed that more than 85% of the tracer was present as 2-DG-6-PO_4, the remainder being 2-DG.

One of the major aims of this study was to determine whether ^{3}H-2-DG-labelled retinas embedded in plastic would provide resolution at the single cell level[14]. Figure 1 shows an autoradiograph from an experiment in which dark-adapted goldfish retinas were incubated with ^{3}H-2-DG in darkness (*a*), under flashing red light (700 nm) (*b*), and under flashing white light (*c*). This figure demonstrates that our modification of the 2-DG technique can be used to obtain resolution at the cellular level. Additionally, although we have not yet completed a detailed analysis of all the labelled cell types in these retinas, our preliminary analysis reveals several stimulation-dependent differences in the labelling patterns, possibly reflecting the functional dependence of ^{3}H-2-DG labelling. Dark-adapted retinas incubated in the dark with ^{3}H-2-DG (Fig. 1*a*) show considerable labelling in most rod and cone photoreceptors, heavy labelling in H1 horizontal cells (luminosity- or L-type) but not other horizontal cells, and heavy labelling in some presumptive type *a* (centre-hyperpolarising) but not type *b* (centre-depolarising) bipolar cells (terminology used is that of refs 4 and 6). Retinas incubated under flashing red light (Fig. 1*b*) show less labelling in photoreceptors, little labelling in H1 horizontal cells, but heavy labelling in H2 and/or H3 horizontal cells (chromaticity- or C-type) and in some type *b* bipolar cells. Retinas incubated under flashing white light (Fig. 1*c*) show almost no labelling in photoreceptors, only diffuse labelling in all horizontal cells, and heavy labelling in some type *b* bipolar cells. In all cases, very few amacrine or ganglion cells are labelled, possibly because the mode of stimulation was not optimal for labelling these cells.

In many cases, single 1-μm longitudinal sections through the retina were not very useful for identifying individual cell types. Accordingly, tangential and serial longitudinal sections were cut, prepared for autoradiography and exposed for longer periods of time. Figure 2 shows that these techniques permit the identification of specific cells in the retina. Tangential sections through the inner nuclear layer of the goldfish retina reveal the morphology of the horizontal cells which have taken up ^{3}H-2-DG. Figure 2*a* shows an H1 horizontal cell identified by this method, and for comparison, Fig. 2*b* shows an isolated H1 horizontal cell obtained by enzymatic dissociation[15]. Similar identifications can be made for other retinal neurones. To identify bipolar cells, autoradiograms of serial 1-μm longitudinal sections were traced in sequence and the tracings superimposed to demonstrate the cell's morphology (Fig. 2*c*). When these reconstructions are compared with isolated cells (Fig. 2*d*) or with cells impregnated by the Golgi technique (Fig. 2*e*) they can be readily identified as bipolar cells. In this particular case, the functional, positional and morphological characteristics reveal these cells to be type *b* bipolar cells[3–6]. The identification of the other labelled retinal neurones by these methods is now in progress. Thus, ^{3}H-2-DG incorporation under specific stimulus conditions can be used to demonstrate the functional morphology of certain retinal cell types.

Increased 2-DG labelling has been shown to reflect increased functional activity of neural tissue[16]. For retinal neurones, our autoradiographic results are consistent with the idea that accumulation is influenced by the membrane potentials of the cells, with depolarisation leading to enhanced labelling and hyperpolarisation to decreased labelling. The strongest evidence for this suggestion is the intense labelling of type *b* bipolar cells under red and white stimulation, conditions which are known to depolarise these cells. Further evidence lies in the increased labelling of H1 horizontal cells and presumptive type *a* bipolar cells in the dark, conditions in which they are depolarised; the H1 cells accumulate little label under red stimulation, a condition in which they are hyperpolarised. Thus, it seems that in all three incubation conditions, neurones in a depolarised state accumulate more label than those in a hyperpolarised state. This method may therefore be useful for the elucidation of neuronal connections and functional architecture in the retina, especially in retinas not easily amenable to intracellular recordings, as well as in other areas of the central nervous system.

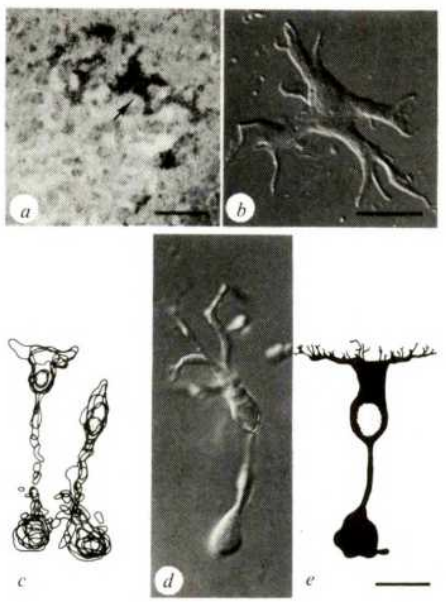


Fig. 2 Identification of ^{3}H-2-DG-labelled cells in the goldfish retina. *a*, Tangential section through the inner nuclear layer after dark incubation with ^{3}H-2-DG and subsequent autoradiographic preparation. An H1 horizontal cell is filled with label (arrow). Scale bar, 30 μm. *b*, For comparison, an isolated H1 horizontal cell prepared according to the dissociation technique of Lam[15]. Scale bar, 20 μm. *c*, Camera lucida tracings of serial longitudinal sections of a retina labelled under red light were superimposed to produce reconstructions of type *b* bipolar cells with axon terminals in sublamina *b* of the inner plexiform layer. Stippled areas identify the nuclei. *d*, An isolated type *b* bipolar cell prepared by cell dissociation. *e*, A type *b* bipolar cell impregnated by the Golgi technique (redrawn from Stell *et al.*[4]). Scale bar for *c*, *d* and *e*, 10 μm.

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Glucocorticoids inhibit endorphin synthesis by pituitary cells

ENDORPHINS in the pituitary gland[1,2] are specifically localised in the intermediate and anterior lobes[3,4], whereas the posterior lobe contains much less opioid activity[4]. Recent results have indicated that β-endorphin and adrenocorticotropic hormone (ACTH) are synthesised by pituitary cells from a common precursor polypeptide[5–8] and that they both appear in the circulation after stressful stimulation[9]. It is well known that an increased potassium concentration stimulates the release of ACTH from pituitary cells in a calcium-dependent fashion[10]. Similar potassium-evoked release of endorphins was indicated in AtT/20 cells which originated in the anterior lobe of mouse pituitary tumours[11]. In addition, a basal release of endorphins was indicated, suggesting a possible dual regulation of endorphin secretion from the pituitary[11]. Coordinated release of ACTH, β-endorphin and β-lipotropin was reported[12] while the present study was in progress. This study was undertaken to determine whether glucocorticoid hormones, which are known to regulate ACTH, also regulate pituitary endorphins. Although it has recently been shown[9] that dexamethasone inhibits *in vivo* elevation of the content of pituitary endorphins after adrenalectomy, the mechanism of the action of this synthetic glucocorticoid remains unclear. Here, we describe data suggesting a direct inhibitory role of glucocorticoids in the synthesis of endorphins by pituitary cells.

The results shown in Fig. 1*a* indicate that the content of endorphins in AtT/20 pituitary cells was decreased when the cells were cultured with the synthetic glucocorticoids, dexamethasone and prednisolone. This effect is dose dependent and shows half-maximal response at 1.1±0.5 nM and 1.6± 0.6 nM for dexamethasone and prednisolone, respectively. The inhibitory role of the glucocorticoids was observed at these physiological concentrations only when the serum used for cell culture was extensively dialysed (Fig. 1). If non-dialysed serum was used, dexamethasone had significantly lower activity, decreasing the cellular content of endorphins by only 35±7% and 60±12% with 100 and 1,000 nM, respectively. These findings agree with previous studies which showed that the effect of steroids could be observed at physiological concentrations only after dialysis or charcoal absorbance, apparently through their removal of free steroids or some other form of inhibition.

As AtT/20 cells release endorphins spontaneously[11,12], we needed to know whether the decreased cellular endorphin content observed after treatment with prednisolone or dexamethasone reflected a shut-off of the synthesis of these peptides or whether the hormones caused the fall in the cellular levels by potentiating the release system. Therefore, the level of endorphins in the culture medium was also determined. Figure 1*b* shows that concomitantly with the drop in the cellular endorphin content seen with increasing concentrations of prednisolone or dexamethasone, there was a dose-dependent fall in the amount of endorphins in the culture medium. The effect of dexamethasone (10 nM) on the release of endorphins at different days after addition of the hormone showed a simple

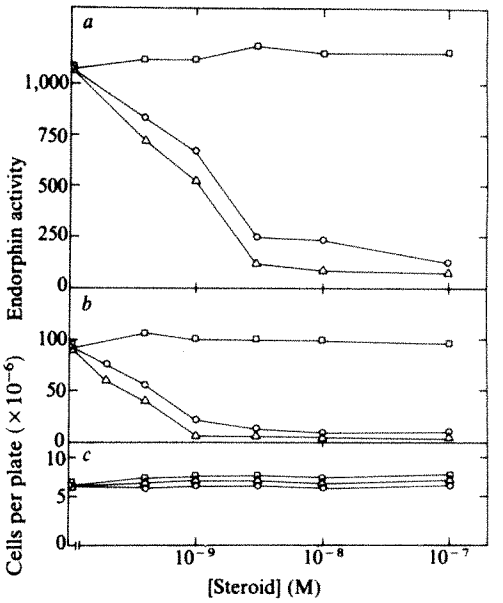


Fig. 1 Effect of steroid hormones on cellular and released endorphins from pituitary cells. AtT/20 mouse pituitary cells were cultured as described previously[11] except that the fetal calf serum (H-21, GIBCO) was extensively dialysed for 4 consecutive days against 50 volumes (each day) of 0.15 M NaCl. Cells were seeded for the experiments at 5×10^5 cells in 5 ml of culture medium containing 10% dialysed serum in a 60-mm Petri dish (Nunc). The cultures were treated on the day of inoculation and 48 h later with 5 μl of ethyl alcohol containing oestradiol benzoate (□), prednisolone (○) or dexamethasone (△) to give the indicated final hormone concentrations. Control dishes obtained 5 μl of ethyl alcohol. 48 h after the last hormone addition the cells were collected with the culture medium and the plates washed with 3 ml of phosphate-buffered saline, *p*H 7.4, which was added to the media containing the cells. This mixture was divided into two portions and centrifuged for 3 min in a low-speed centrifuge (500*g*). The medium of one portion was collected and stored at −20 °C and the cells were suspended in 0.1% EDTA in phosphate-buffered saline (PBS) and counted (C). The medium of the other portion was discarded and the cells were extracted in 3 ml of 0.1 M HCl. The homogenate was neutralised with 1/5 volume of 0.5 M Tris base, centrifuged for 10 min at 40,000*g* and the supernatant stored at −20 °C. Endorphin activity of the cell extracts (*a*) or the media (*b*) was quantitated by the opiate radioreceptor assay. We used this method rather than radioimmunoassay for quantitation of endorphins to ensure that all the endorphins were detected and also to avoid any possibility of misinterpretation due to cross-reaction between endorphin antibodies and other pituitary peptides. In these conditions, β-lipotropin was inactive. Rat brain tissue was used as the source of opiate receptors and details of the assay are described elsewhere[11]. Aliquots of 50–100 μl of the AtT/20 cell extracts or culture media were tested in duplicate and their activity was calculated from standard curves of Met-enkephalin (1–500 nM). Endorphin activity is expressed as pmol Met-enkephalin equivalents per 10^6 cells. Data presented are the average of three experiments carried out with duplicate plates for each hormone treatment.

time-dependent drop with no indication of any increases (data not shown). It was possible that the decreased endorphin levels in the presence of the glucocorticoids were a result of inhibition or other modification in cell proliferation. However, this was ruled out because (1) cells of each plate were counted and the cellular as well as the released endorphins were calculated per cell, and (2) there were no significant changes in cell multiplication after treatment with 0.2–100 nM prednisolone or dexamethasone (Fig. 1*c*).

The specificity of the effect of the steroids on AtT/20 cell endorphin content was also studied. Figure 1 indicates that 0.4–100 nM of Oestradiol benzoate had no significant effect on either the cellular content or the amounts of endorphins released. Two other sex hormones, progesterone and testosterone, and two other steroids, 17-α-epitestosterone and Δ^4-androsten-11β-3,17-dione, had no effect at 10 and 100 nM (Table 1). The specificity of the effect of the glucocorticoids was further indicated by the fact that incorporation of radiolabelled ^{3}H-dexamethasone into the nuclei of AtT cells was selectively inhibited by dexamethasone or prednisolone at nanomolar

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concentrations. In the same conditions, oestradiol benzoate, testosterone, 17-α-epitestosterone and Δ^4-androsten-11β-3,17-dione at 10 or 100 nM did not inhibit ^{3}H-dexamethasone incorporation by these cells (Table 1).

The inhibitory role of adrenal glucocorticoids on ACTH secretion seems to be mediated both by inhibition of the release of hypothalamic corticotropin-releasing factors and by a direct effect on the pituitary cells (ref. 13 and refs therein). The present study suggests that adrenal glucocorticoids at physiological concentrations inhibit endorphin synthesis by anterior pituitary AtT/20 cells in cultures that lack added hypothalamic factors. A previous study with AtT/20 cells[12] suggested that dexamethasone inhibits the stimulatory effect of hypothalamic factors which cause an increased release of endorphins. However, as the cells were cultured for 48 h with a high dexamethasone concentration (1,000 nM), one should reconsider the possibility that dexamethasone acted by decreasing the cellular endorphin content. This is supported by studies which indicated a concomitant decrease of cellular content and the basal release of ACTH after treatment of AtT cells with dexamethasone[14].

Recently, we have observed fluctuations in the content of pituitary endorphins during the normal oestrous cycle of female rats[15]. Interestingly, the lowest level of pituitary endorphins occurred at the day of proestrus, which was also found to be the day in which there was release of adrenal corticosterones[16]. It is conceivable, therefore, that physiological and experimental conditions that increase glucocorticoid levels in the blood can inhibit the synthesis of endorphins by pituitary cells and thereby decrease the amount of endorphins available for release to the peripheral circulation or other target tissues. Acute injection of morphine to experimental animals stimulates ACTH and adrenal hypersecretion. The fact that removal of the source of glucocorticoids in the body (by adrenalectomy) renders rats more susceptible to morphine is well known[17]. Thus, the possibility that glucocorticoid-induced suppression of endorphin synthesis in the pituitary gland may fulfil a role in chronically treated subjects deserves further study.

Table 1 Specificity of the effect of various steroids on cellular content of endorphins and on incorporation of ^{3}H-dexamethasone to AtT/20 pituitary cells

Steroid	Concentration (nM)	Cellular endorphins	^{3}H-Dexamethasone incorporation (fmol per 10^7 cells)
None	—	1,043±165	28.4±4.6
Dexamethasone	0.4	725±110*	NT
	1.0	520±85*	26.5±3.6
	10.0	92±60*	23.8±1.1†
	100.0	80±35*	16.4±0.1*
Prednisolone	0.4	835±115†	NT
	1.0	670±100*	27.2±5.1
	10.0	198±60*	24.7±3.2†
	100.0	105±55*	17.5±0.2*
Oestradiol benzoate	10.0	1,147±190	29.7±3.6
	100.0	1,260±220	23.4±4.4
Progesterone	10.0	1,061±160	28.4±5.5
	100.0	985±260	27.6±3.2
Testosterone	10.0	1,140±117	25.7±4.0
	100.0	1,031±125	33.6±5.5
17-α-Epitestosterone	100.0	1,275±178	27.7±3.1
Δ^4-Androsten-11β-3,17-dione	100.0	1,074±125	27.1±3.7

Cellular endorphin content was determined as described in Fig. 1. Data are pmol Met-enkephalin equivalents per 10^6 cells. Studies of the incorporation of ^{3}H-dexamethasone (23 Ci mmol^{-1}, Radiochemical Centre) to nuclei of AtT/20 cells were carried out as described elsewhere[18], with minor modifications. Cells were dispersed with 0.1% EDTA in PBS, counted, washed with culture medium without serum and dispersed at 2×10^7 cells per ml of the same medium. The cells were then incubated for 40 min at 37°C in a humidified incubator with 4×10^{-8} M ^{3}H-dexamethasone with or without the indicated concentration of unlabelled steroids. Nuclei were prepared and radioactivity was determined[18]. 10 μM dexamethasone was taken as the baseline of maximal displacement. All data are the average ±s.e. of duplicate samples from 2–4 experiments carried out independently. NT, Not tested.

*$P<0.001$; †$P<0.01$ compared with control samples.

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Continuous culture of T cells cytotoxic for autologous human leukaemia cells

CYTOTOXIC T lymphocytes (CTLs), capable of lysing autologous and HLA-identical human leukaemia cells, can be generated *in vitro* by culturing lymphocytes from patients in remission from leukaemia with X-ray irradiated autologous leukaemia cells and irradiated allogeneic normal cells in 'three-cell' mixed leukocyte culture (MLC) protocols[1–3]. Additionally, T cells cultured with X-ray irradiated normal lymphocytes pooled from 20 unrelated individuals can differentiate into CTLs capable of lysing autologous Epstein–Barr (EB) virus-transformed lymphoblastoid cell lines and autologous human leukaemia cells, but not autologous normal cells[4,5]. Because *in vitro* generated CTLs directed against syngeneic tumour cells can inhibit tumour development in some animal tumour systems[6–9], it is likely that if large numbers of human CTLs cytotoxic for autologous malignant cells could be generated *in vitro*, these CTLs could be effective in eliminating residual malignant cells in patients who have received chemotherapy. Recent findings that normal human T cells[10,11] and allosensitised mouse[12] and human CTLs[13,14] grow continuously in medium containing T-cell growth factor (TCGF) derived from supernatants of mitogen-stimulated lymphocytes, have raised the possibility that large numbers of *in vitro* generated CTLs cytotoxic for autologous human malignant cells could be propagated by growing CTLs in TCGF. We report here that *in vitro* generated CTLs cytotoxic for autologous lymphoblastoid cell lines (LCL) and human leukaemia cells can be increased in number by several millionfold by growth in TCGF and that the continuously growing CTLs are highly cytotoxic for autologous abnormal cells.

Experiments were undertaken to determine whether large numbers of T cells cytotoxic for autologous EB virus-transformed LCL cells could be obtained by culturing T cells that had been sensitised to pooled allogeneic normal cells (pool$_x$) in TCGF. T lymphocytes stimulated with the 'pool' for 7 days lysed autologous LCL cells but not autologous normal cells; however, both the LCL cells and normal lymphocytes were lysed by pool-sensitised T cells from unrelated individual A (Table 1). After 27 days growth in TCGF, the CTLs had increased in number by nearly 10^7-fold; the resulting cells were at least threefold more cytotoxic for autologous LCL cells than were

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Table 1 Lysis of autologous LCL cells by continuous cultures of T cells sensitised to pooled allogeneic normal cells

Effector cells	Days after sensitization	Days grown in TCGF	Effector: target ratio	% Specific ^{51}Cr release ± s.d. J's LCL cells	J's normal lymphocytes	H's LCL cells	H's normal lymphocytes
J pool$_x$	7	0	30:1	20.0±5.2	0.9±5.4		
			10:1	11.8±1.6	0.4±4.8		
	34	27	30:1	53.4±5.5	−4.3±3.9		
			10:1	34.0±1.5	−6.4±3.8		
J J$_x$	34	27	30:1	13.9±2.4	−4.0±4.2		
			10:1	4.1±1.6	−5.2±3.8		
H pool$_x$	7	0	30:1			19.0±9.3	−4.3±4.1
			10:1			15.4±1.9	−3.3±4.1
	34	27	30:1			29.4±7.0	−1.8±2.0
			10:1			22.7±3.4	−2.5±1.8
H H$_x$	34	27	30:1			9.5±5.0	−1.8±2.1
			10:1			3.5±2.9	−4.7±2.3
A pool$_x$	7	0	30:1	71.0±2.1	58.8±6.1	60.7±5.4	41.1±2.1

T cells from the peripheral blood of normal individuals J, H and A were isolated by rosetting Ficoll–Hypaque purified mononuclear cells with sheep red blood cells (SRBC) by a method reported elsewhere[16] which was modified as follows. Briefly, 3 ml of 0.5% SRBC in phosphate-buffered saline (PBS) were mixed with an equal volume of mononuclear cells (5×10^6 cells ml^{-1}) and 0.6 ml heat-inactivated pooled human AB serum which had previously been adsorbed with SRBC as described previously[16]. The cell mixture was incubated at 37°C for 5 min, centrifuged at 50*g* for 5 min and incubated at 4 °C for 60 min. The cells were gently resuspended, layered on 10 ml of ice-cold Ficoll–Hypaque and centrifuged for 20 min at 400*g*. The pellet of rosetted cells was resuspended and SRBC were lysed by hypotonic shock. The T cells were washed with PBS and 5×10^6 responding T cells were cultured with equal numbers of X-ray irradiated (2,500 R) allogeneic lymphocytes pooled from 20 normal individuals (designated pool$_x$) or with equal numbers of X-ray irradiated autologous normal lymphocytes (designated J$_x$ and H$_x$) in 50 ml Falcon tissue culture flasks containing 5 ml of RPMI-1640 medium supplemented with 25 mM HEPES buffer and 20% heat-inactivated normal human serum, as previously described[4]. On day 7 after the onset of MLC, some of the effector cells were seeded in 50 ml Falcon tissue culture flasks at 1×10^5 cells ml^{-1} in 10 ml RPMI-1640 medium supplemented with 25 mM HEPES buffer, 20% heat-inactivated normal human serum and 10% TCGF (Associated Biomedic Systems). Before use TCGF was concentrated fourfold against polyethylene glycol powder, dialysed against 10 volumes of serum-free medium and assayed for optimal concentration as described elsewhere[15]. When the cells reached a density of 8×10^5–1×10^6 cells ml^{-1} they were re-seeded at 1×10^5 cells ml^{-1}. On day 7 after the onset of MLC (before growth in TCGF) and 34 days after the onset of MLC (after 27 days growth in TCGF) the effector cells were washed extensively and tested for their ability to lyse ^{51}Cr-labelled autologous LCL cells and normal lymphocytes in 6 hr ^{51}Cr release assays as previously described[4]. The % specific ^{51}Cr released from target cells was calculated as follows

$$\frac{\text{c.p.m. test release} - \text{c.p.m. spontaneous release}}{\text{c.p.m. maximal release} - \text{c.p.m. spontaneous release}} \times 100$$

where spontaneous release = c.p.m. released from target cells in medium alone and maximal release = c.p.m. released from target cells in detergent.

CTLs 7 days after pool sensitisation. Non-sensitised T cells from individuals J and H, following 27 days growth in TCGF, mediated low levels of cytotoxicity against autologous LCL cells.

Similar experiments to propagate CTLs cytotoxic for autologous leukaemia cells (Fig. 1) indicate that 7 days after pool sensitisation, T cells from a patient with hairy cell leukaemia mediated 27% specific ^{51}Cr release from the autologous leukaemia cells; after 26 days growth in TCGF 80% lysis of autologous leukaemia cells occurred whereas the patient's non-sensitised T cells grown in TCGF for the same length of time caused only 9.5% specific ^{51}Cr release. Normal autologous lymphocytes were not lysed by pool-sensitised T cells before or after growth in TCGF. In this and other experiments, CTLs grown for 3–5 weeks in TCGF were 3–4.5-fold more cytotoxic for autologous leukaemia cells than were CTLs 7 days after pool sensitisation. These findings are consistent with previous reports that mouse CTLs directed against syngeneic leukaemia cells are more cytotoxic after growth in TCGF[12,15].

With some leukaemia patients we have failed to detect cytotoxicity against autologous leukaemia cells after pool sensitisation, or after sensitisation in three-cell protocols with autologous leukaemia cells and allogeneic normal cells. In at least some of these cases, it is possible that CTLs are generated against the autologous leukaemia cells but that the level of cytotoxicity is below that detectable by our assay. We thus investigated whether growth of 'sensitised' cells in TCGF in such cases would result in detection of anti-leukaemic CTLs, as sensitised cells grown in TCGF are more cytotoxic than CTLs 7 days after sensitisation. T cells from a patient in relapse with acute myelogenous leukaemia mediated no cytotoxicity against autologous leukaemia cells on day 7 after sensitisation with the pool or after sensitisation in the three-cell protocol (Table 2a). In contrast, after the sensitised cells had been grown for 11 days in TCGF, the effector cells were highly cytotoxic. When, later, with the patient in remission, T cells were isolated and subjected to pool sensitisation or sensitisation in the three-cell protocol, once again no cytotoxicity was detected on the leukaemia cells (Table 2*b*). However, after 19 days of growth in TCGF, high levels of cytotoxicity were detected against autologous leukaemia cells but not against autologous remission bone marrow cells which were, however, lysed by pool-sensitised T cells from unrelated individual N.

That lysis of autologous LCL and leukaemia cells by sensitised lymphocytes following growth in TCGF is mediated by CTLs rather than allo- or TCGF-activated natural killer (NK) cells is supported by the following findings. First, 92–95% of the effector cells grown in TCGF formed rosettes with sheep red blood cells (SRBC). Second, NK cells express receptors for the Fc portion of IgG and can be removed by adsorption to SRBC monolayers coated with anti-SRBC IgG[17,18]. Nonsensitised and pool-sensitised human lymphocytes cultured in fetal calf serum are markedly cytotoxic for NK-sensitive target cells and most of this cytotoxic activity can be removed by depletion of Fc receptor-bearing cells (ref. 19 and J.M.Z., unpublished observations). In contrast, we have found that depletion of Fc receptor-bearing cells from pool-sensitised cells generated in human serum does not reduce cytotoxicity for allogeneic NK-sensitive targets or autologous abnormal cells[18]. Third, we wondered whether leukaemia cells would be lysed by autologous NK cells activated by interferon. Although interferon treatment augments NK-cell activity against allogeneic NK-sensitive leukaemia cells[18,20], cells treated with interferon were not cytotoxic for autologous leukaemia cells (J.M.Z., unpublished observations), indicating that even if TCGF can activate NK cells, the

Table 2 Lysis of autologous acute myelogenous leukaemia cells by *in vitro* sensitised T cells after growth in TCGF

Responding T cells	Stimulating cells	Days after sensitisation	Days grown in TCGF	Effector: target ratio	% Specific ^{51}Cr release ± s.d. Leukaemia cells	% Specific ^{51}Cr release ± s.d. Remission marrow cells
a						
P (patient)	L_x (Patient's leukaemia cells) +	7	0	40:1	−2.1 ± 3.3	
	A_x (normal allogeneic lymphocytes)	18	11	40:1	41.3 ± 8.3	
				10:1	15.5 ± 6.6	
P	$pool_x$	7	0	40:1	−2.0 ± 3.5	
		18	11	40:1	47.7 ± 5.3	
				10:1	12.4 ± 3.8	
N (normal individual)	$pool_x$	7	0	40:1	47.5 ± 4.2	
b						
P (patient)	L_x (patient's leukaemia cells) +	7	0	30:1	−7.2 ± 6.5	1.2 ± 2.4
	B_x (normal allogeneic lymphocytes)	26	19	30:1	19.2 ± 6.5	3.2 ± 5.6
P	$pool_x$	7	0	30:1	−2.5 ± 4.9	−6.4 ± 5.4
		26	19	30:1	30.2 ± 9.8	4.0 ± 3.6
N (normal individual)	$pool_x$	7	0	30:1	38.0 ± 4.7	44.6 ± 6.1

T cells were isolated as described in Table 1 from the peripheral blood of patient P who was in relapse (*a*) and remission (*b*) from acute myelogenous leukaemia. T cells (5×10^6) were cultured with 5×10^6 X-ray irradiated (4,000 R) autologous leukaemia cells (designated L_x) and 5×10^6 X-ray irradiated (2,500 R) allogeneic normal lymphocytes (designated A_x) or with 5×10^6 X-ray irradiated (2,500 R) allogeneic normal lymphocytes pooled from 20 unrelated individuals (designated $pool_x$) as described in Table 1. On day 7 after the onset of MLCs, the sensitised cells were collected and seeded in medium containing 10% TCGF, as described in Table 1. On day 7 after sensitisation and following growth in TCGF, the cells were tested for their ability to lyse ^{51}Cr-labelled autologous leukaemia cells and remission bone marrow cells in 6-hr ^{51}Cr-release assays as detailed in Table 1. The leukaemia cells and remission bone marrow cells, which had previously been control-rate frozen and stored in liquid nitrogen, were thawed and cultured in medium for 12 h before use as target cells. 92–94% of the patient's sensitized cells, after growth in TCGF, formed rosettes with SRBC.

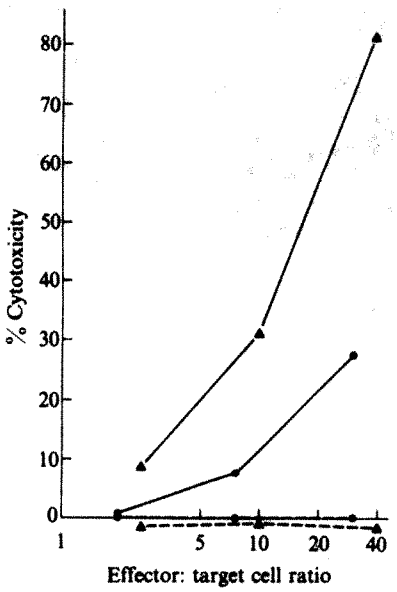


Fig. 1 Lysis of autologous hairy cell leukaemia cells (———) and autologous normal T lymphocytes (– – – –) by T cells sensitised for 7 days with X-ray irradiated pooled allogeneic normal cells before growth in TCGF (●) and after 26 days growth in TCGF (▲). The T cells were isolated, sensitised, grown in TCGF and assayed for cytotoxicity as described in Table 1. The leukaemia cells were isolated from the patient's spleen[5] and were control-rate frozen and stored in liquid nitrogen until 12 h before use as target cells, at which time they were thawed and cultured in medium. 93% of the pool-sensitised cells grown in TCGF formed rosettes with SRBC. Non-sensitised T cells, grown in TCGF for 26 days, mediated only 9.5% ^{51}Cr release from autologous leukaemia cells at an effector: target cell ratio of 40:1.

leukaemia cells do not seem to be susceptible to lysis by activated autologous NK cells.

The results presented here may be important for the following reasons. First, past studies aimed at generating CTLs *in vitro* against autologous or HLA-identical leukaemia cells have demonstrated that in some cases no cytotoxicity is detected following sensitisation[2,3,5]. Our finding that such sensitised T cells cultured in TCGF exhibit significant cytotoxicity for autologous leukaemia cells suggests that the leukaemia cells of at least some of these patients express target antigens against which autologous CTLs can be generated. This approach may also be useful in other systems in which CTLs may be stimulated but in numbers that cannot be detected before growth in TCGF. Second, for basic studies and possible immunotherapeutic application, it is desirable to have large numbers of T lymphocytes highly cytotoxic for autologous leukaemia cells. Growth of *in vitro* generated anti-leukaemic CTLs in TCGF would be a useful approach to this problem, as the number of CTLs can be increased at least 5×10^6 times by 3 weeks growth in TCGF. As continuous cultures of CTLs cytotoxic for syngeneic mouse leukaemia cells inhibit leukaemia development *in vivo*[21], it is possible that continuous cultures of human CTLs cytotoxic for autologous leukaemia cells may be of immunotherapeutic value in leukaemia patients.

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Thy-1 antigen on astrocytes in long-term cultures of rat central nervous system

THE THY-1 ANTIGEN has been exploited by immunologists as a marker for thymus-derived lymphocytes in mice[1]. Since its first description by Reif and Allen, it has been known to be present in equivalent amounts in brain and thymus[2]. However, in contrast to thymus, where adult levels of Thy-1 are present at birth, Thy-1 in mouse and rat brain increases with physiological maturation of the brain from 1–2% at birth to 100% of the adult level by 3–6 weeks of age[2–5]. It is widely assumed that neurones are the principal cell type which express Thy-1 and by implication that the increase in brain Thy-1 during neonatal development is related to increases in neuronal membrane as a result of dendritic arborisation[5]. I describe here the use of monoclonal anti-Thy-1 antiserum in combination with other recently defined neural cell markers[6] to study the distribution of Thy-1 on cells grown in long-term cultures of rat central nervous system. I have found that Thy-1 appears on astrocytes as well as neurones and fibroblasts but not on oligodendrocytes, and that the rate of appearance of Thy-1 on cultured astrocytes parallels that of Thy-1 in brain *in vivo*. This is the first report to identify unambiguously Thy-1 on astrocytes. It settles a controversial point about the Thy-1 distribution on neural cells and clearly demonstrates that previous implications (see discussion) that neurones are the major Thy-1-positive cells in the brain is incorrect.

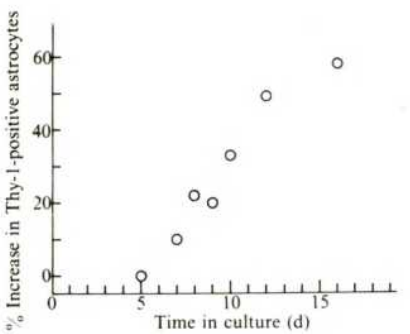


Fig. 1 Increase in percentage of Thy-1-positive astrocytes with time in culture. Cells from 5–6-d old rat corpus callosum were grown and studied by indirect immunofluorescence[6]. Data were collected from three separate experiments counting at least 150 GFAP-positive cells per coverslip.

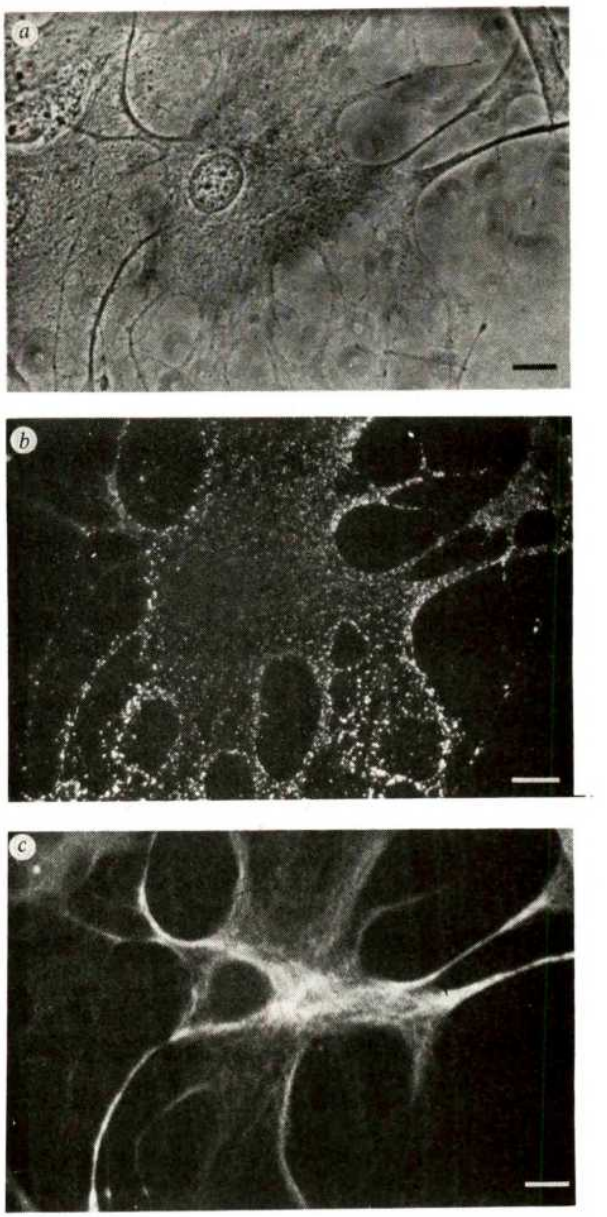


Fig. 2 Presence of Thy-1 on 'protoplasmic' astrocytes. Cells were labelled using mouse anti-Thy-1, followed by goat anti-mouse Ig–rhodamine, fixed, then labelled with rabbit anti-GFAP followed by goat anti-rabbit Ig–fluorescein[6] and viewed with phase contrast (*a*); rhodamine (*b*), or fluorescein optics (*c*). Scale bar, 10 μm.

Cells were cultured from the cerebellum and corpus callosum of 5–6-d old rats and examined by indirect immunofluorescence[6] using the antisera listed in Table 1. In agreement with the previous report[6], no Thy-1-positive astrocytes were seen during the first week of culture. However, by 7 d 10% of the cells positive for glial fibrillary acidic protein (GFAP), a specific marker for astrocytes, were Thy-1 positive; and after 16 d in culture slightly over half the astrocytes were Thy-1 positive (Fig. 1). Thy-1 was found on astrocytes having a range of morphologies including putative fibrous and protoplasmic[6] astrocytes (Figs 2, 3). The possibility that fibroblasts were developing GFAP with time (that Thy-1-positive cells were becoming GFAP positive) was ruled out by the finding that none of the GFAP-positive cells in these cultures was positive for fibronectin, a marker found on the vast majority of fibroblasts in these cultures[6]. Throughout these studies, the one major central neural cell type which remained Thy-1 negative was the oligodendrocyte. Even after months in culture neither primary nor secondary corpus callosum-derived oligodendrocytes

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developed Thy-1. The Schwann cell in the peripheral nervous system has also been reported to remain Thy-1 negative even after months in cluture[7,8].

An important question remaining from the study of long-term cultures derived from neonatal rats is whether or not all astrocytes become Thy-1 positive. By 3 weeks these cultures had become too confluent to count accurately and the percentage of Thy-1-positive astrocytes varied between 30 and 60%. Similar results were obtained when the monolayers were dissociated and assayed for Thy-1 and GFAP as single cell suspensions. Although the percentages of Thy-1-positive astrocytes obtained using cell suspensions tended to be somewhat lower, they are within the range of variability seen in monolayer cultures older than 16 d (30% compared with 58%). To investigate whether all astrocytes in adult brain are Thy-1 positive, cultures of cerebellum and corpus callosum were prepared from a 7-month old rat using the same method as for neonatal tissue[6]. Again, only 30–60% of the astrocytes were Thy-1 positive when the cells were examined by immunofluorescence 9 d after plating. Whether Thy-1-negative astrocytes in the adult and long-term neonatal cultures represent a small pool of immature astrocytes with an increased proliferative/survival capacity in culture remains to be determined. Alternatively, the percentage of Thy-1 negative and positive astrocytes may reflect functionally distinct subclasses *in vivo*.

Table 1 Antisera used to identify neural cells in culture

Antiserum	Dilution	Neural cell type	Source
Mouse anti-Thy-1*	1:2,500	$\frac{1}{m}$†	E. A. Clark & P. Lake
Rabbit anti-GFAP‡	1:100	Astrocytes[6,22]	—
Rabbit anti-galacto-cerebroside	1:40	Oligodendro-cytes[6,22]	M. C. Raff
Rabbit anti-fibronectin	1:75	Fibroblasts[6]	E. Ruoslahti
Goat anti-rabbit Ig-fluorescein	1:100	—	Nordic
Goat anti-mouse Ig-rhodamine§	1:60	—	Nordic

*Monoclonal IgM antiserum pooled from hybridoma tumour-bearing mice. The hybridoma was raised from spleen cells of mice immunised with rat thymocytes and screened for cytotoxity against AKR (Thy-1.1) versus AKR/Cum or CBA (Thy-1.2) thymocytes. A description of the preparation and specificity will be described elsewhere[25].

†Previously found on neurones and fibroblasts [6,17].

‡Glial fibrillary acidic protein (GFAP) and antiserum were prepared by the method of Dahl and Bignami[23,24]. The specificity for cultured astrocytes has been described previously [6,22].

§Absorbed with Sepharose-coupled rabbit immunoglobin.

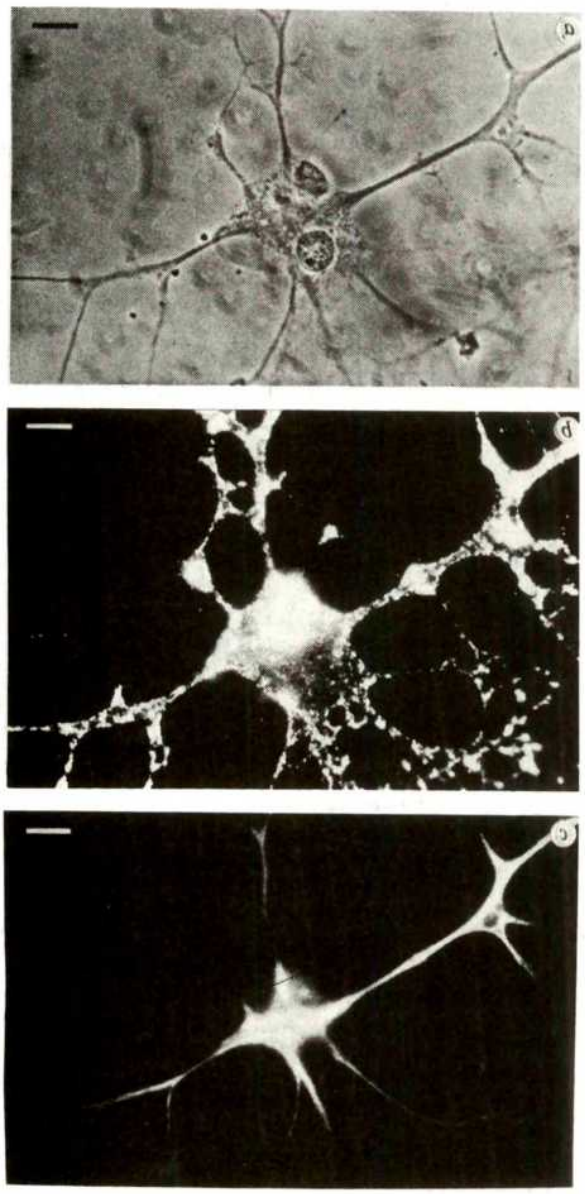


Fig. 3 Presence of Thy-1 on 'fibrous' astrocytes. Cells were labelled as described in Fig. 2 and viewed using phase contrast (*a*), rhodamine (*b*) or fluorescein optics (*c*). Scale bar, 10 μm.

This is the first direct demonstration of Thy-1 antigen on glial cells. Previous attempts to establish which cells of the nervous system are Thy-1 positive have used absorption studies [5,9–12], and immunofluorescence on brain sections[5,9,13] and cell lines[14–16]. Most of these studies have suggested that Thy-1 is primarily expressed by neurones. The absorption studies have largely ignored the presence of contaminating plasma membranes from non-neuronal elements, and immunofluorescence microscopy on brain sections are technically limited in their ability to localise Thy-1 to the membranes of specific cell types within a Thy-1-positive region. Thus, although these types of studies have associated Thy-1 with neurones they could not eliminate the possibility that glia could be Thy-1 positive. Examination of neural tumour-derived cell lines has suffered from ambiguity due to the lack of criteria for classifying the various cell types[14–16]. Previous studies using primary central nervous system-derived cultures confirmed that neurones and fibroblasts are Thy-1 positive but failed to demonstrate Thy-1 on glial cells[6,17]. This failure was probably due to the inability to distinguish astrocytes from fibroblasts on morphological grounds[17], whereas in other studies the animals and/or cultures were of insufficient age[6,17]. Thus, the astrocyte can be added to the growing list of Thy-1-positive cell types, which in addition to lymphocytes, neurones and astrocytes include epidermal cells[18], fibroblasts [19], breast epithelium [20] and myoblasts[21]. The time course of Thy-1 development on astrocytes in culture was similar to that reported for whole neonatal rat brain[4,5], days 5–16 in culture corresponding to postnatal days 11–22. These findings suggest astrocytes as well as neurones contribute to both the ontological appearance and the large amount of Thy-1 present in brain.

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Characteristics of *Aedes albopictus* cells persistently infected with dengue viruses

DENGUE VIRUSES, which have four serotypes, belong to the genus flavivirus of Togaviridae and are transmitted by mosquitoes such as *Aedes aegypti* or *Aedes albopictus*. Human infection with dengue viruses is manifested by disease varying in severity from mild undifferentiated fever to grave haemorrhagic fever, which is widespread in Southeast Asia[1–3]. Persistently infected cultures can be established when cultured mosquito cells are infected with Togaviridae, either alphaviruses or flaviviruses[4–7]. Analysis of mosquito cells persistently infected with alphaviruses has shown characteristics such as the generation of small-plaque and temperature-sensitive (ts) viruses[7–9], and resistance to superinfections with the homologous but not the heterologous viruses[6,10]. In contrast, little is known about mosquito cells persistently infected with flaviviruses[4,11,12]. Here, in experiments with *A. albopictus* (Singh) cells persistently infected with dengue viruses, I show that such cultures produce ts viruses and become resistant to all the types of dengue viruses.

Dengue virus type 1 (DEN-1) Hawaiian, type 2 (DEN-2) New Guinea B, type 3 (DEN-3) H-87, and type 4 (DEN-4) H-241 strains were used to prepare stock viruses[13]. Infectivities were assayed on BHK21 cells by the fluorescent focus counting method[13,14] and were expressed as focus forming units (FFU). Replicate cultures of Singh's[15] *A. albopictus*, clone C6/36 cells, were prepared in 2-ounce bottles by seeding 5×10^5 cells in 5 ml of cell growth medium (10% fetal calf serum in Eagle's medium in Earle's saline supplemented with 0.2 mM each of non-essential amino acids) in each bottle[13]. After 3 days of incubation at 28 °C, medium was removed and dengue viruses were inoculated at input multiplicities (MOI) of 0.1 FFU per cell for DEN-1, DEN-3 and DEN-4 viruses, and 1 FFU per cell for DEN-2 virus. After 2 h of adsorption, cells were covered with maintenance medium (cell growth medium in which serum was reduced to 2%), and were incubated at 28 °C. Every culture showed some cytopathic effect after 2–6 days of infection. Seven days after infection, medium was replaced by fresh cell growth medium and the cultures were further incubated. When surviving cells grew out, they were transferred weekly at 1 : 20 dilution, and virus titres in the medium before the cell transfer were assayed on BHK21 cells at 37 °C and 28 °C. As shown in Fig. 1, the difference between the titres assayed at these two temperatures was not significant in the case of DEN-1, DEN-2 or DEN-4 infected cells up to 50 weeks of infection. However, with DEN-3 infected cells, infectivity at 37 °C was significantly lower than that at 28 °C after 22 weeks of infection, and virtually no infectivity was detected at 37 °C after 44 weeks of infection.

Medium was collected from the persistently infected cells after 50–60 weeks of infection and virus infectivities were assayed on BHK21 cells at various temperatures and by intracerebral inoculation into suckling mice (SMB). Table 1 shows that viruses obtained from persistently infected cultures were essentially temperature sensitive compared with standard viruses used to initiate the infections. Except for DEN-1, these ts viruses were not able to kill suckling mice and seemed less virulent than the standard viruses, such as found in other Togavirus systems[4,16–18]. These viruses from persistently infected cells were neutralised to the same extent as the standard viruses by rabbit antiserum raised against the homologous standard virus.

Table 1 Infectivities of standard dengue viruses (std) and viruses produced in *A. albopictus* cells persistently infected with dengue viruses (PI)

Assay host	Temperature (°C)	DEN-1 std	DEN-1 PI	DEN-2 std	DEN-2 PI	DEN-3 std	DEN-3 PI	DEN-4 std	DEN-4 PI
BHK	39	4.9	1.0	6.7	<1.0	4.6	<1.0	6.2	2.2
	38	5.8	1.7	6.9	1.9	6.3	<1.0	6.4	3.3
	37	5.7	4.3	7.4	4.0	5.9	<1.0	6.8	4.6
	34	5.9	4.2	7.4	4.7	5.9	3.5	6.8	4.6
	28	5.8	4.8	7.0	5.2	5.5	3.7	6.6	4.9
SMB		6.0	4.0	7.1	<1.5	6.8	<1.5	6.9	<1.5

Titres are shown as log(FFU per ml) or log(LD_{50} per ml)

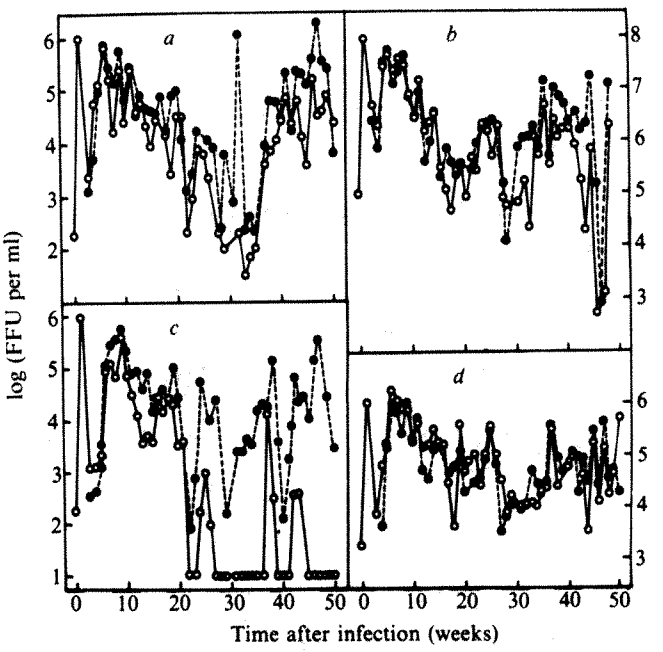


Fig. 1 Infectivity in the culture medium of *A. albopictus* cells persistently infected with dengue virus. Persistently infected cultures were established by infection with DEN-1 (*a*), DEN-2 (*b*), DEN-3 (*c*) and DEN-4 (*d*) viruses as described in the text. Virus infectivity in the medium before cell transfer was assayed at 37 °C (○——○) and at 28 °C (●- - - - - - - ●) on BHK21 cells.

Replicate cultures of *A. albopictus* C6/36 cells or the cells persistently infected with one of the four types of dengue virus were prepared in a Limbro multichamber tissue culture plate (FB-16-24-TC) by seeding 10^5 cells per ml per well. After 3 days of incubation at 28 °C, each well was inoculated with 0.1 ml of standard dengue virus as described above, or chikungunya virus

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Table 2 Yields of standard dengue and chikungunya viruses from *A. albopictus* cells persistently infected (PI) with dengue viruses

Host cells	Standard virus yield				
	DEN-1	DEN-2	DEN-3	DEN-4	CHIK
C6/36	5.71±0.11	7.76±0.22	5.67±0.21	5.61±0.18	7.01±0.34
DEN-1 PI	3.98±0.27	5.91±0.26	4.34±0.39	4.11±0.50	7.11±0.45
DEN-2 PI	4.44±0.41	4.47±0.29	4.66±0.48	4.25±0.50	7.38±0.35
DEN-3 PI	3.38±0.38	5.48±0.45	2.46±0.42	2.72±0.53	6.79±0.50
DEN-r PI	3.75±0.34	5.14±0.45	3.69±0.43	3.34±0.17	6.71±0.49

Titres are shown as average of log(FFU per ml) ± s.d. from 4–6 experiments.

at MOIs of 1 FFU per cell. After 2 hours of adsorption, residual virus was removed and the cells were covered with maintenance medium and incubated at 28 °C. Titres of standard viruses in the media were assayed on BHK21 cells at 38 °C after 2 days of incubation for chikungunya, 3 days for DEN-2 and DEN-4, or 4 days for DEN-1 and DEN-3 viruses, respectively.

Table 2 shows that the yields of standard dengue viruses from persistently infected cells were significantly lower than those from control C6/36 cells. The reduction of the standard virus yields was not only observed with the homologous virus used to initiate the infection, but was also seen with heterologous types of dengue viruses. However, the yield of chikungunya virus was not significantly less in the persistently infected cells than in C6/36 cells. Culture medium was collected 3 days after transfer of persistently infected cells at 60–70 weeks of infection, passed through Millipore type HA filters, and used to inoculate C6/36 cells. The cells were cultured for 1–2 weeks and then tested for the yields of standard dengue and chikungunya viruses after challenge of these viruses as described above. Yields of standard dengue viruses from those cells pretreated with medium of persistently infected cells were significantly lower than those from C6/36 cells and were similar to the yields from persistently infected cells. On the other hand, the yield of chikungunya virus was not reduced by these treatments. The dengue-resistant state in the treated cells persisted for at least 6 weeks. This transfer of dengue resistance to C6/36 cells by the culture medium from persistently infected cells did not occur when the treatment media were preheated at 56 °C for 30 min.

It is not known whether production of ts dengue viruses is unique to mosquito cells or one of the general phenomena of the persistently infected cultures[19]. The lower temperature of mosquito cell cultivation probably favoured the selection of ts viruses. The experimental results indicate that infection of mosquito cells with one type of ts dengue virus renders the cells resistant to all types of standard dengue viruses.

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The X-linked α-chain gene of *Drosophila* LSP-1 does not show dosage compensation

IN many species of higher organisms there is compensation for the different number of 'doses' of X-linked genes in the two sexes. This can be demonstrated by studies on enzymes which show that the level of activity of an X-linked enzyme is the same in the sex with one chromosome as in the sex with two. Dosage compensation therefore represents a system which can be used to study the control of gene activity in eukaryotes. Dosage compensation in *Drosophila melanogaster* has been extensively reviewed[1], and although it has been studied mainly in adult flies it does occur in larval stages[2–5] and in cultured cells[6]. The one published example of an X-linked gene which does not show complete dosage compensation is the white-eosin mutant[7] where there is more pigment in the eye of the female homozygote than in the eye of the male hemizygote. Here we report a second example, that of the X-linked α-chain gene of *Drosophila* larval serum protein-1.

Larval serum protein-1 (LSP-1)[8] of *Drosophila* is a family of proteins made up of the random association of three polypeptides[9] which we have called the α-, β- and γ-chains (D.B.R. and S.E.-R., in preparation). These chains are similar in their immunological properties[9] and amino acid composition[9] and in their peptide pattern after partial digestion with protease (H. Brock and D.B.R., unpublished results). These data suggest that they have evolved by gene duplication followed by mutation. We have mapped the coding sequences of these polypeptides (D.B.R. and S.E.-R., in preparation); the α-chain coding sequence is X-linked (39.5) whereas the other two coding sequences are found on the autosomes. Table 1 shows the amounts of α-chain (expressed as a fraction of the sum of the amounts of β- and γ-chains) found in the haemolymph of male and female larvae of two different wild-type stocks and in female larvae from a cross which generates normal diploids and larvae

Table 1 Amounts of α-chain found in the haemolymph of *Drosophila* larvae

	$\alpha/\beta+\gamma$*		α, β and γ as % of total protein†	
	Mean	s.d.	Mean	s.d.
Oregon R females	0.42	0.04	60	4.1
Oregon R males	0.21	0.02	55.5	4.2
Samarkand females	0.51	0.06	58.1	2.6
Samarkand males	0.23	0.05	52.4	3.6
w/FM7 females	0.43	0.10	54.2	5.3
Df H368/FM7 females	0.18	0.01	52.4	5.2

Haemolymph was extracted from third instar larvae[8] and electrophoresed on Cellogel at *p*H 9.3. In these conditions LSP-1 dissociates into monomers and the α-, β- and γ-chains are separated (D.B.R. and S.E.-R., in preparation). The Cellogels were stained with amido black 10B and washed thoroughly with 5% acetic acid/47.5% methanol before the three LSP-1 bands were cut out separately. The Cellogel containing each band was dissolved in 80% acetic acid and the optical density of the solution was read on a spectrophotometer at 600 nm.

* The optical density of the α-chain is expressed as a fraction of the sum of the β- and γ-chain optical densities.

† LSP-1 α-, β- and γ-chains expressed as a percentage of the total protein stained on the cellogel. Male and female larvae were sexed according to Bodenstein[11]. The deficiency heterozygotes were obtained by crossing flies heterozygous for the deficiency HF 368 (11A2-11B9, G. Lefèvre, personal communication) and the balancer chromosome FM7 (ref. 12) with male flies hemizygous for an X-chromosome marked with white (*w*). The two classes of female larvae were distinguished by the white malpighian tubules of the *w*/FM7 females compared with the normally pigmented malpighian tubules of the deficiency HF 368/FM7 larvae.

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which are heterozygous for a deficiency of the α-chain gene. The results show that the LSP-1 α-chain gene shows no dosage compensation. Where there is one copy of the gene, whether it is in male larvae or in female larvae heterozygous for the deficiency, there is 50% of the quantity of α-chain found in normal females.

A possible explanation for this absence of dosage compensation is that the α-chain gene has only 'recently' arrived on the X-chromosome, either by classical translocation or as part of the process of duplicating the ancestral gene, and that it has not acquired the regulation for dosage compensation. An experimentally translocated autosomal segment on the X-chromosome does not show dosage compensation[10]. The α-, β- and γ-chains of LSP-1 apparently perform essentially similar functions. As they are synthesised in approximately equimolar amounts (Table 1) the ratio between the amount of these polypeptides in male and female larvae will be 5:6. This will result in less selection pressure for compensation than for a gene where the ratio of products in males and females is 1:2.

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Cellular protein synthesis and inhibition of cell division are independent of butyrate-induced histone hyperacetylation

SODIUM BUTYRATE has many effects on mammalian cells—inducing the biosynthesis of new proteins[1–6], changing the relative synthetic rates of specific proteins[7–10], increasing various enzyme activities[7,9–13], inducing morphologic changes in cultured cells[9,12,14–19], and inhibiting DNA synthesis and cell division[14,15,20]. The mechanism of these effects is not known. Sodium butyrate induces massive hyperacetylation of histone in cultured mammalian cells[21] via inhibition of histone deacetylase[22–24]: this hyperacetylation can be mimicked to varying degrees by other salts of short-chain fatty acids[22]. As histones interact so intimately with DNA, and as the change in extent of histone acetylation is so great, we sought to determine whether the changes in histone modification are involved in causing the changes in synthesis of specific proteins or the cessation of DNA synthesis and cell division. In hepatoma tissue culture (HTC) cells, the increased amount of histone acetylation is maximal 6–12 h after administration of sodium butyrate[22], and we have used two-dimensional gel techniques[25] to establish whether different proteins are made during this period. Our results suggest that cellular protein synthesis and inhibition of cell division are independent of butyrate-induced hyperacetylation.

Control and butyrate-treated HTC cells were labelled with ^{35}S-methionine during 2-h pulses at the following times after butyrate administration: 0, 3, 6, 9, 12 and 24 hours. The cells were collected by centrifugation, dispersed in a lysis buffer containing 9 M urea, 5% mercaptoethanol, and 2% nonionic detergent and analysed by two dimensional electrophoresis[25]. The protein patterns of zero time and 24-h control cells are shown in Fig. 1*a*, *b* and that of 24-h butyrate-treated cells in Fig. 1*c*. Although the growth rate of control cells is significantly slowed after 24 h as they increase in density and approach stationary phase, the autoradiograms of labelled proteins from the control culture at these two times are almost identical in terms of the number and position of proteins and in terms of relative intensity of the spots.

Comparison of panels *a* and *b* of Fig. 1 with panel *c* shows that almost all of the spots are present in both systems, but that a significant number of proteins have changed their relative rate of synthesis (or breakdown). Proteins which are presently only in butyrate-treated cells are indicated by arrows in panel *c*. Only six proteins which are synthesised in the presence of butyrate cannot be distinguished in the control *a* and *b* after the period of autoradiographic exposure used. Proteins which are relatively more intense in untreated cells are indicated by circular lines in panels *a* and *b*, and proteins more intense after butyrate treat-

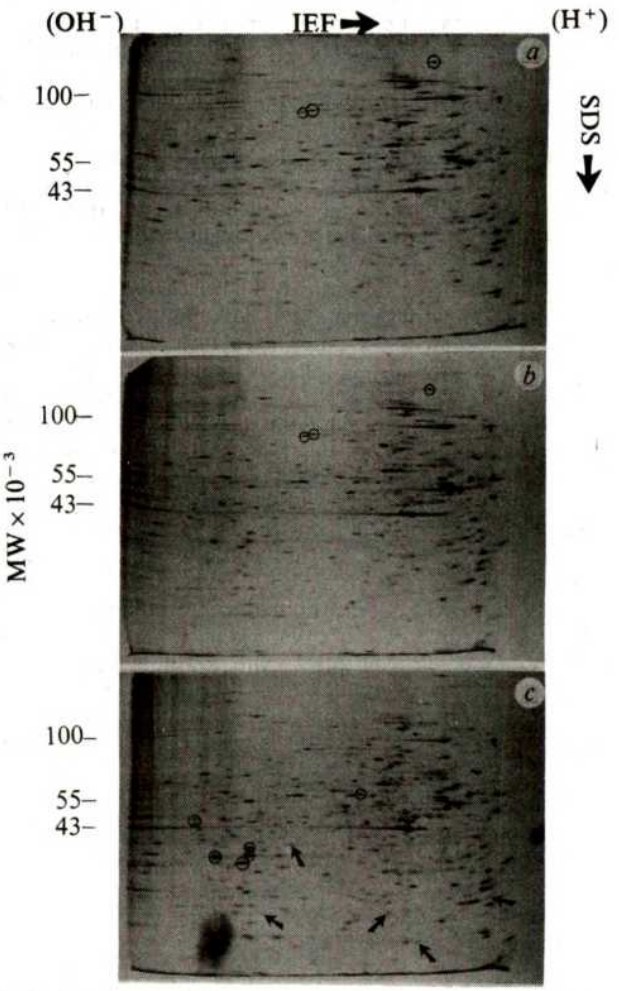


Fig. 1 Proteins synthesised in HTC cells in the presence of sodium butyrate. HTC cells, maintained in suspension culture, were incubated in Swims-77 medium in the presence of ^{35}S-methionine (100 μCi, 740 mCi mmol^{-1}, NEN) for 2.5 h. The cells were collected by centrifugation, washed twice in phosphate-buffered saline and disrupted in a buffer containing 9.2 M urea, 5% (w/w) 2-mercaptoethanol, 2% NP-40, 1.6% *p*H 5–7 ampholines and 0.4% *p*H 3–10 ampholines[25]. Samples were analysed simultaneously on two-dimensional gels as described by O'Farrell[25]. Equivalent amounts of trichloroacetic acid-precipitable c.p.m. of ^{35}S-methionine were loaded on to the gels with up to 30 μg of total cell protein loaded per gel. The gels were dried and autoradiographed for 7 days. Panels *a* and *b* represent proteins synthesised in log phase HTC cells at zero time (300,000 cells per ml) and 24 h later. Panel *c* is the same as panel *b* except that 6 mM sodium butyrate was present during the 24 h before labelling. Spots indicated by an arrow are present only in that panel. Spots indicated by circles are present in all panels but are more intense in the panel where circled.

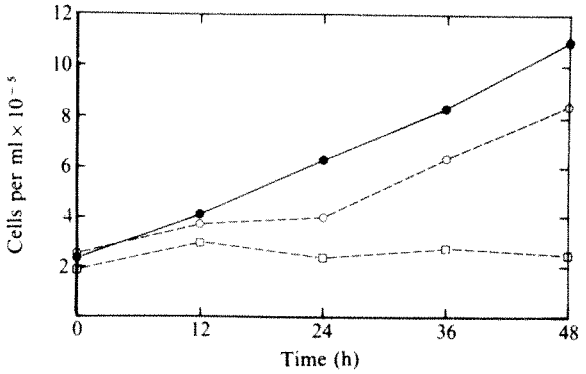


Fig. 2 Effect of salts of short chain fatty acids on cell replication. HTC cells were suspended in Swims-77 medium at a cell density of approximately 2×10^5 cells per ml in the presence of either 6 mM sodium butyrate, (□); 6 mM sodium propionate (○), or no added salts, (●). The cells were counted in a haemocytometer at the times indicated. Each point is the mean of eight determinations.

ment are shown similarly in panel *c*. Seven protein spots are definitely more intense in panel *c* relative to both *a* and *b*. This type of analysis was performed four times and only those differences which were consistently observed have been reported. Thus, out of approximately 500 proteins visualised in both control and butyrate-treated HTC cells, we have observed significant variations in the rate of synthesis or breakdown for only 16 proteins.

The concentration of butyrate administered to the cells inhibits cell growth[22], so the minor changes observed could reflect a cellular adjustment to the inhibition of DNA replication rather than response to butyrate treatment. However, comparison of 24-h butyrate-treated cells with control stationary (density inhibited) cells showed that non-growing cells appeared very similar overall to both control logarithmically growing cells and butyrate-treated cells (data not shown). Some characteristic differences were observed but they were not the same variations seen between the experimental groups analysed above.

Thus, we see that for 12–18 h after the attainment of the full extent of histone acetylation there are only minor shifts in the kinds and steady state levels of proteins synthesised, at least within the *p*H 5–7 isoelectric focusing range. A similar analysis has been conducted at the basic end of the protein *p*I scale using *p*H 6–10 ampholines[26] with results that were essentially the same and accordingly such gel patterns have not been shown as they simply confirm the type of results shown above in the lower *p*I range. Thus there is apparently no immediate cause and effect relationship between hyperacetylation and major changes in gene expression at the translational level.

In order to find whether the hyperacetylation of histones is related to the inhibition of cell division by sodium butyrate we used other agents which affect levels of acetylation and studied their effect on cell division. As previously reported, other short chain fatty acid salts also induce hyperacetylation to almost the same extent as sodium butyrate[22]. However, the effect of sodium propionate induced hyperacetylation on cell division is radically different from that of sodium butyrate (Fig. 2). Although 6 mM sodium propionate causes a decrease in rate of cell division it simply amounts to a decrease of generation time from 16 to 20–22 h. In contrast, 6 mM sodium butyrate inhibits cell division after 10–20 h. Subsequently cells exposed to this level of butyrate begin to admit vital stain and some cell death ensues.

We conclude that bulk hyperacetylation of the non-H1 histones *per se* does not lead to cessation of DNA synthesis and cell division. Evidently these processes continue quite efficiently even when 70% of the H3 and H4 histones are acetylated (the level of modification induced by sodium propionate). However, as the proportion of acetylated H4 histone is consistently slightly higher (80%) after treatment with sodium butyrate[21,22], and in particular the level of tetra-acetylated H4 is somewhat greater, we cannot exclude the possibility that the extra margin of hyperacetylated histone might exert a highly deleterious effect on cell division. In this regard we note that histone synthesised in the presence of butyrate is a major contributor to the high levels of modification[22], and that it might be through this fraction of the total histone complement that critical differences could be developed. Finally, we note that propionate causes no major change in types of proteins synthesised as evidently by a two dimensional analysis of the type shown in Fig. 1 (data not shown).

Perhaps the most surprising thing about the recent observations on hyperacetylation of histones is not so much the dramatically increased amounts of the hyperacetylated forms of histone, but rather the apparent minimal effect on chromatin function and replication as detected by the apparent health of the cells. In view of the rapid rates of modification and hydrolysis of the monoacetyl histones in untreated cells it seems that this is likely to be the most critical modification step and that in untreated cells the small amount of the more highly modified pre-existing histones are inconsequential results of low-level recognition by the acetylating enzymes. They apparently exert no overtly deleterious effect as such, and this may account for the results described in this paper.

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Micro- and macro-stabilities of globular proteins

PROTEIN stability is usually expressed in terms of the energies required for the micro- and macro-unfolding of its structure, determined from hydrogen exchange and denaturation experiments. Both these characteristics represent qualitatively different properties of a protein macromolecule which can be classified as the rigidity of the structure and the stability of the macroscopic state. In this report, the dependence of these properties on the parameters specifying protein structure is analysed.

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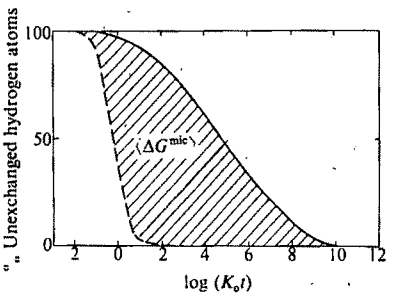


Fig. 1 Relaxation spectrum of an abstract protein (solid line) and that of a random coil polypeptide (dotted line).

There are two experimental approaches to a quantitative determination of protein stability: the first is based on the study of peptide hydrogen exchange in native protein and the other on the study of protein denaturation. The first uses the assumption that exchange of peptide hydrogen from the internal part of the protein with the hydrogen from the surrounding water takes place as a result of local or micro-unfolding of a structure[1]. In this case the rate constant, K_i, of hydrogen exchange from the ith part of a macromolecule is expressed through the rate constant, K_o, of hydrogen exchange from the unfolded conformation by

$$K_i = \exp\left(\frac{-\Delta G_i}{RT}\right) K_o \qquad (1)$$

where ΔG_i is the Gibbs energy of the corresponding micro-unfolding of the compact structure. Kinetic studies showed that different hydrogens in a protein are exchanged at different rates; the energy of unfolding of different parts of the protein varies from 0 to 14 kcal mol^{-1} (ref. 2). The variation of the energy of micro-unfolding in proteins is usually represented by a 'relaxation spectrum' (refs 3, 4), which is a plot of the relative number of peptide hydrogens unexchanged at time t versus log $K_o t$ (Fig. 1). In this plot the area enclosed between the curves for the protein and the unfolded polypeptide corresponds to the average energy of micro-unfolding required for the exchange of one peptide hydrogen in the protein. This quantity, $\langle \Delta G^{mic} \rangle$, is usually considered as a measure of the micro-stability of a protein, that is, its tendency to undergo micro-unfolding.

The second approach to the determination of protein stability is based on the assumption that denaturation of a protein is a cooperative transition from the ordered native state (N) to the disordered denatured state (D), and that both these states are macroscopic, that is, they are described by thermodynamic functions[5]. In this case transition into a disordered state (denaturation) is defined by the difference thermodynamic functions of enthalpy

$$\Delta_N^D H = H^D - H^N$$

of entropy

$$\Delta_N^D S = S^D - S^N$$

and of Gibbs energy

$$\Delta_N^D G = G^D - G^N.$$

These difference functions can be determined experimentally[6] by calorimetric measurements of the change of the enthalpy $\Delta_d H$ and the heat capacity $\Delta_d C_p$ at the temperature of denaturation T_d

$$\Delta_N^D H(T) = \Delta_d H - \int_T^{T_d} \Delta_d C_p \, dT \qquad (2)$$

$$\Delta_N^D S(T) = \frac{\Delta_d H}{T_d} - \int_T^{T_d} \Delta_d C_p \, d\ln T \qquad (3)$$

$$\Delta_N^D G(T) = \Delta_d H \frac{T_d - T}{T_d} - \int_T^{T_d} \Delta_d C_p \, dT + T\int_T^{T_d} \Delta_d C_p \, d\ln T \qquad (4)$$

Figure 2 illustrates these functions using myoglobin as an example[7]. The $\Delta_N^D G$ function which corresponds to the work required for the transfer of a macromolecule from the native macroscopic state to the denatured macroscopic state, that is, for the disruption of all the native structure, is usually regarded as a measure of the stability of the native macroscopic state, or the macro-stability of the protein.

Because, in considering micro- and macro-stabilities, we are dealing with somewhat similar events—with the unfolding of a compact structure—one might be expected that these properties of the protein structure would be interrelated. However, in searching for the relationship between them one should not

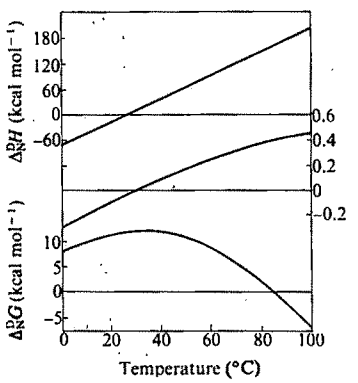


Fig. 2 Enthalpy, entropy and Gibbs energy functions for protein unfolding of myoglobin at a *p*H corresponding to its maximum stability[7].

forget the great gap in the scales of these events which leads to the qualitative difference between these characteristics. It is somewhat misleading that both stabilities formally have the same dimensions, being measured in kcal mol^{-1}. However, the macrostability ($\Delta_N^D G$) is calculated per mol of the cooperative unit, whereas the micro-stability ($\langle \Delta G^{mic} \rangle$) is calculated per mol of the peptide hydrogens. As a result, the macro-stability describes the stability of the whole cooperative structure whereas the micro-stability describes the average stability of the regions of a macromolecule which are on the same scale as the micro-unfoldings. Obviously the average micro-unfolding

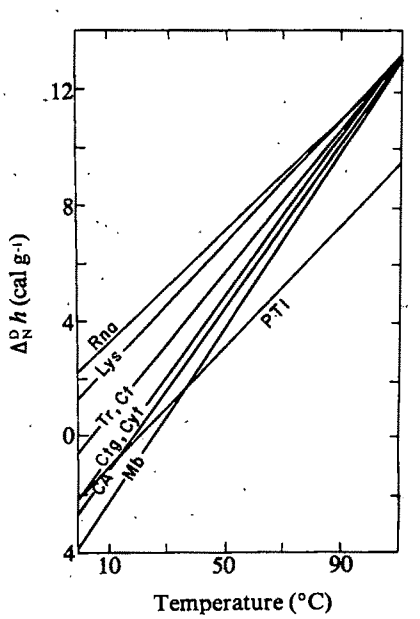


Fig. 3 Enthalpy functions for proteins listed in Table 1. The designations used are: Rna, ribonuclease A; Lys, lysozyme; Tr, β-trypsin; Ct, α-chymotrypsin; Cyt, cytochrome c; PTI, pancreatic trypsin inhibitor; Ctg, chymotrypsinogen; CA, carbonic anhydrase B; Mb, metmyoglobin.

Table 1 Thermodynamic characteristics of some globular proteins at 25 °C

Proteins	$\langle\Delta G^{mic}\rangle$ (Kcal mol^{-1})	Ref.	$\Delta^D_N g$ (cal g^{-1})	$\Delta^D_N h$ (cal g^{-1})	$\Delta^D_N s$ ($\times 10^2$) (cal K^{-1} g^{-1})	$\Delta_d C_p$ (cal K^{-1} g^{-1})	Ref.
Ribonuclease A	2.5	8*	0.8	5.0	1.42	0.090	11
Lysozyme (egg white)	2.7	3	1.0	4.4	1.14	0.100	7
β-Trypsin	3.1	3	0.5	2.6	0.64	0.120	12*
α-Chymotrypsin	3.3	3	0.5	2.7	0.72	0.120	13
Cytochrome *c* (Ferry)	3.4	3	0.7	1.7	0.34	0.130	14
Pancreatic trypsin inhibitor	3.7	4*	0.8	0.0	−0.26	0.110	†
Chymotrypsinogen	4.7	3		2.0		0.130	15*
Carbonic anhydrase B (bovine)	6.6	3 10*	0.4	0.1	−0.10	0.140	†
Metmyoglobin	7.1	3	0.7	0.0	−0.22	0.150	7

$\langle\Delta G^{mic}\rangle$, averaged Gibbs energy of micro-unfolding of protein necessary to exchange one proton; $\Delta^D_N g$, specific Gibbs energy of denaturation; $\Delta^D_N h$, specific enthalpy of denaturation; $\Delta^D_N s$, specific entropy of denaturation.
* Our calculation from published data. † Unpublished results obtained in our laboratory.

cannot be large, as extended unfoldings which lead to the exchange of deeply buried hydrogens are rare. Thus, micro-stability never characterises the stability of the whole macromolecule and it can be regarded only as a characteristic of an average 'local stability' of a structure or of its 'rigidity'. It seems that this micro-stability should be treated as a specific characteristic insofar as the average size of micro-unfoldings for the exchange of one peptide hydrogen is constant for proteins. Consequently, the micro-stability can be compared only with characteristics that are also specific. Thus, we cannot compare the micro-stability directly with the macro-stability $\Delta^D_N G$, but only with the specific Gibbs energy of denaturation which is obtained by dividing $\Delta^D_N G$ by the molecular weight, M, of the cooperative unit, $(\Delta^D_N G)/M = \Delta^D_N g$. However, in using this specific Gibbs energy of denaturation we must remember that it no longer describes the stability of the macroscopic state.

Table 1 lists $\langle\Delta G^{mic}\rangle$ values and the specific thermodynamic characteristics for various proteins. We have restricted the list to globular proteins which can be considered as a single cooperative system. The reason for this restriction is that the average size of micro-unfoldings might be expected to be similar for proteins of one structural class and that the meaning of averaged quantities is more or less clear only for this simplest case. The cooperativity of all the proteins presented has been tested by the thermodynamic criterion of the coincidence of the Van't Hoff enthalpy of denaturation and the calorimetrically measured enthalpy[7].

As seen from Table 1, the micro-stability of all nine proteins does not correlate with the specific Gibbs energy of denaturation, $\Delta^D_N g$, and does correlate with the specific enthalpy $\Delta^D_N h = (\Delta^D_N H)/M$ and with the specific entropy $\Delta^D_N s = (\Delta^D_N S)/M$: $\Delta^D_N h$ and $\Delta^D_N s$ decrease with the increase in $\langle\Delta G^{mic}\rangle$. But these correlations with the specific enthalpy and entropy seem to be in themselves the result of a correlation with the specific heat capacity change at denaturation, $\Delta_d C_p = (\Delta_d C_p)/M$ (equations (2) and (3)).

Table 2 Structural characteristics of some globular proteins

Protein	M	N_H	$\frac{N_H}{M} = n_H$ ($\times 10^3$)	N_φ	$\frac{N_\varphi}{M} = n_\varphi$ ($\times 10^3$)
Ribonuclease A	13,600	81	6.0	90	6.6
Lysozyme	14,300	89	6.2	108	7.5
α-Chymotrypsin	25,200	173	6.9	238	9.4
β-Trypsin	23,800	165	6.9	215	9.0
Cytochrome *c*	12,400	70	5.6	136	11.0
Myoglobin	17,900	133	7.4	213	11.9
Pancreatic trypsin inhibitor	6,500	28	4.3	49	7.5

M, Molecular weight; N_H, the number of internal H-bonds in the protein; N_φ, the number of pairs of nonpolar groups located at a distance less than 4.0 Å in the protein.

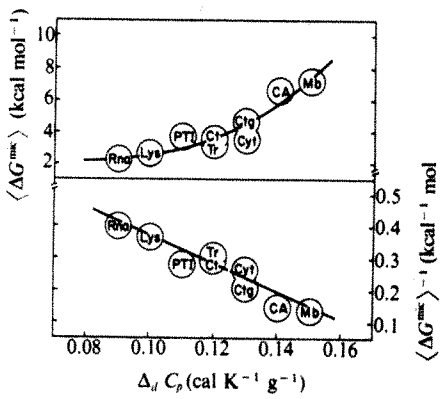


Fig. 4 Plot of the micro-stability ($\langle\Delta G^{mic}\rangle$) and the mobility ($\langle\Delta G^{mic}\rangle^{-1}$) versus the partial specific heat capacity change at denaturation ($\Delta_d C_p$) for various globular proteins. Designations as in Fig. 3.

The predominant role of $\Delta_d C_p$ in the observed correlations follows from the previously established fact that for compact globular proteins with a native structure, equally saturated with hydrogen bonds, the enthalpy and the entropy functions incline to definite points: 13 cal g^{-1} and 3.5×10^{-2} cal K^{-1} g^{-1}, respectively, at 110 °C (ref. 7). A consideration of the known three-dimensional structures of proteins shows (Table 2) that the concentration of hydrogen bonds, that is, the number of hydrogen bonds calculated per unit of mass $n_H = (N_H)/M$, is very similar for all studied proteins, an exception being pancreatic trypsin inhibitor, for which n_H is significantly less. Correspondingly, the denaturational enthalpy and entropy values are also lower for this protein (Fig. 3). Nevertheless, the $\Delta_d C_p$ value also correlates with the $\langle\Delta G^{mic}\rangle$ for this protein, indicating that only this correlation is of primary importance.

The relationship between $\Delta_d C_p$ and $\langle\Delta G^{mic}\rangle$ is somewhat complicated (Fig. 4). However, note that the relationship between $\Delta_d C_p$ and the inverse quantity $\langle\Delta G^{mic}\rangle^{-1}$, which can be regarded as a measure of protein structure 'mobility', seems much simpler.

It has been shown earlier[7] that the denaturational change of the specific heat capacity of proteins is proportional to the concentration of nonpolar contacts in the native structure $n_\varphi = (N_\varphi)/M$, where N_φ is the total number of pairs of nonpolar groups located at a distance of van der Waals contact. The validity of this statement for the proteins considered is evident from a comparison of Table 1 with Table 2. Thus, we conclude that the micro-stability or rigidity of protein structure depends on the saturation of its structure with nonpolar contacts.

As the parameter n_φ reflects the inhomogeneity of distribution of nonpolar groups in the protein, that is, the degree of clustering of these groups, we can rephrase this conclusion by stating that the rigidity of the protein structure is determined by the clusters of nonpolar groups in the protein interior: it increases with the increase of the hydrophobic core of a macromolecule. At the same time hydrophobicity of the protein interior does not noticeably affect the stability of the protein, that is, the Gibbs energy of denaturation. The absence of this dependence can be understood if one remembers that the Gibbs energy is determined by the difference between the enthalpy and entropy terms and not by their similarity, as $\Delta G = \Delta H - T\Delta S$. As a result, n_φ does not affect $\Delta^D_N G$ because it influences the enthalpy and entropy of denaturation to similar extents. But such a factor as the cross-linking of a polypeptide chain, which affects only the entropy of unfolding, can essentially shift the enthalpy–entropy balance and change $\Delta^D_N G$. Thus, the absence of any correlation between the micro-stability and the Gibbs energy of denaturation of a protein is not as surprising as it might seem.

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Membrane crystals of ubiquinone:cytochrome *c* reductase from *Neurospora* mitochondria

HIGH-RESOLUTION X-ray diffraction or electron microscopy studies of membrane proteins are hampered by the difficulty in obtaining crystals or periodic aggregates of the proteins. So far, only two examples of membrane proteins forming highly ordered arrays are known, the purple membrane of *Halobacterium halobium*[1] and a membranous preparation of cytochrome *c* oxidase from beef heart[2]. Ubiquinone:cytochrome *c* reductase, a multiprotein enzyme, contributes about 10% of the inner mitochondrial membrane protein[3]. The enzyme is isolated as a dimer (molecular weight (MW) ~550,000) and the monomeric unit comprises two *b* cytochromes (MW ~30,000 each), a cytochrome c_1 (MW ~31,000), an iron–sulphur subunit (MW ~25,000) and six subunits without known prosthetic groups (MWs ~50,000, 45,000, 45,000, 14,000, 12,000 and 8,000)[3,4]. The enzyme catalyses the reduction of ferricytochrome *c* by reduced ubiquinone and conserves the energy generated by the oxidation–reduction reaction by translocating protons across the inner mitochondrial membrane[5]. Cytochrome *c* reductase is also of interest with respect to the genetics and biogenesis of mitochondria. The cytochrome *b* subunits of this enzyme belong to the small group of proteins which are translated on mitochondrial ribosomes[6] and coded for by mitochondrial DNA[7]. We report here a method for preparing membrane crystals of ubiquinone:cytochrome *c* reductase isolated from *Neurospora crassa*.

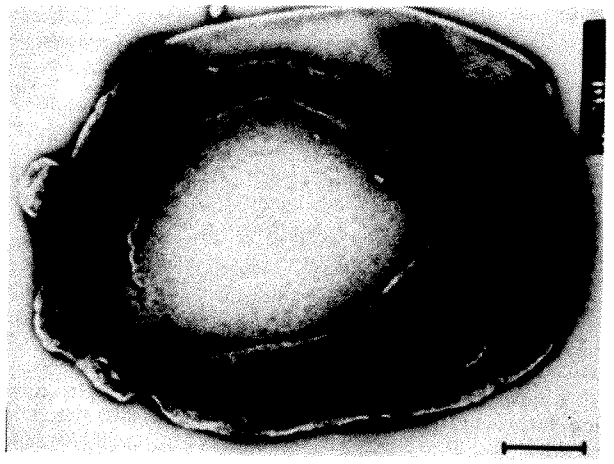


Fig. 1 Negatively stained broken vesicle of crystallised cytochrome *c* reductase. Scale bar, 0.5 μM.

The cytochrome *c* reductase was released from the membranes of *N. crassa* mitochondria by Triton X-100. A micelle (MW ~100,000) of this non-ionic detergent replaces (most of) the phospholipids[4]. The enzyme–detergent complex was then purified by affinity chromatography on immobilised cytochrome *c* followed by gel chromatography. It has a Stokes radius of 86 Å and a sedimentation coefficient $S_{20,w}$ 15.6 (ref. 4).

To obtain membrane crystals from the isolated enzyme, the enzyme-bound detergent was replaced by phospholipid as follows. One ml of a solution containing 2.5–3 mg ml^{-1} enzyme in 0.05% (w/v) Triton X-100, 50 mM Tris-acetate, *p*H 7, and 0.5 mM EDTA was mixed with 1 ml of a solution containing 4 mg ml^{-1} phosphatidyl choline (from soybean) and 1 mg ml^{-1} phosphatidyl serine (from bovine brain) and 0.25% Triton X-100, 50 mM Tris-acetate, *p*H 7, 0.5 mM EDTA and 5 μM butylated hydroxytoluene. Triton X-100 was removed by stirring 0.5 g Bio Beads SM-2 (ref. 8) in the combined solution for 2.5 h at 4 °C. The turbid solution was then subjected to isopycnic centrifugation on a 12-ml 10–50% (w/v) sucrose-density gradient in 50 mM Tris-acetate, *p*H 7, 0.5 mM EDTA and 5 μM butylated hydroxytoluene at 280,000*g* for 20 h. The membranes banded at a density of 1.15 and the excess phospholipid remained close to the top of the gradient. The protein to lipid ratio of the membranes was about 1.7 (w/w).

The membranes were prepared for electron microscopy as follows. The suspension was dialysed overnight against 50 mM Tris-acetate, *p*H 7, to remove sucrose and then brought to 0.5 mg ml^{-1} protein. Copper grids (400 mesh) coated with collodion-reinforced carbon films were rendered hydrophilic by glow-discharge in air. Drops of the lipoprotein suspension were placed on parafilm and grids were touched to these for 25 s, to distilled water for 10 s and then to 2% aqueous uranyl acetate for 45 s. Excess liquid was removed from the grid with filter paper between each stage. The grids were examined in a Philips EM400 electron microscope operating at 80 kV and images were recorded at ×43,000–70,000 magnification. Optical diffraction was carried out as described previously[9].

The electron micrographs showed the presence of negatively stained vesicles up to several microns in diameter which consisted of the cytochrome *c* reductase in highly ordered arrays (Fig. 1). Images of broken vesicles (Fig. 2*a*) showed a 'one-sided' pattern of protein packing whereas closed vesicles showed a moiré pattern (Fig. 2*b*) arising from overlap of the lattices from the two sides of the flattened vesicle. The overlap is clearly demonstrated by the optical diffraction pattern (Fig. 2*d*): the pattern from the image of the closed vesicles shows a mirror-related doubling of all the spots compared with the pattern from the one-sided image (Fig. 2*c*). The angle between the two lattices, as measured by the relative rotation of the spots, was always close to 90°. This restricted angle may result from a folding occurring preferentially along the line of the heavily

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stained groove. Such a folding also explains the finding that the opposite edges of the vesicles are frequently straight and parallel (not shown).

The diffraction patterns extend to the 5th order, limiting the resolution to about 25 Å. This is probably due to the staining and electron beam damage, but the resolution should be extendable by low dose microscopy.

The unit cell is 137 Å × 174 Å. The diffraction pattern (Fig. 2*c*,*d*) and the computer-reconstructed image[9] (Fig. 3) are interpreted as arising from a projection of the membrane crystal corresponding to plane group pgg. If our interpretation of the plane as being a single layer is correct, this symmetry requires that alternate protein molecules are arranged in an up-and-down manner, that is, the glide planes seen in the image correspond, in projection, to two-fold screw axes in the plane of the membrane. This is unlike the membrane crystals reported for cytochrome *c* oxidase[2], in which the protein is arranged unidirectionally. Furthermore, with the cytochrome *c* reductase crystals no interaction seems to occur between the sheet on opposite sides of the vesicles.

The dimensions of one dimeric cytochrome *c* reductase molecule in projection are ~90 Å × 70 Å. These dimensions are only about half the Stokes diameter, 172 Å, of the (dimer) enzyme–Triton X-100 complex estimated by gel filtration[4]. Even when the amount of enzyme-bound Triton X-100—about 0.18 g per g protein—is subtracted, a Stokes diameter much larger than 100 Å would result. This inconsistency between the dimensions of the enzyme in projection and its apparent hydrodynamic diameter in solution, indicates that cytochrome *c* reductase is a rod-like enzyme which spans the membrane with its elongated axis perpendicular to the plane of the membrane.

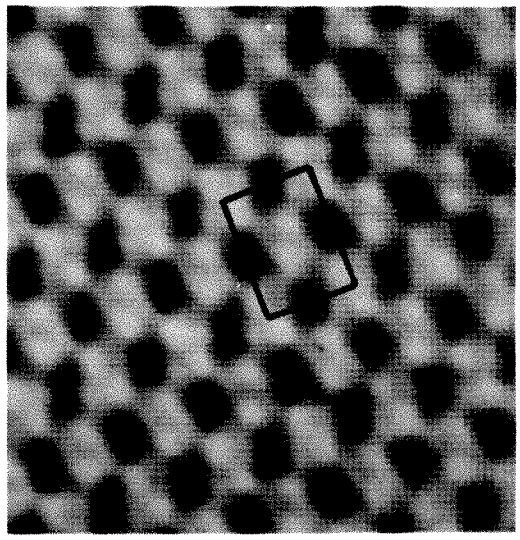


Fig. 3 Computer-reconstructed image of the 'one-sided' membrane crystal shown in Fig. 2*a*. The protein molecules are apparently dimeric. In projection they are arranged with pgg symmetry, so that alternate dimers are enantiomorphic and thus must arise from opposite views. The rectangular unit cell (137 Å × 174 Å) has been drawn in.

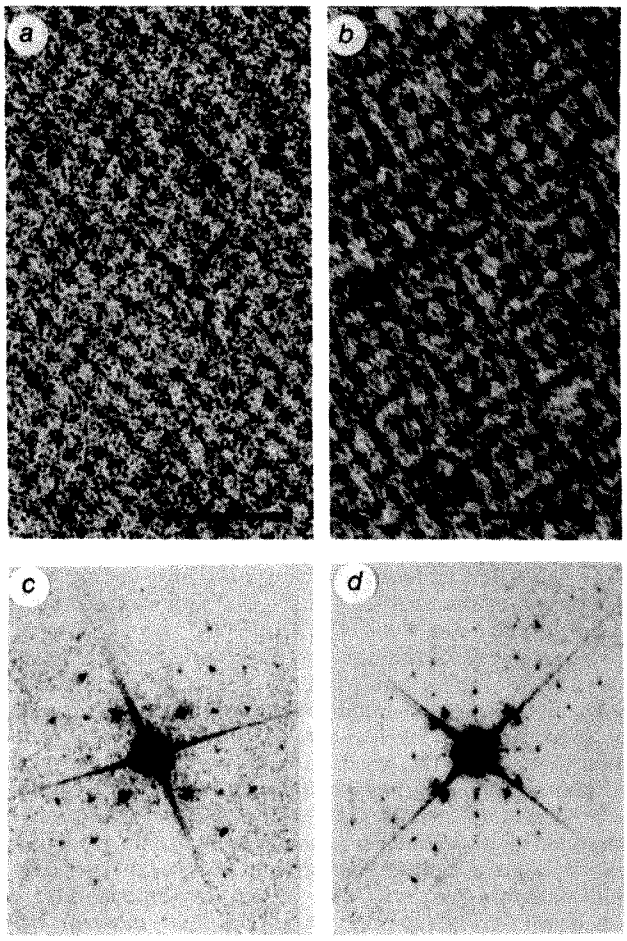


Fig. 2 *a*, *b*, Higher magnification images of the membrane crystals. *A*, 'one-sided' pattern obtained from the central region of the broken vesicle shown in Fig. 1. *B*, Moiré pattern produced by overlap of images for the two sides of a flattened closed vesicle. Scale bar, 50 nm. *c*, *d*, Optical diffraction patterns for the images of the 'one-sided' membrane crystal (*C*) shown in *a* and of the 'two-sided' membrane crystal (*d*) shown in *b*. The absence of the (0, 1) and (0, 3) reflection on the meridian in *c* is consistent with the pgg symmetry. In the case of *d* the two overlapping patterns are rotationally displaced by exactly 90°.

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Adenine N^6-substituent of agrocin 84 determines its bacteriocin-like specificity

AGROCIN 84 (Fig. 1(1)) is the active factor[1] in the biological control of crown gall, a plant cancer induced by certain strains of *Agrobacterium*. Growth inhibition by agrocin 84 has been correlated with pathogenicity[2–5] and the presence of a nopaline (N^2-(1,3-dicarboxypropylarginine)) tumour-inducing plasmid[3–5]. Characterisation of agrocin 84 as a disubstituted adenine nucleotide[6] has been reported and a proposed[7] peptide structure has been retracted[8]. What are those salient features of agrocin 84 that determine its remarkable bacteriocin-like specificity

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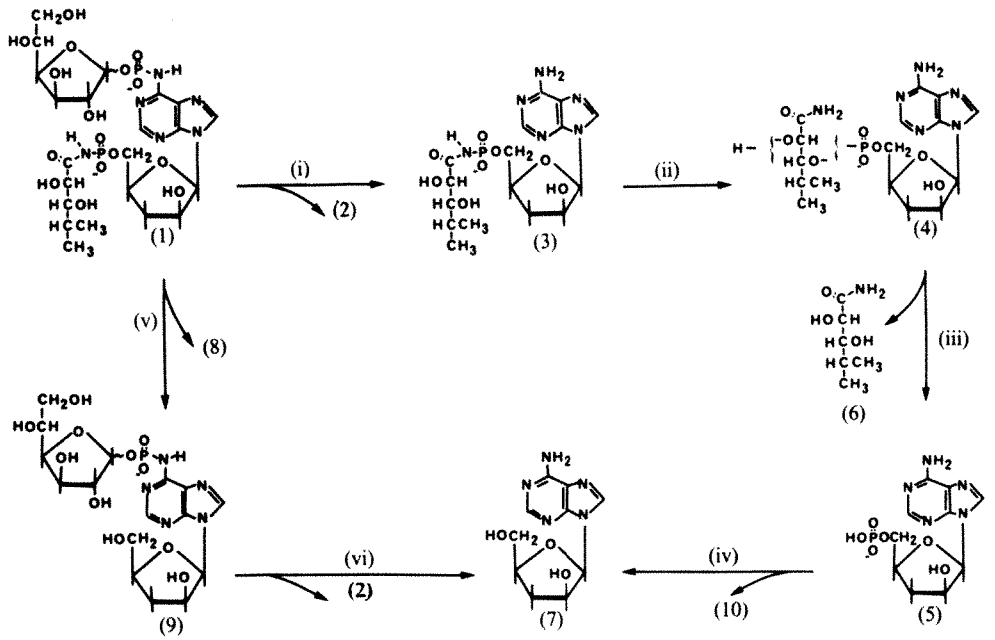


Fig. 1 Agrocin 84, acid and alkaline degradation pathways. Products: (1) agrocin 84; (2) cyclic glucofuranosyl phosphate; (3) nucleoside 5′ phosphoramidate; (4) nucleoside 5′ phosphodiester; (5) nucleoside 5′ phosphomonoester; (6) D-*threo*-2,3-dihydroxy-4-methylpentanamide; (7) nucleoside (9-(3′-deoxy-β-D-*threo*-pentofuranosyl)-adenine); (8) cyclic phosphoramidate of D-*threo*-2,3-dihydroxy-4-methylpentanamide; (9) nucleoside N^6-D-glucofuranosyloxyphosphoramidate; (10) inorganic phosphate. Reaction conditions: (i) 110 °C, *p*H 7, 15 min; (ii) 25 °C, 0.1 M HCl, 17 h; (iii) 25 °C snake venom phosphodiesterase, *p*H 8.8, 3 h; (iv) 25 °C, snake venom 5′ nucleotidase, *p*H 9.0, 1 h; (v) 1 M NH_4OH, 25 °C, 3 h; (vi) 1 M NH_4OH, 110 °C, 1 h.

towards cancer-inducing strains of *Agrobacterium*? The structural evidence reported here shows that a 5′-phosphoryl linkage from the 'fraudulent' nucleoside core 9-(3′-deoxy-β-D-*threo*-pentofuranosyl) adenine[6] to the amide group of D-*threo*-2,3-dihydroxy-4-methylpentanamide (Fig. 1(6)) is required for antibiotic activity, but bacteriocin-like specificity is conferred by a D-glucofuranosyloxyphosphoryl substituent at N^6 of adenine. Agrocin 84 seems to be the harbinger of a series of highly specific nucleotide bacteriocins, which behave as molecular 'Trojan horses'.

The evidence leading to Fig. 1 is summarised as follows: mild alkaline hydrolysis (1 M NH_4OH at 25 °C for 24 h) of agrocin 84 produces nucleotide (9) containing D-glucose, with the characteristic[6] absorption maximum (264 nm, ε 19,500) of an adenine nucleoside N^6-phosphoramidate. In addition, the non-UV absorbing organic phosphate monoesters from the initial cyclic product (8) yield inorganic phosphate and a vicinal glycol after dephosphorylation with *Escherichia coli* alkaline phosphatase. This glycol has now been crystallised (m.p. 192–194; $[\alpha]_D$ −51.6 ± 3) and shown to be virtually indistinguishable in all its measured properties including melting point, mixed melting point, IR spectrum, mass spectrum, optical rotation, and chromatographic and electrophoretic behaviour from a synthetic sample of D-*threo*-2,3-dihydroxy-4-methylpentanamide (6). The racemic *threo*-2,3-dihydroxy-4-methylpentanoic acid (m.p. 108) described by Braun[9] also crystallises as an isomorphous form with a broad m.p. 114–121 and was resolved by way of its quinine salt. The dextrorotatory acid ($[\alpha]_D$ = +13.6 ± 0.2; m.p. 141–142) was converted by way of its methyl ester derivative to yield the required levorotatory amide (6) (m.p. 194–195; $[\alpha]_D$ −52.9 ± 0.6).

Periodate oxidation of agrocin 84 (Fig. 1(1)) yields one mol of formaldehyde and one mol of isobutyraldehyde, whereas periodate oxidation of nucleotide (9) yields only one mol of formaldehyde. As no glycol constituents other than glucose were detectable in nucleotide (9) after further hydrolysis to the nucleoside and inorganic phosphate, it follows that the glucose is present as a glucofuranosyloxyphosphoramidate and that neither the 2, nor the 3 hydroxyls of the D-*threo*-2,3-dihydroxy-4-methylpentanamide is esterified to phosphate in agrocin 84 (1). The nature of the anomeric glucofuranosyl linkage remains uncertain. Further alkaline hydrolysis of nucleotide (9) yields the nucleoside 9-(3′-deoxy-β-D-*threo*-pentofuranosyl) adenine structure (7) and a cyclic glucofuranosyl phosphate (2) which in turn hydrolyses to yield glucose phosphate monoesters.

In an alternative route to the nucleoside core, brief heat treatment (100° C at *p*H 7 for 15 min) of agrocin 84 produces two nucleotides (3) and (4), which do not contain glucose and exhibit absorption maxima at 260 nm. In addition, a cyclic glucofuranosyl phosphate (2) is produced. Nucleotide (3) undergoes periodate oxidation and yields one mol of isobutyraldehyde. Nucleotide (3) also forms a borate complex and is slowly cleaved by snake venom phosphodiesterase to produce the nucleoside phosphate monoester (5). Treatment of the periodate sensitive, borate complexing nucleotide (3) with dilute acid (0.1 M HCl at 25 °C for 17 h) irreversibly isomerises it to the periodate insensitive, non-borate complexing nucleotide (4). The latter is readily cleaved by snake venom phosphodiesterase to produce one mol of the nucleoside phosphate monoester (5) and one mol of D-*threo*-2,3 dihydroxy-4-methylpentanamide (6). Finally, the nucleoside phosphate monoester (5) is smoothly cleaved by a specific snake venom 5′-nucleotidase to yield inorganic phosphate (10) and the nucleoside (7). The latter observation combined with the formation of isobutyraldehyde by the periodate oxidation of nucleotide (3) and agrocin 84 (1), suggests the presence of a second phosphoramidate linkage in agrocin 84 from the 5′-hydroxyl of the nucleoside to the amide nitrogen atom, but the possibility that the linkage to the amide group may be by way of an enolic form to the oxygen atom is not excluded by these data.

The calculated molecular weight (904.4) for the anhydrous *bis*-triethylammonium salt $C_{34}H_{66}N_8O_{16}P_2$ is in accord with the predicted[6] maximum value of 1,100 ± 100.

When the scale of the isolation procedures was increased to process 1,000 l of culture medium, it became evident that a second biologically active component was present. This product was soon shown to be identical to the thermal degradation product nucleotide (3), which lacks the N^6-glucofuranosyloxyphosphoramidate and may be an isolation artefact.

For structure–activity correlations, the potency of a compound may be defined in arbitrary agrocin units per mol. An arbitrary agrocin unit is that amount of agrocin necessary to totally inhibit a zone of radius 1.0 cm (corresponding to the dashed ordinates in Fig. 2) in a lawn of the indicator strain. Using the pathogenic indicator strain 57A in the Stonier[10] bioassay procedure, the logarithmic dose–response plot (Fig. 2*a*) shows that 20 μl 3.49×10^{-7} M solution agrocin 84 corresponds to one arbitrary agrocin unit; this is equivalent to $1.4 \pm 0.3 \times 10^{11}$ agrocin units per mol. Using the same indicator strain, the degradation fragment (3) shows a potency of $3.9 \pm 0.5 \times 10^8$ units per mol. In other words, for the pathogenic strain 57A, agrocin 84 is 360 times more potent than its nucleotide fragment (3). However, Fig. 2*b* shows that the non-pathogenic strain 57 is not inhibited by agrocin 84 at a range of molarities at which the

nucleotide degradation fragment (3) readily demonstrates inhibition, with a measured potency of $3.2 \pm 0.5 \times 10^8$ agrocin units per mol.

Thus, it can be seen that the nucleotide fragment (3) without the N^6-D-glucofuranosyloxyphosphoramidate substituent shows the nonspecific growth inhibition of a simple antibiotic, but only agrocin 84 (1) with its unusual N^6 substitution shows the strain specificity of a bacteriocin for the pathogenic strain 57A.

Over similar concentration ranges, neither strain was inhibited by nucleotides (4) and (9) or the nucleoside (7). The absence of detectable activity using either indicator strain with the N^6-substituted nucleotide (9) shows that although this is a necessary condition for selectivity, it is not a sufficient requirement for antibiotic activity. For detectable antibiotic activity in the bioassay, using initial well concentrations of less than 10^{-3}M, a phosphoramidate linkage to the amphiphilic 2,3-dihydroxy-4-methylpentanamide (6) seems to be mandatory.

The pathogenic *in planta* transconjugant strain 57A was originally obtained[2] using the non-pathogenic strain 57 as the recipient and the pathogenic strain 27 as donor. Examination by agarose gel electrophoresis[11] of the isolated plasmid DNA[12] from strains 57, 57A and 27 confirmed that strain 57A differed from strain 57 solely by the presence of a band corresponding in mobility to the 1.5×10^8, molecular weight[13] donor strain 27 tumor-inducing plasmid (pTi27). Murphy and Roberts[14] have used this subtle difference in plasmid makeup to show that the virulence plasmid (pTi27) encodes an energy-dependent transport mechanism which selectively transports intact agrocin 84 into strain 57A. The absence of the virulence plasmid (pTi27) in strain 57 leads to exclusion of agrocin 84, and it is noteworthy that agrocin 84 does not bind irreversibly to the cell surface of susceptible strains. The analogy of a molecular 'Trojan Horse' seems apt.

A cytosine 'nucleotide bacteriocin', agrocin 108 is also under investigation (Elvin and M. E. T. unpublished) and we suggest that these cell-specific nucleotides may be more abundant in nature than is generally realised.

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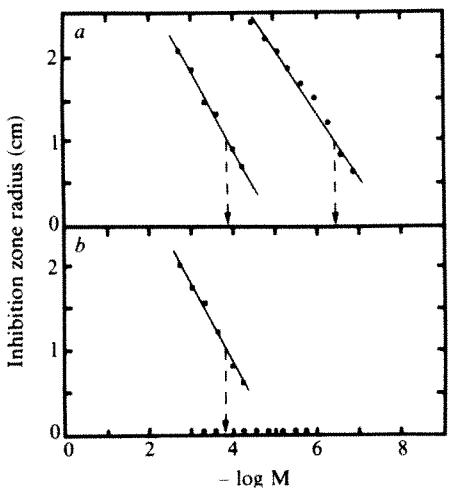


Fig. 2 Least squares fit for the negative logarithm of centre well (20 μl) molarity (initial) against inhibition zone radius using the pathogenic *Agrobacterium radiobacter* var. *tumefaciens*, strain 57A. Comparison of agrocin 84 (●; Fig. 1(1)) with the nucleoside 5′ phosphoramidate of D-*threo*-2,3-dihydroxy-4-methylpentanamide (■; Fig. 1(3)). *b*, As for *a*, but with the non-pathogenic *Agrobacterium radiobacter* strain 57 as the bacterial indicator lawn in the Stonier[10] bioassay. Dashed ordinates indicate the negative log molarity (initial) for an arbitrary agrocin unit of potency. Note that whereas the nucleoside 5′ phosphoramidate (Fig. 1(3)) shows antibiotic activity towards both strains, only agrocin 84 (Fig. 1(1)) with an N^6-D-glucofuranosyloxyphosphoramidate substitution shows specific growth inhibition of the pathogenic strain 57A. Molar absorptivities of 19,860 at 264 nm for agrocin 84 (ref. 6) and 15,400 at 260 nm for the nucleotide 5′ phosphoramidate were used for initial concentration measurements on electrophoretically and chromatographically homogeneous samples.

Erratum

In the letter 'Inhomogeneous distribution of filipin–sterol complexes in smooth muscle cell plasma membrane' by R. Montesano, *Nature* **280**, 328–329, Fig. 2 on page 329 was printed with poor contrast. The figure is reprinted below.

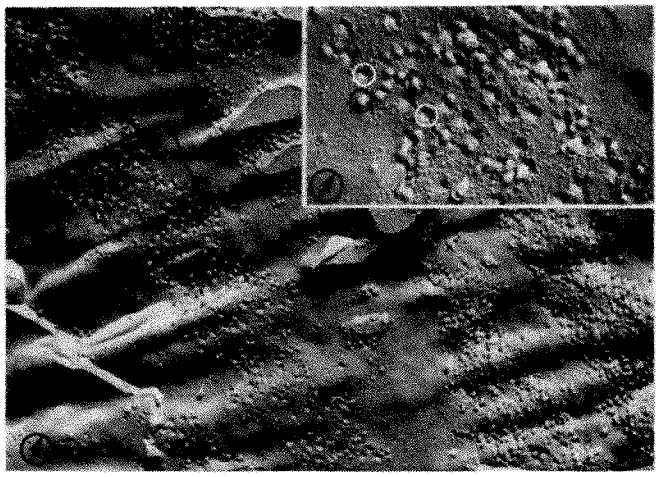


Fig. 2 Freeze-fracture replica of a smooth muscle cell fixed in glutaraldehyde-filipin mixture. The detail of a typical band of invaginations (black rectangle) is shown at high magnification in the inset. Numerous filipin–sterol complexes (black circles) are interspersed among the microinvaginations (white circles), whereas they are virtually absent outside the invaginated membrane regions. Filipin–sterol complexes appear as 25–30 nm protuberances which can be distinguished from the contiguous microinvaginations by the opposite polarity of platinum deposition (the direction of the shadowing is indicated by the encircled arrow). ×22,000. Inset: ×44,000.

matters arising

Dihydroergocryptine is a non-selective antagonist for human platelet α-adrenoreceptors

BINDING of ^{3}H-dihydroergocryptine (DHEC) which is suppressible by phentolamine is widely used as a measure of the α-adrenoreceptor content of tissues[1]. Recently, Kunos *et al.*[2] have reported that phenoxybenzamine, an irreversible α_1-selective adrenoreceptor antagonist, suppressed only a portion of the specific DHEC binding to rabbit uterine membrane fractions although this antagonist had caused total blockade of noradrenaline-induced contraction in the rabbit uterine strips from which the membrane fractions had been prepared. These data suggest that not all the α-adrenoreceptors present in rabbit uterus may be involved in mediating contraction induced by noradrenaline but do not define the nature of the additional α-receptors detected[2].

Using appropriate selective α-adrenoreceptor agonists and antagonists we have recently demonstrated in a functional test system that human platelets carry both α_1- and α_2-adrenoreceptors[3]. In appropriate conditions these cells can therefore be used to analyse the selectivity of α-agonists and antagonists for which this property has not been defined. In the case of an antagonist, selectivity is most appropriately analysed by comparing the potency of the drug as an inhibitor of the clonidine (α_2-adrenoreceptor-mediated) and methoxamine (α_1-adrenoreceptor-mediated) stimulation of the response to ADP. When such studies are carried out using DHEC, the data obtained (Fig. 1) clearly demonstrate that this antagonist has very little ability to discriminate between the platelet α_1- and α_2-adrenoreceptors although it seems to be to be slightly more potent as an inhibitor of the α_1-mediated response. The data of Fig. 1 closely resemble those obtained in similar studies using the classical non-selective α-adrenoreceptor antagonist, phentolamine. Peroutka *et al.*[5] have reported that DHEC is also non-selective with respect to the proposed 'agonist' and 'antagonist' conformations of the α-adrenoreceptor in calf brain.

If the lack of α-selectivity of DHEC as defined here for human platelets is applicable to other tissues, as seems to be the case for uterine smooth muscle[2], caution should be exercised when this ligand is used to detect a correlation between a specific biological effect and receptor content. Thus, if the tissue contains significant concentrations of both α_1- and α_2-adrenoreceptors, estimation of receptor number from the extent of ^{3}H-DHEC binding will give misleading results unless selective α_1- and α_2-antagonists are used to indicate specificity. Similar considerations apply in studies which report changes in the number of α-adrenoreceptors present resulting, for example, from prior exposure of the cells to catecholamines[6].

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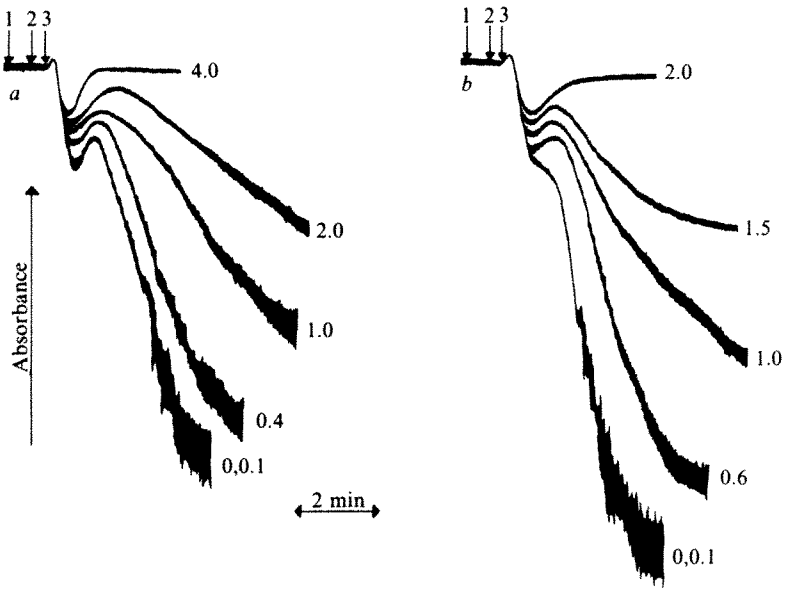


Fig. 1 Inhibition by DHEC of the stimulation of the response of human platelets to ADP induced by clonidine (*a*) or methoxamine (*b*). Aggregation was measured in 0.25-ml samples of human platelet-rich plasma prepared as described previously[4]. Additions were made as follows: at 1, DHEC at the concentrations (μM) indicated by the numbers on the trace; at 2, 5.7 μM clonidine (*a*) or 80 μM methoxamine (*b*); at 3, 1 μM ADP. Unpublished studies similar to those published previously[3] demonstrate that methoxamine stimulation of the response to ADP is mediated by an α_1-adrenoceptor. DHEC at concentrations up to 5 μM had no effect on the response to 1 μM ADP added alone.

Are cardiac muscle cells skinned by EGTA or EDTA?

MILLER[1] re-examined criteria that have been used in defining the state of frog cardiac membranes treated with EGTA or EDTA. From these data, he concluded that 'supposedly' skinned cardiac cells are not skinned by EGTA/EDTA treatments.

To assess whether a preparation is skinned or not, it is helpful to have an objective definition of a skinned fibre preparation. This is especially necessary when using chemical methods of preparation that do not, as in mechanically skinned preparations, remove the major portion of sarcolemmal membranes. A definition we have found useful is that a fibre is skinned when normally impermeable solutes such as MgATP, EGTA, EDTA and other large molecular weight ions gain free access to the myofilament space.

Under this definition, critical physiological tests to determine whether a fibre is skinned must include one or more of these normally impermeable solutes, and a means for evaluating whether the solute has gained free access to the myofilament space. Examples that meet requirements of a 'critical test' for whether a preparation is skinned are (1) development of rigor and relaxation from rigor should occur within seconds of MgATP removal or addition, respectively (time interval predicted for ATP diffusion into or out of the fibre core)[2]; (2) the response of chemically skinned fibres to variations in low MgATP concentrations (0.1–50 μM) should be identical to that of mechanically skinned fibres and consistent with data[3] obtained on extracted myofibrillar preparations; (3) the responses of chemically skinned fibres measured in (1) or (2) should not be quantitatively affected either by mechanical removal of the sarcolemma or by treatment of the pre-

paration with non-ionic detergents that destroy the sarcolemma[4].

We therefore suggest that a third criterion for determining whether a preparation is chemically skinned should be added to the two used by Miller of sensitivity of the muscle to Ca and lack of resting potential: that a preparation is chemically skinned if tension responses affected by MgATP are consistent with tension responses of mechanically skinned or non-ionic detergent-treated preparations. Because the behaviour of EGTA-treated mammalian skeletal muscle fibres[4-7] meets this criterion, there can be no doubt that they come under the definition of a chemically skinned preparation[5].

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MILLER REPLIES—Reuben and Wood have defined a critical test of whether a muscle fibre may be considered skinned by treatment with EGTA or EDTA. This test provides an admirable basis against which to check all types of fibre, in addition to the skeletal fibres on which their own work has been carried out. However, their criteria differ markedly from those of Winegrad[1] which are still generally accepted (for review see ref. 2) and applied[3], and which I contend to be ambiguous[4]. Indeed, additional criteria, such as the acceleration of the rate of tension development at higher Ca-buffer levels[1], are also met by unskinned preparations[5] where extracellular exchange is the limiting process.

My own investigations on frog heart (on ventricular muscle, with which the technique was established[1], and auricle) demonstrated that (1) the method fails to produce the effects claimed for it, and (2) there are alternative explanations for the observed phenomena[4]. Subsequent work with the EGTA/EDTA method has, therefore, been founded on an incorrect interpretation and its validity ought to be re-examined in that light. Where additional evidence of skinning, preferably along the lines indicated by Reuben and Wood, has not been provided, the conclusions remain potentially open to criticism. This is not to say that all such preparations will prove, like frog heart, not to have been skinned, but that evidence broadly based on Winegrad's original criteria alone is not compelling. Wood *et al.*[6] had reported ultrastructural evidence of skinning by EGTA in skeletal muscle and the detailed results published more recently[7] amply confirm their evidence of skinning. However, the feature of the method responsible for the skinning effect remains obscure as prolonged exposure to EGTA (80 mM) in itself does not lead to membrane disruption[8,9].

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MILLER[1] has challenged the notion that the permeability of the sarcolemma of cardiac muscle can be markedly enhanced by treating the tissue with either EDTA[2] or EGTA[3,4]. He has claimed that the two major criteria generally used to infer the existence of the high permeability state, that is, low transmembrane potential and increase in force at very low Ca concentrations, can be produced without an accompanying increase in membrane permeability, and he has concluded that only microdissection of the sarcolemma[5] can produce a cardiac cell in which one can be sure that the membrane barrier to the movement of ions has been removed and the sarcoplasmic reticulum and mitochondria retained.

Although Miller's conclusions may apply to his own studies on EDTA-treated frog ventricle[1], it is less clear that they apply to my EDTA-treated frog ventricular preparation, as I found that the resting potential was not restored for many minutes after the re-introduction of normal Ringer's bathing solution (compare ref. 1, Fig. 1 with ref. 2, Table 1). In addition, in my preparation at *p*Ca 5.4, tension was almost 90% of maximum whereas in Miller's it was less than 50% of maximum in spite of the same Na concentration. Miller's results do highlight the difficulty of producing a hyperpermeable state in frog heart with EDTA and focus on the reason for developing a better preparation.

Miller's conclusions are incorrect for hyperpermeable cardiac fibres that can be produced in rat ventricular muscle by treatment with EGTA as there are at least six different properties of this preparation that favour the existence of a high membrane permeability to small ions and molecules.

(1) The amount of force and the rate at which the force develops at any given concentration of Ca are very stable and reproducible[4].

(2) A stable rigor state is rapidly initiated when ATP has been removed from the bath and rapidly reversed when ATP is restored. This occurs in spite of the maintenance of the concentrations of Na and K and the elevation of the concentration of imidazole to keep the ionic strength constant. In the absence of ATP, the addition of phosphocreatine rapidly relaxes the rigor state only if ADP is present[4]. The relaxation therefore does not depend on a change in the concentration of Na.

(3) The membrane potential of individual cells was stable at −8 mV for long periods when all the KCl had been replaced with NaCl as well as in the presence of Ca concentrations as high as 0.03 mM. According to Miller and Mörchen[6], reduction of Ca to 0.02 mM prolongs the action potential in frog only 20–200%, with little change in resting potential. In rat ventricle Garnier *et al.*[8] found the duration of the action potential prolonged to only a few seconds by Ca-free solutions. Because in our study the same cell was impaled for several minutes, the depolarised state could not be due to a long plateau of an action potential.

(4) The Ca-induced Ca releases from the sarcoplasmic reticulum that were produced by lowering the concentration of EGTA to 30 μM and raising Ca to sub-threshold concentration were very rapidly terminated by raising the EGTA concentration to 3 mM[4]. The very large increase in tension produced by caffeine-induced Ca release from the sarcoplasmic reticulum in 0.03 mM EGTA was totally blocked by 3 mM EGTA[4].

(5) Although treatment of the EGTA-treated cells with detergent for 30 min destroys the function of the cell membranes, it does not alter either the rate of development of force at similar levels of tension or the maximum Ca-activated force.

(6) Possibly most important in refuting Miller's argument is the fact that the amount of force generated at a given concentration of Ca is not altered by changing the Na concentration in the bathing solution. The Na concentration was varied from 10 mM to 40 mM over a range of *p*Ca of 9.0–4.5 with no change in the force generated at any concentration of Ca. These results indicate that Ca, ATP and EGTA all cross the membrane very rapidly and that gradients of K and Cl cannot be maintained across the membrane.

We have described the EGTA-treated cell as hyperpermeable because it is not the same as a mechanically skinned cell. The cell membrane remains, and it is still a diffusion barrier for large molecules such as creatine phosphokinase and glutamate oxaloacetate transaminase. This is not a limitation, but rather an advantage, as it allows the direct probing of the contractile proteins with Ca in a preparation that more closely resembles the intact cell, and regulatory mechanisms that are lost in the mechanically skinned fibre are retained in

the EGTA-treated hyperpermeable cells.

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MILLER REPLIES—To answer Winegrad's conclusion that frog heart is rendered 'hyperpermeable' by EDTA/EGTA treatment: the discrepancy in time scale for the restoration of normal resting potential between his study and my own[2,3] can have several causes. First, my preparations are considerably smaller, are rapidly superfused and display tension responses within seconds[2,4] rather than minutes[1] of solution changes. Slow extracellular exchange will contribute to delayed resting potential recovery. Second, Winegrad reported on the recovery of the resting potential after prolonged exposure to the full 'skinning' medium. It seems most likely that the intracellular ionic levels could alter in these circumstances (high $[K]_0$, low $[Na]_0$, depolarisation, low $[Ca]_0$) and contribute to the slow redevelopment of resting potential in normal Ringer's solution. Third, Winegrad's results were obtained with "an exploring microelectrode" (ref. 1, p. 79). This makes a reliable measurement of time course difficult as substantial repolarisation must occur to give a clear indication of a successful impalement with the microelectrode.

The differences in tension levels at *p*Ca 5.4 noted by Winegrad are hardly indicative of qualitative differences between preparations. He has reported elsewhere that tension developed at this (or any other) *p*Ca is strongly influenced by the immediate history of the preparation (for example, see ref. 5, Fig. 4). Quantitative differences among frog species[4] and between auricle and ventricle (ref. 2 and my unpublished observation) do occur. However, in thin, rapidly superfused ventricle trabeculae exposed to a regimen identical to that of Winegrad, rapid ($t_{1/2} <$ 5 s), complete relaxation always occurs when $[Na^+]_0$ is raised to 120 mM and $[K^+]_0$ reduced to 3 mM (*p*Ca 5.4). This finding signifies more than the "highlighting of the difficulty of producing a hyperpermeable state".

I will discuss the six properties which Winegrad considers "favour the existence of a high membrane permeability to small ions and molecules" in rat ventricular muscle. Properties (1) and (3) have been dealt with already (see above and refs 2, 3). With respect to property (2), removal of ATP as reported in Winegrad's publications had seemed to be associated with Na removal (ATP present as Na_2ATP[1,5–7]). In experiments of the type that I previously reported[2], removal of Na_2ATP evokes a submaximal transient tension development (direct comparison is difficult as Winegrad's records are not calibrated in absolute or relative tension). After this the preparation becomes insensitive to *p*Ca steps[2,3]. The tension transient is blocked if NaCl (10 mM) is included in the ATP-free medium. These effects are also seen in frog trabeculae exposed to EGTA treatment overnight. Clearly, ATP removal does not produce rigor tension in frog. These comments are not necessarily appropriate to Winegrad's experiments in the rat. However, two points can be made.

First, tension development in these conditions is not necessarily rigor tension. Stiffness measurements would form one potentially definitive line of evidence for or against the occurrence of rigor and hence the hyperpermeable state. Second, if the rat cells are not 'hyperpermeable' then the effects of ATP removal, and ADP and phosphocreatine addition[6], would be extracellular. Extracellular ATP was responsible for qualitative changes in the behaviour of the frog preparations[2]. The concentrations of ATP ($MgATP^{2-}$, ATP^{4-}?) involved await detailed analysis. ADP and phosphocreatine have yet to be investigated, but they could act in tandem outside the cell. However, as Winegrad has noted[7], leakage of creatine kinase is increasingly used as an index of myocardial damage. This raises the possibility that a low, but significant, level of extracellular ATP is produced by both nonspecific sites and by creatine kinase leakage from damaged cells. Certainly, this type of experiment is potentially capable of differentiating between skinned and non-skinned cells (see also Reuben and Wood's comments above).

To discuss property (4), even with rapid superfusion of very small preparations, $\geq$0.1 mM EGTA (in nominally Ca-free media) is required to prevent tension development in Na-poor solutions[4]. Tension oscillations in these poorly Ca-buffered conditions cannot readily distinguish between Ca-induced Ca-release phenomena in intact and skinned cells as they are triggered by local $[Ca^{2+}]$ fluctuations in both cases: extracellular $[Ca^{2+}]$ near the sarcolemma for intact cells and $[Ca^{2+}]$ adjacent to the sarcoplasmic reticulum for skinned cells. Extracellular $[Ca^{2+}]$ steps produce Ca release in K-depolarised frog heart[8] and oscillatory responses have been reported[9].

Referring to property (5), the comparability of both the rate of tension development and the maximum force in EGTA- and detergent-treated cells is not a definitive property. Ca-exchange would be limited by the gross morphology of the preparations whether the cell membranes are 'hyperpermeable' or not (see also first paragraph). However, the Ca-sensitivity of detergent-treated preparations differs markedly from that of EGTA-treated cells, half-maximum tension occurring more than 1 *p*Ca unit lower in the latter[6,7]. Winegrad attributes this to a modifying effect of fragments of the sarcolemma persisting after EGTA treatment and subsequently removed by detergents. This idea contrasts markedly with the criteria suggested by Reuben and Wood (see above). Similarly, Winegrad's interpretation of the effects of 5 mM theophylline as occurring through phosphodiesterase inhibition[7] rather than Ca release from sarcoplasmic reticulum (like caffeine and other methylxanthines[10,11]) seems unduly selective.

Considering property (6), the sharp sensitivity of EDTA/EGTA-treated frog heart to $[Na^+]$ provides a clear-cut distinction from that of skinned fibres. In frog, the curve relating tension to $[Na^+]$ is shifted more than one order of magnitude towards higher $[Na^+]_0$ by Winegrad's media compared with 'simple' Na-poor solutions (see ref. 2, Fig. 3 and ref. 4, Fig. 4). As these effects have not been quantified for the mammal one cannot assume that any particular $[Na^+]$ will produce relaxation, but levels higher than 40 mM are worth trying. (Note: $K_{Na}^{app} \cdot [Na]_0^4$ in Fig. 3, ref. 3, is given as

$$\frac{1}{K_{Ca}[Ca]^2} + \frac{K_{Na}}{K_{Ca}[Ca]^2} \cdot [Na]^4$$

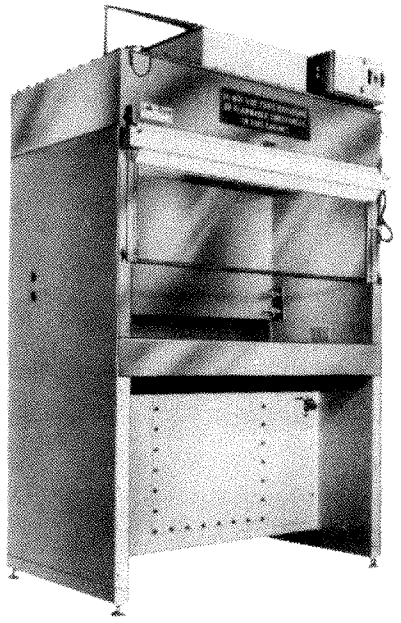
which does not significantly affect the shape of the curve.)

The failure of the EGTA 'skinning' technique has been demonstrated for frog heart, and a plausible mechanism presented to account for the findings which had proven misleading[2]. The criteria for successful skinning as previously applied to frog are, therefore, inadequate. As a result, a definition of skinning in other tissues is not at all straightforward, but the ideas presented by Reuben and Wood should form a useful starting point.

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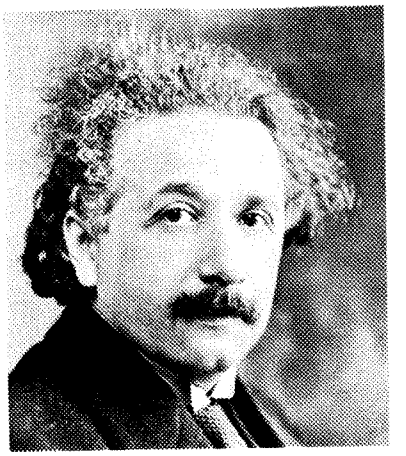
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reviews

General Relativity after Einstein

Robert Wald

General Relativity: An Einstein Centenary Survey. Edited by S. W. Hawking and W. Israel. Pp. 831. (Cambridge University Press: Cambridge, 1979.) £37.50.

MARCH 14, 1979, marked the one hundredth anniversary of the birth of Albert Einstein and numerous activities commemorating this event have taken place or are planned for the near future: the erection of a statue, the convening of conferences and the publication of memorial volumes, such as this book. I do not wish to speculate on how Einstein would have viewed all these activities; in any case, in the final analysis their value must be judged on their own scientific (or other) merits, not on their proclaimed connection to Einstein. Judged in this light, the Hawking-Israel volume is, in all respects, a marvellous book.

When two distinguished scientists edit a book consisting of sixteen articles contributed by other distinguished scientists, it is almost impossible for a bad book to be produced. But the Hawking-Israel volume is quite a bit more than merely "not a bad book". The articles cover the complete spectrum of progress in relativity theory in the past decade, and almost all are very well written. Most of them should be accessible to graduate students in relativity and yet almost all contain some new results or new points of view that should be of interest to the specialised researcher. Of course, no book is perfect and in this volume there is some disparity from article to article in the technical expertise expected of the reader. But this fault — which is almost unavoidable in a book of this nature — is about the only one important enough to be worthy of mention.

Very little work on general relativity was done during the 1940s and 1950s, but there has been a great deal of activity and progress since then, both in development of the theory and in experimental (or observational) verification of its predictions. With regard to the latter, for many years the empirical basis of general relativity rested on the fact that its predictions were in accord with observations in the three classical tests: the gravitational redshift, the bending of light, and the precession of Mercury's orbit. In recent years, however, the use of techniques such as long baseline radio interferometry (to measure the gravitational deflection by the Sun of radio signals from a quasar) and radar (and laser) ranging (to measure gravitational time delay of signals and determine orbits of bodies) have greatly improved measurement accuracy; and the discovery of a pulsar in a close binary orbit has given us an apparently clean system where effects of strong gravity are important and can be studied. In the meantime, a theoretical frame has been developed for analysing alternative theories of gravitation and determining which aspects of each theory the various experiments and observations actually test. All these developments are comprehensively reviewed in the chapter by Will, who concludes that, so far, general relativity has passed all empirical tests, but more stringent tests will confront it in the near future.

One important prediction of general relativity that has not yet been directly confirmed (although it can now be inferred from changes in the orbit of the binary pulsar) is the existence of gravitational radiation. The chapter by Douglass and Braginsky estimates the charactertistics of gravitational radiation expected from the various possible astrophysical sources and discusses the schemes for detecting this radiation. The problems of detection are formidable, as even the radiation from a supernova within our galaxy is estimated to produce fractional relative displacements of only $\sim 10^{-17}$. Nevertheless, the authors are optimistic that an unambiguous discovery of gravitational radiation will occur in the near future.

A key ingredient in the renaissance of theoretical work in general relativity beginning in the 1960s was the use of techniques of modern differential geometry. These techniques were used to prove theorems about the global structure of spacetime, in particular the inevitability of singularities in certain conditions and important results in the theory of black holes. These 'global methods' are reviewed in the chapter by Geroch and Horowitz. Their review focuses on the basic ideas and issues and illustrates how a theorist goes about proving a theorem or constructing a counterexample. The 'expert' who wants complete proofs of the strongest known theorems would be best off consulting other sources, such as the textbook of Hawking and Ellis. But the non-expert — or the graduate student who wishes to become an expert — should find this article to be the best available introduction to the subject.

Progress in the analysis of Einstein's equation is the subject of the chapter by Fischer and marsden. The main topics of their review are the initial value formulation of general relativity, linearisation stability (that is, the question of whether a linear perturbation corresponds to an exact solution), and other issues involving the manifold structure of the space of solutions. In contrast to the Geroch-Horowitz chapter, this chapter *is* written for the expert and is rather demanding of the reader. Most relativity theorists will also have difficulties following the notation, as index notation for tensors is not used.

One of the most exciting areas of progress in the past decade has been the full development of the theory of the black hole, the "region of no escape" which results from the gravitational collapse of a body. Among the marvellous results that have been obtained are the proof of uniqueness of black hole stationary states, the theory of energy extraction, and the analogy between the laws of black hole mechanics and the laws of thermodynamics. These and other results are reviewed in the chapter by Carter.

The marvellous properties of black holes do not end with the above general theory. Studies of the equations governing gravitational, electromagnetic, and other perturbations of the Kerr solution — the unique stationary black hole — have shown that decoupled equations can be obtained and can be solved by separation of variables. Furthermore, numerous differential identities exist between solutions. The chapter by Chandrasekhar provides a thorough introduction and summary of his work on this problem.

The role of black holes in astrophysics is the subject of a very well written review by Blandford and Thorne. These authors summarise the theoretical scenarios where black holes may occur and the astrophysical effects they would produce, as wel as the observational evidence for them. In addition, Blanford and Thorne do a good job in conveying a sense of the nature of the assumptions which go into the various models. Thus, the relativity theorist or other non-astrophysicist who wants to known how seriously to take reports of discoveries of black holes will find this chapter to be a valuable source.

The other area besides black holes where the effects of general relativity play a dominant role is cosmology. A brief chapter by Dicke and Peebles presents some interesting — though rather

sketchy — speculations on issues related to the apparently finite origin of our Universe. Zel'dovich describes the history of the early Universe, including discussions of primordial black holes, quantum effects, and implications of particle physics for cosmology (and *vice versa*). The chapter by MacCallum provides a thorough review of homogeneous, anisotropic cosmological models as well as the rather meagre known results concerning inhomogeneous cosmology.

One of the highlights of the book is Penrose's beautifully written, thought provoking chapter on time asymmetry. Penrose considers the much discussed question of the origin of the arrow of time, but his ideas are quite original, though, as he continually points out, well within the realm of conventional physics. Penrose's main thesis is that at least some, and possibly all, of the various arrows of time — such as entropy increase and the retardation of radiation — can be explained by assuming the initial state of the Universe is one of low entropy. He speculates that this conclusion should be incorporated into physical law, perhaps in the form: The Weyl tensor vanishes at all initial singularities. (The Weyl tensor is a rough measure of gravitational clumping, so the intent of the proposed law is to state that initial gravitational entropy is small.) Whether or not this speculation is correct, the reader should find Penrose's discussion of cosmic censorship, his criticisms of Hawking's "black hole is a white hole" hypothesis and his fundamental criticism of chaotic cosmology, as well as numerous other side discussions, to be of great interest.

The final four chapters of the book deal with quantum aspects of general relativity. Virtually all researchers agree that classical general relativity cannot be an exact description of nature; that the gravitational field, like all other fields, must be quantised. But major conceptual issues arise when one attempts to do so, resulting mainly from the fact that the quantity which describes the gravitional field — the spacetime metric — also describes the background structure of spacetime. If one ignores this issue and tries to quantise gravity by procedures analogous to those used for other fields, one finds that the theory is non-renormalisable, that is, in perturbation theory new terms must be introduced in order to cancel the ultraviolet divergences. Presumably, an infinity of new, undetermined, parameters would appear if one went to arbitrarily high order in perturbation theory.

These difficulties have been known for a long time, but two recent developments have made theorists considerably more optimistic that they can be overcome. The first is the success achieved in particle physics in quantising gauge theories and unifying the fundamental interactions. Gravity would fit very naturally into further developments of this programme. The second is Hawking's calculation of thermal particle creation by black holes due to quantum effects. The beauty of this result has provided the psychological equivalent of experimental verification that the theory is on the right track.

Almost all of the solidly grounded results on quantum effects in strong gravitational fields have been obtained in the approximation where the metric can still be treated classically. These results include calculations of particle creation (such as Hawking's black hole calculation) and 'back-reaction' of the particles on the gravitational field. This work is nicely reviewed from the Cambridge viewpoint by Gibbons. The chapter by DeWitt also contains an extensive review of this work, including a good discussion of the observer dependence of particle detection. Proposals for dealing with gauge problems in full quantum gravity are also discussed by DeWitt.

The chapter by Hawking is probably the best available introduction to his current ideas on quantum gravity. Hawking uses the path integral approach and proposes a solution to the problem of the indefiniteness of the gravitational action (which causes convergence problems in the path integral). His ideas for going beyond the lowest approximation lead him to a picture of "spacetime foam", a highly complex topological structure of spacetime on Planck length (10^{-33} cm) scales. Much of this work is highly speculative and the logic is not always airtight; but the beauty of many of the ideas should convince the reader that there must be much fundamental truth contained in this work.

The chapter by Weinberg takes a much more conventional viewpoint on quantum gravity. Weinberg reviews the evidence that quantum gravity is non-renormalisable, but points out that if the condition of "asymptotic safety" (defined and discussed in the chapter) holds for the theory, then all but a finite number of the infinity of parameters occuring because of non-renormalisability could be fixed in a natural way. This would tame the non-renormalisability problem and could make the theory acceptable without further modifications. Some evidence that quantum gravity might be asymptotically safe is provided by a model calculation in $2+\varepsilon$ dimensions.

As the above discussion should indicate, the Hawking-Israel volume provides a dramatic demonstration of recent progress and future prospects of general relativity theory. The time is ripe for a book of this kind, and it is fortunate that the anniversary of Einstein's birth has provided the impetus for publishing it.

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Control of prokaryotic gene expression

The Operon. Edited by J. H. Miller and W. S. Reznikoff. Pp. 449. (Cold Spring Harbor Laboratory: Cold Spring Harbor, New York, 1978.) $42.

The Operon is a collection of review articles that summarise much of what is known about the control of prokaryotic gene expression. The first half of the book describes aspects of regulation in the *Escherichia coli* lactose operon: the system that is best understood. The remainder of the book focuses on other systems which contrast with the *lac* operon: regulation by temperate phage λ repressor, the regulation of λ repressor synthesis, dual control of the galactose operon, the synthesis of 3′,5′ cyclic AMP, positive regulation of the arabinose operon, attenuation control of the tryptophan biosynthetic operon, autogenous regulation of the histidine degradation operon, and the mechanism of phase variation in *Salmonella*. The book is highly successful in that the articles are well written and provide a very broad education. It would be suitable for graduate courses and useful as a source of information for instructors in molecular genetics.

Jonathan Beckwith's first article describes the genetic methods used to study the *lac* operon. This chapter is sufficiently clear and general to allow the reader to understand microbial genetics in a broader sense. Beckwith provides a second chapter on methods for fusing the β-galactosidase gene into foreign regulatory units, so that the simplicity of the β-galactosidase assay can be used to probe very complex regulatory systems as well as the mechanism by which proteins are exported from the cytoplasm to the cell membrane and into the medium.

Jeffrey Miller's chapter gives a concise history of genetic studies on the lactose repressor (*lacI*) gene. It also contains a detailed description of the genetic methods used to create mutant repressors with interesting physical and chemical properties. An extra attraction of this article is its summary of how the mechanism of mutagenesis was worked out, using the known DNA sequence of the *lacI* gene, in combination with a variety of genetic manipulations.

The chemical properties of normal and mutant *lac* proteins are thoroughly discussed by I. Zabin and K. Beyreuther. I was interested to learn that both the specific and nonspecific DNA-binding properties of the *lac* repressor are

confined to the amino-terminal 53 amino acid residues, whereas the ability to form tetramers and to bind inducer requires the carboxy-terminal part of the repressor molecule. In a similar fashion, λ phage repressor seems also to contain two domains: an amino-terminal DNA-binding region and a carboxy-terminal domain needed for oligomerization and derepression.

Terry Platt has contributed a fine article on the tryptophan biosynthetic operon, which is regulated by a combination of repression at the operator and antitermination at the nearby 'attenuator'. Transcription termination provides a versatile and sensitive form of regulation, as the *trp* attenuator responds to changes in nucleotide sequence, to termination factor *rho* and even to the structure of tryptophan tRNA. Platt mentions the fact that the histidine biosynthetic 'operon' has no repressor nor operator and is controlled almost entirely by antitermination. Thus, the histidine operon would be worthy of a seperate article.

The chapter on phase variation provides the greatest novelty in the book, as the inversion of several hundred nucleotide base-pairs determines whether the H-1 or H-2 flagellar antigen is synthesised. **Richard Calendar**

Richard Calendar is Professor of Molecular Biology at the University of California at Berkeley.

Sexuality in man and animals

Sex, Hormones and Behaviour. Ciba Foundation Symposium No. 62. (New Series.) (Exerpta Medica: Amsterdam, Oxford and New York, 1979.) $41; Dfl.84.

THE purpose of this symposium was to explore, and extend, the zone of overlap between studies of human sexuality and experimental research on sexual behaviour in animals. The book contains fourteen papers, dealing mainly with aspects of sexual development, homosexuality, and the neuroendocrine regulation of hetero-sexual behaviour.

In many respects, the overlap between human and animal research in these areas is extensive and this is particularly apparent where studies of the non-human primates are concerned. The degree to which androgens and experiential factors influence the development of sex differences in human behaviour remains a controversial issue, as indicated by contributions from Ehrhardt, Meyer-Bahlburg and Green. Fresh insights into these problems could emerge from research on monkeys. Abbott and Hearn describe experiments on common marmosets which indicate that sexual differentiation of the brain by androgen occurs, at least partly, during postnatal life. In future it may be possible to use marmosets as models to study how androgens affect the developing brain of male primates.

Very little is known about the neuro-endocrine control of human sexual behaviour in adulthood, or the extent to which steroid hormones, brain monoamines and peptides influence such behaviour. Contributions by Nieschlag, Bancroft and Skakkebaek describe the effects of androgens on human sexual behaviour, and Everitt presents new findings concerning monoamines and sexual behaviour in rhesus monkeys. The interplay between social factors, neuro-endocrine mechanisms and sexual behaviour constitutes an important field of research which is explored by Keverne, in his paper on laboratory groups of talapoin monkeys.

Satisfactory animal models with which to study certain facets of human sexuality have yet to be defined. Homosexuality and transsexuality are specific examples. It is evident that approaches to the study and treatment of homosexuality advocated by Dörner at this symposium were not endorsed by some participants. In his paper, Beach provides thorough guidelines on the potential value and limitations of animals as subjects for research on homosexuality and heterosexual behaviour in man.

This is an excellent book. One minor criticism is that some contributors present purely qualitative papers, whereas the reader might find a quantitative treatment more useful. Discussion sessions between the participants are reported fully, as in other Ciba Foundation Symposia, and total almost 150 pages. These discussions greatly enhance and amplify the value of the papers, making this volume a valuable addition to the literature on sexual behaviour in man and animals.

Alan F. Dixson

Alan F. Dixson is a Research Fellow at the Wellcome Laboratories of Comparative Physiology, Zoological Society of London, UK.

Atomic absorption spectroscopy

Atomic Absorption Spectroscopy. Second edition. By M. Salavin. Pp.193. (Wiley: New York and Chichester, UK, 1979.) £14; $26.

THIS monograph is the second edition of Volume 25 of a series on analytical chemistry and its applications edited by P.J. Elving, J.D. Winefordner and I.M. Kolthoff. As the first edition was written by Walter Slavin, son of the present author, Morris, they join that select company of father-and-son books so prized by collectors, but in reverse order.

In his preface the author states that this should be regarded as a new book on atomic absorption spectroscopy from a different perspective. The organisation and layout of the book, however, follow lines similar to the first edition but, naturally, in view of the developments that have occurred in the intervening eleven years, the material content of the sections has been brought up to date and in many cases completely rewritten. The additional areas covered include a section on electrothermal atomisation and greater emphasis is given to the chemical preparation of samples and to environmental applications. The author rightly draws attention to the neglected area of high precision analysis for major elemental constituents but offers little fresh guidance on how to achieve it. An appendix on commercial instrumentation contents itself with a list of eleven sources of relevant commercial equipment of which only four are actual suppliers of atomic absorption instruments. The information in this section is cursory and refrains from mentioning any European manufacturers.

The different perspective follows inevitably from the author's obvious experience in optical emission spectroscopy which by the same token demonstrates his unfamiliarity with the conventional terminology of atomic absorption spectroscopy. This makes the book slightly upsetting for practitioners of the art but more importantly sets a poor example for the newcomer or for the bench worker in atomic absorption spectroscopy, for whom the book is intended.

The book is written in an off-hand, chatty style, which would be refreshing enough were it not casual to the point of inaccuracy. The use of jargon from other disciplines — who outside the field of emission spectrometry knows what a direct-reader is? (p14) — is less than helpful. Many of the terms used are positively misleading, for example, the sample is "injected" (p11) and the flame is described as being "scanned" by a beam from a hollow-cathode discharge lamp (p9 and p27). Other errors are to be found — nitrous oxide is hardly a fuel (p11), reference 33 should be to Zaidel and in Table 3:1 (p53) 50% O_2–50% N_2 would hardly make a satisfactory flame.

The book is easy to read and can be regarded more as an essay on the subject than as a satisfactory laboratory handbook especially in a field where a plethora of excellent textbooks already exists.

Allan M. Ure

Allan M. Ure is a Principal Scientific Officer in the Department of Spectrochemistry at the Macaulay Institute for Soil Research, Aberdeen, UK.

Neural modelling

Neural Modelling: Electrical Signal Processing in the Nervous System. By R. J. MacGregor and E. R. Lewis. Pp. 474. (Plenum: New York, 1979.) $30.

NEURAL MODELLING is an activity carried out by theoretical biologists, mathematicians, bioengineers, computer types and some physiologists, the aim of which is to provide insights into how brains work. As such, it has aroused considerable misgivings, to say the least, in the minds of many, if not most, experimental neurobiologists. One distinguished neurophysiologist maintains, perhaps with tongue in cheek, that neurobiology is "totally non-mathematical"; another that mathematical neurobiology is mostly wrong, long on formal theory, but short on facts, and that what is not wrong is trivial. One can perhaps understand, if not sympathise with such feelings, given that most neurobiologists are profoundly ignorant of advanced mathematics, and have formed a rather stereotyped view of the neurotheorist, perhaps not unjustified, as one not really interested in, nor capable of distinguishing 'real' facts.

It is against such a background that this book should be seen. MacGregor and Lewis have tried to cover everything from the biophysical properties of the neuronal membrane to the computational aspects of neocortical nets, in some fifteen chapters comprising 380 pages. Chapters 1 and 2 provide a quick introduction to the neurone doctrine, that the brain is made up of a coupled nerve cells interacting across specialised synapses, and to the notion of the unit compartment: that the critical element in modelling electrical signal processing in cells and in nets is a patch of membrane, rather than an entire cell.

Chapters 3 and 4 cover in some detail the biophysics associated with the generation of transmembrane resting potentials. Readers will find in here a good discussion of the Nernst-Planck and Goldman equations, of Donnan equilibria, of ionic and electrogenic pumps, and the like.

Chapters 5—7 follow this up through the Eccles equivalent circuit for postsynaptic membrane potential changes, the Fuortes—Hodgkin—Baylor models for photoreceptor transduction, Rall's work on cable electrotonus, the Hill—Monnier—Rashevsky theories of suprathreshold excitation, to the Hodgkin—Huxley equations, but not beyond.

There follows a radical change of pace and style, as the authors begin the treatment of neuronal nets. Chapter 8 describes current work on analogue computer simulations of small nets of idealised neurons, called neuromines, and their utilisation in the modelling of invertebrate neuronal structures. Chapters 9 and 10 describe applications of stochastic process theory to model neuronal firing patterns, and the various statistical techniques which have been used to discern regularities in the firing patterns of single and paired cells. Chapters 11 — 13 deal with the dynamical properties of large-scale nervous activity, seen through Monte Carlo simulations, continuum field theories, or digital computer simulations. Chapter 14, which is very short, discusses briefly, slow potentials and the EEG.

Chapter 15 deals with a number of attempts to understand the functioning of such neuronal structures as the visual neocortex by Pitts and McCulloch, and by Marr; the generation of thalamic and hippocampal rhythms, by Anderson *et at.*; and the brain-stem reticular formation; and there is a brief mention of lateral inhibition in the retina. Finally, chapter 16 closes with a few methodological comments about modelling in general, and brain modelling in particular.

In my opinion, the authors have failed to provide a sufficiently penetrating account of the field of neuronal modelling, up to mid-1977. It is, of course, always a problem in seeking to convey the nature of a discipline, to choose between breadth or depth. To my taste, only chapters 3 and 4 cover the material in sufficient depth as to be interesting. The others are so superficial as to be relatively boring for anyone who knows anything about the subject matter. In addition to this, I feel that the book is written from the wrong perspective, from the bottom up as it were, instead of from the top down. Over the past 5 years or so, it has become apparent to many neurotheorists, that trying to deduce what is going on in neuronal circuits from the properties of neurones and synapses is not very profitable, and that one has to understand in some detail the computational problems which have to be solved by the system and the algorithms used, before one can get to questions of how such algorithms are embodied in neuronal circuits. This is only hinted at in the later chapters of this book. A really good book on the subject of neuronal modelling remains to be written.

Jack Cowan

Jack Cowan is Professor of Theoretical Biology at the University of Chicago, Illinois.

Chromatographic methods

Laboratory Handbook of Chromatographic and Allied Methods. Edited by O. Mikeš. Pp. 764. (Ellis Horwood/Wiley: Chichester, UK, 1979.) £38.50.

THIS is an excellent addition to the numerous practically oriented books in the Ellis Horwood Series in Analytical Chemistry. Dr Mikeš has enlisted the help of 13 of his colleagues, all employed in the Czechoslovak Academy of Sciences, to produce a completely rewritten version of his *Laboratory Handbook of Chromatographic Methods* (1966). I consider the present book to be a worthy successor to E. and M. Lederer's *Chromatography* (1953, 1957).

The "allied methods" alluded to in the title are countercurrent distribution and electromigration, and one chapter is allocated to each. The chapter on counter-current distribution is clear about matters of principle. However, it ignores the dramatic usefulness of the technique in determining the structure of the antibiotic peptides and in the purification of transfer RNAs by Holley and colleagues. Moreover, no mention is made of Alderweireldt's approach, which culminated in the development of 'steady-state' machines; the example given of use of such a machine is undocumented and fails completely to illustrate its capabilities for finding needles in haystacks. The chapter covering electrophoresis is unclear on matters of principle and inadequate in coverage.

By contrast, the 10 chapters relating to chromatography proper provide excellent coverage both of principle and of manipulative practice. Analytical applications described in the text are by way of example only, but the references to reviews and original papers make it easy to find out how any particular technique has been used to deal with particular classes of organic or inorganic substances. There are some notable omissions. Liquid-liquid chromatography on columns and preliminary concentration from crude mixtures by displacement are scarcely mentioned. Although carrier displacement is discussed in principle, no mention is made of Sanger's brilliant application of it to oligonucleotides. Indeed, there is little practical advice in the whole work about detecting or measuring radioactive substances in chromatography. However, in general, the treatment of chromatography is excellent and the practical detail and careful and extensive bibliography will make this book a good investment for the library of every establishment where chemical analyses are carried out, and particularly for those establishments where versatility is demanded.

R.L.M. Synge

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edited by **A. Meister**, *Cornell University Medical College.*

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obituary

Giulio Natta, 1903—1979

GIULIO NATTA, the eminent Italian Chemist, who died on 1 May 1979, at the age of 76, will be remembered for his discovery of isotactic polypropylene, and for his outstanding researches on the synthesis and structure of stereo-regular polymers. Seldom have such scientific contributions aroused such profound fundamental interest, and his work sparked a volume of research on polymerisation never before approached.

Natta was born in 1903 in Imperia, a small seaside town in Liguria. His father was a well known judge, but contrary to the traditional profession of his family, he did not take up law studies. He was fascinated by chemistry from his school days, and after attending the 'Christopher Columbus' school in Genoa, he studied mathematics at the University of Genoa. He changed disciplines when he moved to the Milan Polytechnic Institute where he took his 'Dottore degree' in chemical engineering at the early age of 21, and his 'Libero Docente' three years later. He was assistant lecturer in chemistry at Milan before he moved in 1933 to the University of Pavia, as Professor of General Chemistry, and two years later to Rome as Director of the Institute of Physical Chemistry. In 1937 he became Professor of Industrial Chemistry at Turin, and finally from 1938 until he retired in 1973, Professor and Director of the Milan Institute of Industrial Chemistry.

Natta's varied professional duties and wide range of research interests equipped him for the remarkable contributions he was to make to polymer science from 1954 onwards. He ascribed the particular conditions, which enabled his research school at Milan to achieve such rapid and conclusive results in the genesis and structure of new classes of macromolecules, to his early experience with X-ray studies of the structure of crystals and the resolution of chemical and structural problems.

At first his investigations were directed to the study of the structure of low molecular weight inorganic substances, but in 1932, following a chance meeting with Professor Hermann Staudinger, he was attracted to the study of the lattice structure of linear polymers. This aspect of his work did not develop very rapidly and he concentrated his X-ray work on the structure of heterogeneous catalysts used for certain important industrial applications. At the same time he investigated the processes for the synthesis of methanol, higher alcohols and formaldehyde and in 1938 he was invited by the Italian Government and some industrial companies to initiate a research programme on the production of synthetic rubber. The industrial production of butadiene-styrene copolymer rubbers was realised in Italy within a few years and he also developed a purely physical commercial process for the separation of butadiene from butene-1. At the same time he began a series of researches on the applications of petroleum derivatives, and in particular the use of olefins and diolefins as raw materials for chemical synthesis such as the oxo-synthesis and polymerisation.

In 1952 he attended a lecture by Karl Ziegler[1], the Director of the Max Planck Institute at Mulheim, and was stuck by the fact that on reacting an olefin with an organo-metallic compound, Ziegler obtained a dimerisation which resulted substantially in a single product and not a mixture. Afterward he said of the meeting "the knowledge acquired in the field of polymerisation enabled me to appreciate the singularity of the methods that Ziegler described and my interest was aroused".

Natta suggested to Montecatini, Italy's largest chemical company, for whom he was a consultant, that a direct link should be established with Ziegler, so that his work and ideas could be developed. The outcome was that Montecatini purchased rights for the commercial development of Ziegler's work in Italy and Natta acquired access to Ziegler's researches on the transformation of olefins and started researches in Milan on the polyaddition of ethylene with organic aluminium compounds.

In 1953 Ziegler made the momentous discovery of the polymerisation of ethylene to a high molecular weight linear polymer and immediately thereafter concentrated his research on the development of better and more efficient catalysts, whereas Natta, on the other hand was firmly convinced that the Ziegler catalyst contained the key to the regular polymerisation of olefins, dienes and other unsaturated compounds.

It was in March 1954 that he made his great discovery of the stereo-regular polymerisation of propylene, butene-1 and styrene. Feverish research resulted within a short time in the polymerisation of many olefins to isotactic polymers, propylene to the syndiotactic polymer, ethylene-propylene copolymers and the stereo-specific polymerisation of non-hydrocarbon monomers. The work in his laboratory was organised so as to obtain maximum collaboration between the different disciplines, engineers worked alongside chemists and physicists in a group consisting of as many as 60 scientists of different levels and status.

Natta was the architect and dealt with all things which were to be done, or had to be, or could be done. In scientific matters he favoured experiment rather than theory, facts rather than hypotheses and relied very much on his exceptional intuition. In all these matters he combined the dedication of the pure scientist with the practical logic of the engineer.

About 1956, Natta developed the symptoms of Parkinson's disease. He accepted his illness as a fact of life and tried to continue as though it was not there. His wife and colleagues were a great help to him at this time, and when he received his Nobel Prize from the King of Sweden in 1963, he was supported by his son Guiseppi and his Nobel Lecture was read by a colleague. He gave up work finally in 1973 and lived near his daughter Franca in complete privacy.

Away from his laboratory he was a keen nature lover, and an active mountain climber. His main hobby was collecting fossils, which he successfully displayed in his home in Milan. Another hobby which he pursued for many years was collecting wild mushrooms and he had an extensive knowledge of edible fungi.

Those who knew Natta well attest to his brilliance of mind and exceptional capacity for work. He was a quietly dressed, soft spoken man of short stature and showed courtesy and consideration to visitors from all over the world who came to Milan, either out of scientific interest, or out of interest in the practical and industrial development of his discoveries. His work was internationally recognised by awards in many countries, and he was co-winner with Karl Ziegler of the 1963 Nobel Prize for Chemistry for his work on olefin polymers.

C.E.H. Bawn

1. Obit.Not.Fell.R.Soc.Lond.21,569-584 (1975).

Elias Melin

WITH THE death of Elias Melin on 22 March 1979, at the age of 89, experimental botany and, more especially, mycorrhizal research has lost a pioneer who held a leading position within his field. His experimental life spanned a period of over 60 years, during which he kept abreast of new developments in his area. Indeed, up to a year before his death, he was still working slowly but productively in his laboratory. His interests were wide and covered aspects of ecology and physiology of higher plants and of fungi.

Melin started his scientific career as a student of the well known plant ecologist Rutger Sernander who in the early decades of this century attracted a great number of gifted students, creating a school of plant sociology which played an important role in the development of Scandinavian botany. At first Melin became interested in the taxonomy and biology of the genus *Sphagnum*, and his doctoral thesis (1917) comprised a study on the plant sociology of North Scandinavian peat bogs. During these studies he observed that young seedlings of pine and spruce grew well on drained peat bogs only if they became infected by mycorrhizal fungi. After this discovery, Melin devoted his life to studies on the mycorrhiza (the symbiotic association between tree roots and fungi)of forest trees.

As a university lecturer at the Royal College of Forestry in Stockholm, he described the structure and the fungal components of the mycorrhizas of pine, spruce, larch, birch, and aspen. He worked out methods for the isolation in pure culture of the fungal symbionts and for the synthesis of mycorrhiza of forest tree seedlings under controlled laboratory conditions. He proved that various species of *Boletus, Amanita, Cortinarius, Lactarius, Russula*, and *Tricholoma* are symbionts of the forest trees.

He also turned his interest to the physiological interactions between the trees and their fungal partners. In pure culture experiments with mycorrhizal fungi, performed in liquid media after the addition of minute amounts of exudates from germinating seeds of pine and spruce, he found that these fungi required simple sugars for growth but were unable to decompose cellulose. He concluded that the mycorrhizal fungi obtain sugars from the tree roots,and that on the other hand, the fungi are especially important to the trees in transferring nitrogen from the soil to the roots. These earlier works and results were summarized by Melin in his monograph *Untersuchungen über die Bedeutung der Baummykorrhiza* (Jena 1925) which is a classic of mycological literature.

In 1930 Melin became professor of botany at the university of Uppsala, where he introduced the study of modern plant physiology. A large number of students, not only from Sweden but also from abroad, were attracted by Melin and his ideas, and under his leadership an intense activity developed in the field of mycorrhiza and physiology of fungi. The laboratory facilities necessary for this development were created under his leadership in the modern institute for plant physiology at the university of Uppsala.

Melin quickly appreciated the possibilities of isotope tracer techniques in mycorrhizal research. In co-operation with H. Nilsson he used this method to prove the transfer of products of photosynthesis from pine seedlings to the fungal symbiont, as well as the transfer of nitrogen, phosphorus, and calcium from the fungus to the plant.

In his later years, Melin still retained his keen interest in the problems of the mycorrhiza, especially the stimulating effect of the plant roots on the growth and spore germination of mycorrhizal fungi. He concluded that this effect was due to exudates from the roots and made considerable efforts to elucidate the chemical nature of this so called 'M-factor'.

Besides his scientific activity, Melin was deeply interested and involved in the social life of the students both at his department and at his student union.His election in 1946 as inspector (master) of this union gave him the greatest pleasure and emphasises the affectionate esteem in which he was held by the students. Colleagues and pupils in several countries are deeply indebted to him for his outstanding leadership and his never-failing friendship and support.

G. Lindeberg

Scott R. Mazzur

SCOTT RUIGH MAZZUR, well-known for her work on social and cultural factors affecting the transmission of hepatitis, died in Washington, DC on 2 March 1979.

Scott Mazzur was born on 30 September 1932 in Princeton, New Jersey and obtained her PhD in 1966 at the University of Pennsylvania working with Dr Werner Henle. Following this, she directed a viral diagnostic laboratory for the New Jersey State Department of Health until 1969 when she joined the laboratory of Dr Barry Blumberg at the Institute for Cancer Research in Philadelphia. In 1973, she was appointed Head of the Viral Epidemiology Section of American Red Cross Blood Services.

Scott Mazzur was fascinated by the interaction and mutual pressure exerted by evolution on human society and human disease. She recognized that a virus like rubella, which has no dormant state, must pass from person to person to maintain itself and that this would be possible only in stable, larger communities which would be able to provide a sufficient number of new contacts. This means that many of the diseases that we know today have probably existed only for the last five or six thousand years when urban communities began to develop in Mesopotamia.

Much of her work was carried out in a series of field trips to a remote area of the Solomon Islands in the South Pacific where the incidence of hepatitis carriers is about five hundred times greater than in the United States. The unique feature of her approach was the prospective study of the total human population of the island, thereby eliminating the elements of chance and selection that have biased the statistical analysis of many studies. The simplicity of her approach was remarkable: she was entirely self-sufficient and did not take any assistants with her on her four field trips to the South Pacific between 1972 and 1977. She took a full census of the residents, drew blood samples and tested them in her field laboratory. She learned to speak Pidgin English and was perfectly at ease in communicating with 'my people' as she like to call them, even though head-hunting and cannibalism was practiced in these islands as recently as the second world war. She loved the islanders and her love was reciprocated by them, as shown by the fact that she was able to collect blood samples even from infants in the various communities.

Scott Mazzur used subtypes of hepatitis B surface antigen extensively as an epidemiological tool and had recently described a new antigenic marker 'e' and used it to subdivide other specificities. Her studies in the South Pacific provided new insights into hepatitis B virus epidemiology. The practice of separating male and female children in the Solomon Islands' culture enabled her to reject the hypothesis that there is a genetic basis for the HBsAg carrier state and to show that high frequency of carriers in males was due to a higher rate of infection, chiefly due to cultural practices. She confirmed earlier suggestions that children under six years have a tendency to become asymptomatic chronic carriers if infected with HBV, while older children and adults often react with an immune response.

Scott Mazzur saw her studies of hepatitis as being a model for other infections of similar epidemiology which might also mimic genetic patterns, for example, in the so-called polyclonal birth defects. This kind of distribution could result from the prolonged presence of infectious carriers combined with the limited dissemination of the infectious agent. Her work bridged anthropology and epidemiology and to each of these she brought new insights. She was a warm and generous friend who met with courage the adversities of her husband's death and her own illness.

G.A. Jamieson

new products for chromatography

These notes, prepared from material provided by the manufacturers, are intended to give an outline of the range of products on the market. For more details use the Reader Enquiry Card bound inside the cover.

A feature on electrodes in analysis will appear in the 20 September issue.

MICROMERITICS

The new Micromeritics microprocessor-based HPLC system, the 7500 gradient liquid chromatograph, incorporates unique design concepts. A chromatographer may now select a system suitable for his application without buying more instrumentation than necessary. Gradient and isocratic models are capable of performing both research and routine HPLC analyses. The compact 7500 can accept all keyboard operating parameters — such as flow rate, pressure limits, solvent concentration and column temperature from the microprocessor-based 740 control module. An alpha-numeric display continually informs the operator of entry status. A printer/plotter located in the 740 produces a 'total analyses report', including gradient/solvent conditions, flow rate, pressure, temperature and operational status. An optional data reduction package prints complete report information describing sample retention times, peak area and height, per cent of concentration, sample and injection numbers. Automation for unattended analyses is achieved by the Micromeritics 725/726 autoinjector, capable of initiating 192 analyses of up to 64 samples. Other 7500 system components which allow flexibility include an all new 753 ternary solvent mixer for more accurate and reliable low pressure solvent blending, a precision heated column compartment for more stable and speedy analyses, a universal injector and a choice of detectors, including a 786 variable wavelength detector, 791 fixed wavelength detector and 771 RI detector.



Micrometrics 726 individual sample programmer

The 726 individual sample programmer and the 725 autoinjector form a system that considerably enhances the versatility of unattended HPLC analyses. The 726 individual sample programmer allows variability in number of injections per sample, rinse functions and indexing on an individual basis. Remote location external events may also be controlled or initiated. The 725 and 726 combine to form an automated system capable of executing either repetitive or random commands to a 64 sample (up to 192 injections) carousel tray. Individual vials may be randomly programmed for 1, 2 or 3 injections of sample or rinse at any time interval (up to 99 min). The 725/726 system maintains all the design criteria previously established for the 725 autoinjector unit: cost-efficient design simplicity, adaptability to all HPLC and computer systems as well as portability, reproducibility and accuracy.

A new solvent delivery system for HPLC has been developed, solving the problem of providing constant flow, continuous delivery and elimination of pulses without affecting cost or performance.

The Micromeritics 750 solvent delivery system is a dual-piston, reciprocating design, incorporating a hypercompensated cam and electronic flow multiplexing synchronisation to give a high degree of flow precision. Typical flow precision is better than ± 0.50% at flows from 0–20 ml min^{-1} and pressures up to 6,000 p.s.i. The absence of pulse dampeners in the 750 gives a reduction in total system volume which allows for single-pump, low pressure gradient programmes to be formed with minimum lag time. Low volume makes unattended recycling (easily connected on the front panel) more efficient. All major pumping system components are easily accessible through the front panel to facilitate maintenance operations. The 750 solvent delivery system is universally adaptable to other HPLC components and may be upgraded to microprocessor control.

Circle No. 69 on Reader Enquiry Card.

WHATMAN

The new, modified Magnum 9 columns permit yields of up to 2.5 g of highly purified compound. They can be fitted to any analytical LC and obviate the need for preparative instruments for laboratory scale preparative HPL. The Magnum 9 columns allow simple scale-up from corresponding Partisil analytical columns. Only the flow rate need be controlled to maintain an equivalent linear velocity in this scale-up. Columns are available packed with any Whatman Partisil 10-μm packing material, offering the chromatographer the possibility of preparative scale separations on media including: silica gel, C_{18} reverse phase, amino-cyano, anion exchange and cation exchange forms. Dimensions, 9.4 mm i.d. × 12.7 mm ($\frac{1}{2}$") o.d. × 50 cm long, 316 stainless steel with LDV outlet to fit any liquid chromatograph.

Whatman produce a wide range of HPLC products that are ideally suited for use in pesticide analysis. Whatmans publication 8122 PA L is a useful guide to column packing and solvent mixtures required for optimum separation of specific compounds together with particular chromatograms obtained in a number of separations. A list of references is also provided. Circle the enquiry card number below for a free copy of this publication.



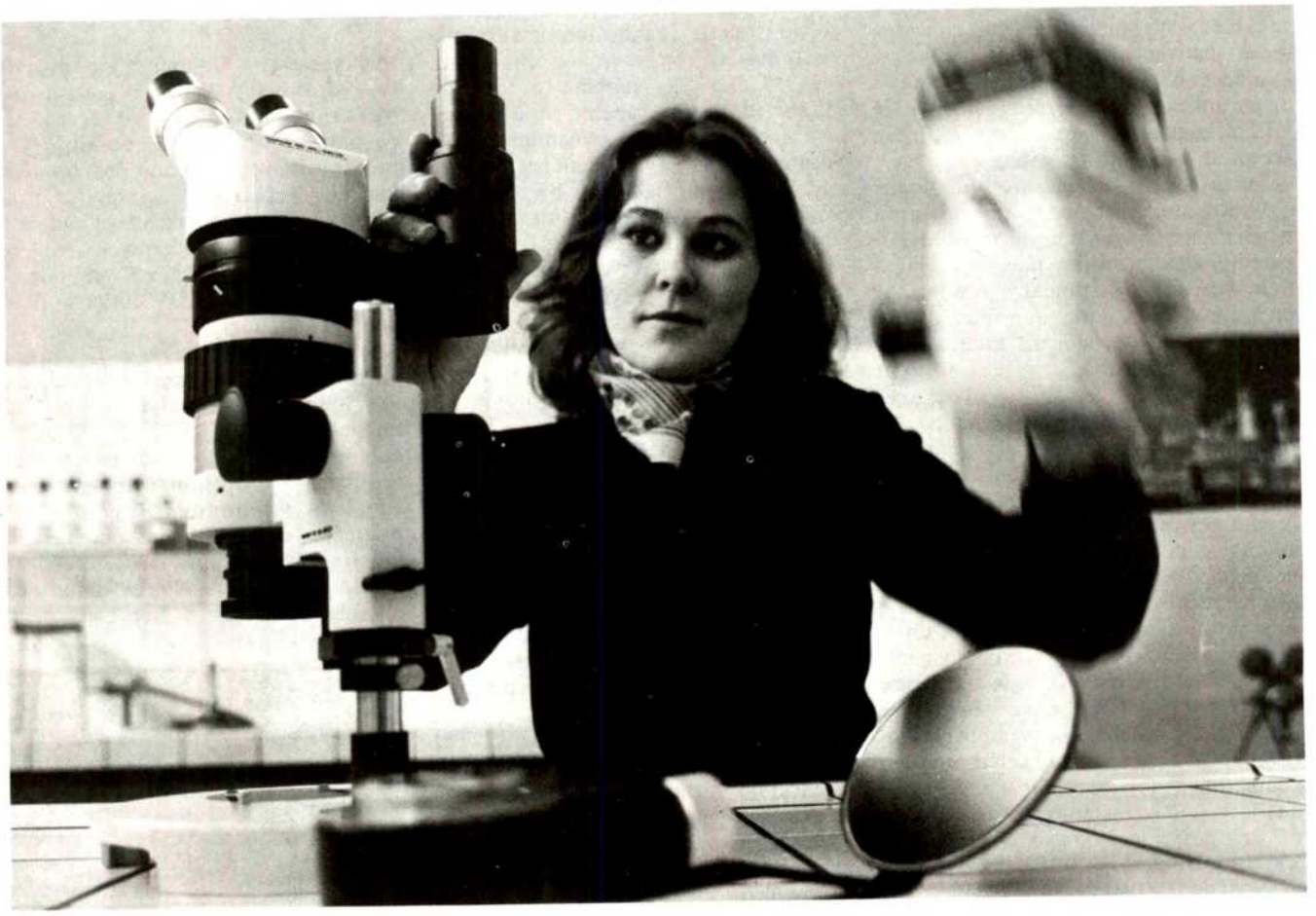
Whatman laboratory filter papers and accessories

A new catalogue (800 PPC) of products for laboratory filtration and paper chromatography is now available from Whatman. Whatman filters are in daily use in laboratories in over 80 countries. Their wide range of filters includes every grade size and combination. Cellulose filter papers in the Whatman range include the ashless quantitative grades as well as qualitative grades. Sizes range from semi-micro 1 cm circles to 50-cm diameter circles and sheets. Whatman glass microfibre filters are also described, along with extraction thimbles, *p*H indicator papers, lens cleaning tissue and disposable phase separators.

Circle No. 70 on Reader Enquiry Card.

x

Adaptability is getting more important.



or stereomicroscopes, too.

I don't have to explain to students the textile weaves which they see on the **closed-circuit TV** monitor.

I don't study the behaviour of small organisms in **dark field,** and as for the inspection of integrated circuits in **coaxial incident light** – well, I just wouldn't know what to look for. But, if I wanted to, I could use my Wild stereomicroscope and its accessories for all of these things.

Minerals are my line. With my Wild instrument I can see crystal structures **in three dimensions;** with the MPS Microphoto System I can **photograph them in sharp detail.** I've measured inclusions with the measuring eyepiece and sketched them with the drawing tube: with precision and clarity. Now I want to buy a **rotating polarising stage** for studying my small collection of thin sections. I really thank my lucky stars that I chose a Wild stereomicroscope; it would have been disastrous to have been unable to add all of these accessories. Have a look at them – most can be used on any Wild stereomicroscope, and they are shown in brochure M1 180e.

Circle No. 21 on Reader Enquiry Card.

Wild Heerbrugg Ltd.
CH-9435 Heerbrugg/Switzerland

WILD
HEERBRUGG

SCHLEICHER & SCHUELL INC.

A new transfer medium, Transa-Bind, designed for the transfer of RNA in 'Northern' blots (Alwine, *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 12 (1977)) is being introduced by Schleicher & Schuell, Inc. New techniques illustrate its expanded use in the transfer of separated proteins from gels. Activation of diazotisation of the transfer medium results in the covalent bonding of RNA or proteins to the paper medium. RNA can then be hybridised with labelled DNA or RNA, autoradiographed and subsequently identified. This can be accomplished without loss of the RNA from the transfer medium. Transferred proteins such as antigens can be detected by probing with antisera. This ensures freshly prepared transfer media, sheet-to-sheet consistency and significant time saving in laboratory preparation. Transa-Bind medium is available in two chemical forms: nitrobenzyloxymethyl cellulose (NBM) paper and aminobenzyloxymethyl cellulose (ABM) paper. Complete instructions include: preparation of the agarose gel; activation of NBM- or ABM-paper before transfer of RNA; transfer; treatment of paper before hybridisation; hybridisation; reuse of blot, and references. Special packaging assures protection in transit and on receipt in the laboratory. An opaque bag has been designed to reduce light penetration and each piece of the medium is enveloped with dry nitrogen to exclude air.

Circle No. 71 on Reader Enquiry Card.

PACKARD INSTRUMENT

The Packard Instrument Co. produce an extensive range of gas chromatography systems. The model 427 is an economical system which is modular and can be upgraded from single to dual column operation. Features include glass-lined injection ports and electron capture detectors, and accurate temperature control. Models 421, 419 and 417 are all-glass GCs for dual column and up to four U-column (model 420) operation. These are especially suitable for the life sciences, clinical chemistry and drug/toxicology chromatography. Specific detectors are available for trace analysis.

Model 429 is a microprocessor-controlled GC particularly suited to automated analysis procedures and model 428 provides great analytical flexibility, with detector changes taking only a few minutes.

For radiochromatography Packard manufacture the model 894 gas proportional counter. It features a high-sensitivity chamber for ^{3}H and ^{14}C. A complete combustion furnace for GLC effluent permits ambient temperature operation of the chamber. A log linear ratemeter with recorder output is fitted.

A wide range of accessories for TLC as well as for GC and HPLC is listed in the Packard chromatography supplies catalogue. Readers in the UK may obtain a free copy of this catalogue from the Caversham (Berks) division of the company by circling no. on the reader enquiry card. Information on chromatographs manufactured by Packard may be obtained (worldwide) by circling the number below.

Circle No. 72 on Reader Enquiry Card.

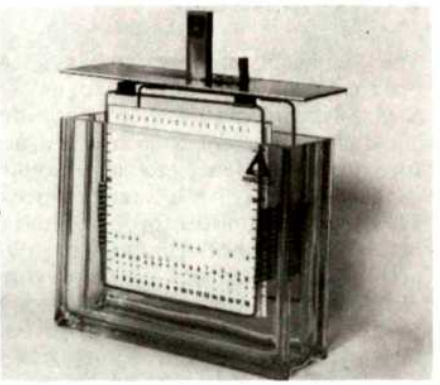
MSE SCIENTIFIC INSTRUMENTS

Now available from MSE is the new ISCO model 1840 UV-visible absorbance monitor for column and liquid chromatography. The model 1840 monitor gives you all the advantages of continuously selectable UV wavelengths, but with sensitivity and price comparable to fixed wavelength detectors. You can often increase detectability for a particular compound several hundred fold by selecting the wavelength of maximum absorption, or increase selectivity by using a wavelength at which interfering solvents or impurities do not absorb. With its ability to detect in the UV down to 190 nm the model 1840 allows you to monitor carbohydrates, non-aromatic amino acids, and many other samples not usually through of as UV-detectable. The ability to select any wavelength allows the use of many standard buffers and procedures that are unsuited for fixed short wavelength detectors operating at 206 or 214 nm. A built-in recorder and optical unit makes the model 1840 complete in a single module, requiring minimal bench space and giving fast, easy set-up. The combination of the model 1840 monitor and model 1850 linear ISCO fraction collector provides the research biochemist with a flexible and economical UV detector/collector combination.

Circle No. 73 on Reader Enquiry Card.

ANALTECH INC.

Analtech have developed a stainless steel TLC plate conditioning apparatus permitting total solvent equilibration in the TLC developing chamber, significantly improving reproducibility of R_f values. The TLC plates (as many as five) with applied samples are held in the developing chamber above the solvent . After solvent equilibration in the chamber, the rack is lowered into the solvent for development. Constructed entirely of stainless-steel, the Analtech TLC plate conditioning apparatus ensures that there is no contamination or deterioration as a result of exposure to the wide variety of solvents used in TLC analyses. The apparatus is supplied complete with stainless-steel lid,



Analtech stainless steel TLC plate conditions

a multiple plate rack which holds up to five 20 × 20 cm TLC plates and a rectangular developing chamber.

The 'Analtechnology' series of technical reports has produced detailed discussion of the methodology used TLC screening of urine samples for the presence of drugs. The report includes discussion of all aspects from extractrion of samples to application of samples to TLC plates and development. Also included are specific reagent formulations and primary references to use of TLC for routine drug screening in urine. The report, *General Procedure for the Analysis of Urine for Drugs*, may be obtained by circling the enquiry card number below.

Circle No. 74 on Reader Enquiry Card.

BRINKMANN

Brinkmann offers a large selection of apparatus and hardware for TLC. Products include applicators, racks and ovens, spotting devices, developing tanks, equipment for drying and visualising separated components, and systems for preserving or documenting TLC separations. Coatings for TLC layers include aluminium oxide, cellulose powder, ion exchange cellulose, ion exchange resins, Kieselguhr, polyamide and silica gel. There are also many special purpose pre-coated layers for unique applications, such as aflatoxin separations, drug screening and so on.

In the HPLC area, Brinkmann products cover packing materials, pre-packed columns, UV-VIS monitors and accessories, pumps and recorders. Labar pre-packed columns are uniformly packed with silica gel 60, a medium porosity adsorbent with mean pore diameter of 60 Å. Narrow particle size distribution means that no peak broadening occurs when working with hydrostatic pressure or low pressure. The Brinkmann fraction collector is compact and extremely useful for a variety of chromatographic applications. It can accommodate any commercially available test tube (o.d. 11-18 mm), culture tube or reagent vessel and can work as low as —20°C.

Circle No. 75 on Reader Enquiry Card.

RAININ INSTRUMENTS

The Rainin Instrument Company have produced a 1979–80 catalogue on chemicals, equipment and accessories for liquid chromatography which is designed for quick reference to all product categories. It includes features, descriptions and prices for the following products: columns/packings, high pressure components, low pressure components, solvents/filters/glassware, pumps, detectors/recorders, valves/fittings, ovens/baths and other accessories. A helpful applications section is included. A free catalogue may be obtained by circling the number below.
Circle No. 76 on Reader Enquiry Card.

HEWLETT PACKARD

The successful Hewlett-Packard 5992 gas chromatograph/mass spectrometer has been up-graded with a new software package. Now with a second disk drive, users can increase library size dramatically and have available virtually unlimited storage. This development allows entire chromatograms to be stored and gives the user a good deal more flexibility. For example, the operator can optimise the system around a specific mass range and choose to save all the data or only the GC peaks where there is a need to maximise the value of collected data. Up to 6 ions can be integrated at any one time and up to 20 ions can be monitored in the selected ion monitoring mode, adding flexibility for quantitative analysis. The extended storage facilities are expected to be of particular value to those assembling drug and pollutant libraries. A 6,500-entry separate library file is available to store information on priority compounds, including names, retention times and response factors.



Hewlett-Packard 5992 GC/MS

New from Hewlett Packard is the versatile HP 5880A gas chromatograph. The simplest version is level 1, a single-detector isothermal instrument with high-speed printer/plotter and retention time labelling. The system can be upgraded through each level to a multi-detector, temperature programmed instrument with data handling and programming capabilities. An optional alpha-numeric keyboard provides report annotation plus BASIC programming. A powerful Hewlett Packard cartridge tape unit allows off-line storage of information.

For liquid chromatography the new HP 1081B for HPLC features several modes of operation. It may function as a free-standing instrument, as a data source for an integrator or data aquisition system, or as an analysing extension of a laboratory automation system. Analytic procedures developed on the more sophisticated HP 1084B can be transferred to the HP 1081B with comparable precision.
Circle No. 77 on Reader Enquiry Card.

APPLIED SCIENCE LABS

The April/May issue of Applied Science Labs Gas-Chrom Newsletter has details of their latest products. Three new GC liquid phases have been introduced on the market, using substituted silicones. Details of the performance of GC separation of *cis/trans* isomers using the SILAR-IOC is also included. The latest newsletter, which normally contains information about new products and their performance capabilities, can be obtained by circling the enquiry card number below.
Circle No. 78 on Reader Enquiry Card.

DUPONT

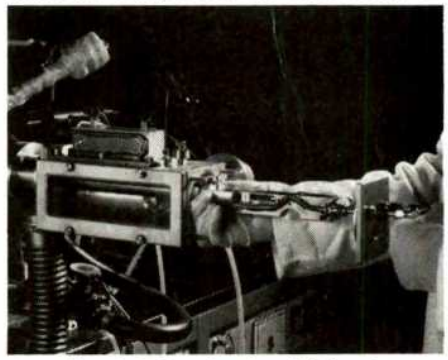
DuPont today offers a broad selection of HPLC systems that embody the latest developments in HPLC, including advances such as microprocessor control, advanced tri-piston pumping systems, state-of-the-art detectors, automatic samplers and other accessories. The systems are readily expandable for further applications.

One of the new DuPont HPLC systems is the model 850. This machine solves the interface problem by giving an on-line display of all key operating parameters while the system is running. Special design features include: precise gradient programming; precision constant-volume tri-piston pump; versatile microprocessor; self-contained memory permitting ready recall and up to eight stored analytical programs; built-in diagnostics for easy service; and universal ports to communicate with system accessories and peripheral equipment. A catalogue describing DuPont's HPLC systems can be obtained by circling the enquiry card number below.

The DuPont DP 102 fully integrated analytical mass spectrometer system is one of their most advanced systems. It is a complete system that incorporates a research type GC with a unique, all glass GC/MS interface; a proven mass analyser with rapid scan and versatile selected ion monitoring capability; and a high-speed computor, fully programmed for instrument control, system management and data handling. The whole system is controlled from a single keyboard.
Circle No. 79 on Reader Enquiry Card.

VG-ORGANIC

Liquid chromatography/mass spectrometry (LC-MS) and pyrolysis/mass spectrometry (Py-MS) are important new combined techniques which extend the scope of mass spectrometry. VG Organic have produced a liquid chromatography interface and a Curie point pyrolysis probe which can be fitted to any of their range of organic mass spectrometers and which do not interfere with other accessories. LC-MS is particularly useful where thermally sensitive and involatile substances are under investigation, and in many cases permits analyses without the need to prepare derivatives. Py-MS is now being used for the analysis of high molecular weight materials. Even complete organisms, such as different strains of bacterial species, have been rapidly identified by this method. The LC interface is based on a moving belt which carries the effluent from the LC directly into the ion chamber. Sample/solvent enrichment is



Assembling the LC interface from VG Organics

achieved by a two stage vacuum lock and heater. Another heater rapidly vaporises the sample to minimise decomposition. The interface has several advantages; being usable in both electron impact (EI) and chemical ionisation (CI) mode; handling probe samples more gently than conventional inlets as well as effluents from the LC; and achieving high transfer efficiency whilst coping with up to 1 ml min^{-1} of volatile solvents and 0.5 ml of involatile solvents. The pyrolysis probe operates by inducing an alternating magnetic flux in the pyrolysis wire on which the sample is applied. Hysteresis losses in the wire heat it very rapidly to its Curie point, the volatile products of the pyrolysis passing into the ion source for mass spectral analysis. A range of wires giving pyrolysis temperatures from 290°C to 980°C is available.

VG-Organic announce the 12-12F quadrupole mass spectrometer, primarily developed for high throughput routine organic analysis. Special features include full microprocessor control and dual analogue/digital display terminal with keyboard for command entry. Minimal contamination is assured by use of a low resolution prefilter thus considerably reducing the need for cleaning of the filter rods. Accessories include an FD/EI source

as well as the standard CI/EI source; a range of gas chromatographs with interfaces for either or both capillary and packed columns; a high efficiency (above 80%) liquid chromatography interface suitable for both normal and reverse phase columns; a probe for Curie point pyrolysis and a comprehensive range of data systems. A new 12-page brochure describing the 12-12F is available on request.

During the past four years the micromass 70-70F high resolution mass spectrometer has established itself as the leading high performance instruments for general analysis and research. The 70-70F is one of the most sensitive mass spectrometers available — 2×10^{-8} Cmg^{-1} for the molecular ion of methyl stearate. Its resolution has been increased by 25% to 25,000 and the mass range is now 2–700 at 4 kV. The 70-70F is capable of scan rates of up to 0.3 s per decade giving, for example, a scan cycle time of 0.8 s over the mass range 500 to 25. It is therefore entirely suitable for high resolution capillary GC-MS. Also, the 70-70F can be supplied with either the VG2000/2200 series or Incos 2400 data system. In both cases VG micromass assume total system responsibility and the new data systems can include extensive control facilities, replacing conventional electronics thus reducing cost and simpifying operation.

Circle No. 80 on Reader Enquiry Card.

GALLENKAMP

The Griffin heated column gas chromatograph was designed as an inexpensive teaching instrument, but its performance specification has come closer to that of far more expensive research and analytical gas chromatographs. As well as being useful in the education field, the Griffin chromatograph has been proved suitable for project work, routine analysis and for quality control applications. The unit can be operated at any fixed temperature between 25°C and 150°C and comes complete with its own flame ionisation detector. The chromatograph is supplied with a standard 1-m column, filled with silicone oil on Celite and also has a heated injection port. A separate control unit incorporates heater controls and an amplifier which is capable of giving an output signal of up to 1 V, together with a baseline zero off-set.

Gallenkamp have recently added a new multisheet tank to their existing range of chromatography equipment. The tank can be used for one- or two-way ascending or descending chromatography and is designed to cater for a large number of samples at one time. Provision has been made for up to ten 250 × 250 mm papers on the aluminium frame for simultaneous ascending developments. Alternatively, two troughs are provided to hold paper for descending chromatography. A further pair of troughs can be used if required, and are available as accessories.

A new hand-held ultraviolet lamp for chromatography has also been recently added to the Gallenkamp range. It is supplied with a stand under which TLC plates may be located during examination. The lamp emits UV radiation at 366 and 254 nm. It contains two long wavelength discharge tubes with integral filters and one short wavelength tube with an external filter. A three-position switch selects the required wavelength or 'OFF'.

Circle No. 81 on Reader Enquiry Card.

SERVA

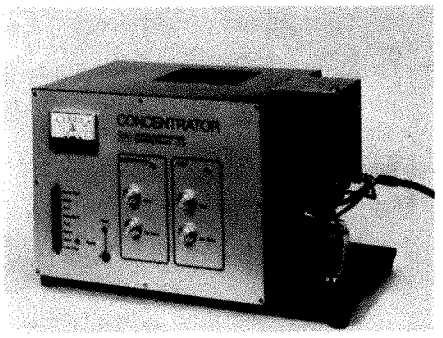
Serva was the first company to develop and produce cellulose ion exchangers, and the quality has been constantly improved. By cross-linking the cellulose fibres with diepoxides they become less prone to swelling and gelling. There are a wide range of Servacel ion exchangers and adsorbants.

Amberlite ground, screened and processed resins are produced in many grades by Servachrom for liquid and gas chromatography. For HPLC, Servachrom produce a range of columns, supports, connectors, precolumns and silica packings. Serva is represented in the USA by Accurate Chemical and Scientific.

Circle No. 82 on Reader Enquiry Card.



Circle No. 12 on Reader Enquiry Card.



The GN concentrator

GN INSTRUMENTATION

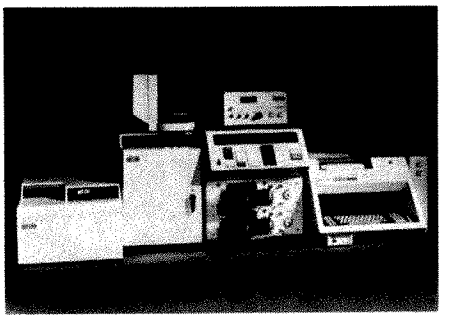
The technique of trapping, or concentration by adsorption, has become well established in the field of industrial hygiene and pollution monitoring. The method is to draw a sample of air through a short tube containing adsorbent, usually Tenax GC, Poropak, or charcoal. The constituents sought are retained by the adsorbent and the tube may then be sealed and returned to the laboratory for analysis. The GN concentrator, a recently developed desorption unit, desorbs the constituents into the chromatograph as a sharp plug of only a few seconds duration. The concentrator uses a two-stage desorption programme. The adsorption tube is first heated in a temperature-controlled oven for a selected time. The desorbate is collected in a cold trap held at —80°C or at any chosen low temperature. The cold trap is then rapidly heated, at the rate of at least 30°C s^{-1}, so injecting the condensed material into the chromatograph as a sharp plug. In this way difficult compounds such as aniline, sulphur-containing and high boiling point compounds have been successfully analysed. Another important application is in the field of water analysis where organic constituents may be concentrated on sorptive traps, either by direct contact or by degassing technique. The concentrator is designed so that it can be piped into the carrier gas supply of a chromatograph without any interference to normal operation; and it is capable of being set up to operate automatically through any desired desorption programme.

Circle No. 83 on Reader Enquiry Card.

RANK-HILGER

Chromaspek is an automated ion-exchange chromatograph with a photometric measuring system designed to analyse amino acids on a routine basis much more quickly than has normally been possible in the past — an analysis can be completed in less than an hour. It does this at low cost, and it is possible to measure down to 200–500 pmol of each amino acid. Chromaspek is unusual in design: it uses only two buffer solutions, with *p*H levels set at each end of the required range. Intermediate values are obtained by ratio mixing of these through a programme controller pre-set by the operator. A 60-position autosampler ensures that the instrument can function automatically over long periods — it can be left unattended over a complete weekend. Chromaspek is bench mounted and consists of reagent compartment, autosampler, peristaltic pump, chromatographic column, monochromator, programme controller and recorder readout. A computer and teletype may also be fitted. No water cooling is required. Results are displayed on a chart recorder in the form of chromatograms and may also be transmitted to a dedicated minicomputer which identifies the individual acids, integrates the areas of the chromatogram peaks, calculates each concentration and then prints the identification and concentration of each acid. The range of this instrument extends beyond the 19 amino acids normally determined in hydrolysate work to the point where it can routinely handle the 40 and more amino acids present in physiological samples. Further increases in sensitivity are possible using the optional fluorimeter attachment.

Circle No. 84 on Reader Enquiry Card.



Perkin-Elmer methods processor

PERKIN-ELMER

Perkin-Elmer's automated methods processor for liquid chromatography is a system concept enabling the user to conduct a complete LC analysis, from injection to processed and printed answer automatically. Using Perkin-Elmer instruments, the processor programs and controls the analysis of up to 42 consecutive samples via a Perkin-Elmer cassette tape program. Additionally, the analyst can generate other programs from the Sigma 10 keyboard. Analytical control features include: pump method, data processor method, sample selection, sample sequence and detector wavelength change. Software includes: error messages, reprompt for non-valid entries and allowance for method modifications without complete re-setup.

The Perkin-Elmer series 3B liquid chromatograph is a dual pump microprocessor-controlled solvent delivery module offering superior pump performance and a degree of control and precise repeatability hitherto unachieved in similar hardware. This module allows the user to refine continually analytical conditions by providing virtually unlimited gradient capabilities, using five segments, each with a variety of concave, convex or linear solvent programs. The series 3B also offers the capability of computer-orientated linkage to data manipulation and reduction apparatus, thereby making it the key element in Perkin-Elmer's new line of liquid chromatographic modules for automated methods processing.

The LC-75 is a new variable wavelength UV spectrophotometric detector which combines high performance with simplicity of operation. As well as being more sensitive, having greater linearity and less drift than previous detectors, the LC-75 has an accessory auto-control unit. This enables the chromatographer to carry out spectroscopic operations such as automatic stop flow scanning, automatic absorbance rationing, wavelength optimisation, autozeroing and baseline correction for gradient runs. Perkin-Elmer's new LC-75 autocontrol module offers expanded capability for users of the new LC-75 UV detector for liquid chromatography. This module allows the use of all the tremendous spectroscopic power designed into the basic model LC-75. The autocontrol combines electronic logic and positive linkages to lead the user step-by-step through the most sophisticated chromatographic peak analyses. Results are presented as clear straightforward digital answers. The new autocontrol module with the LC-75 detector offers: rapid and easy absorbance rationing; pushbutton spectral scanning; background correction of mobile phase absorbance effects during gradient operation; and selection of optimum wavelength for each porter of the chromatogram. Operator benefits include: pushbutton autozero; injection marking; direct LED readout of absorbance, and stored wavelengths, and time; and retention of specific wavelength/absorbance values in absorbance ratio mode.

The model Sigma 10, is a specifically designed to handle data from any instrument in Perkin-Elmer's Sigma series, or any other gas or liquid chromatograph of conventional design. Annotated

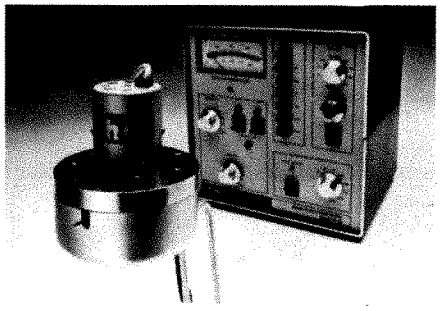
Rocks & Minerals
SPECIMENS FOR RESEARCH — STUDY — DISPLAY
Bulk minerals for all purposes.
Sets of basic rocks, minerals and radioactives. Ground Rock sections.
Advanced collectors' specimens, fluorescents and micro-mounts.
Sets of geological specimens for 'O' and 'A' level examinations.
Send S.A.E. for stock list.
ROCKS AND MINERALS,
4 ROYAL CRESCENT,
CHELTENHAM,
GLOS GL50 3DA.
Tel. 0242 27232

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chromatograms can be obtained from any of four active channels connected to the system, while provision is made for the storage of multiple data files and methods. Six time controlled relays are provided as standard on every channel and ready signals from chromatographs and auto-samplers can be fed back to the system for complete synchronisation of automatic analyses. Accessories available include a basic programmability accessory, which permits the user to write his own programs in BASIC to suit particular application requirements, and a cassette system, using standard magnetic tape cassettes, which extends the system memory by 29K per cassette. Convenient connection to other terminals such as teleprinters and CRTs are provided by the RS-232C input/ouput accessory. The system provides total analytical data on a single report.

A new range of analytical LC columns has just been introduced by Perkin-Elmer. There are eight columns available in the range which will satisfy the needs of most users. Because of their extremely high quality, these columns each have a guaranteed efficiency, and a very long working life. Each column comes complete with an instruction manual, column service kit, original test chromatogram and test certificate.

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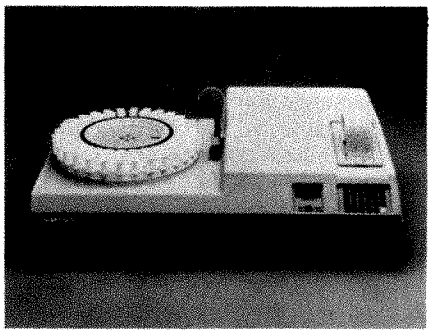


Analysis Automation HNU high temperature chromatograph

ANALYSIS AUTOMATION

A new improved HNU high temperature chromatography detector is now available from Analysis Automation of Oxford, manufacturers and suppliers of analytical instruments for environmental monitoring and industrial safety. The new detector, the PI51 - 02, will operate at temperatures up to 300°C, allowing column temperatures up to 280°C. It retains all the functions of the standard HNU photoionisation detector, particularly in terms of sensitivity, wide dynamic linear range (10^7), broad range of species detected and low noise. However, the higher operating temperature significantly increases the range of analyses that can be performed: new application areas include pesticides, drugs and polynuclear aromatics. The introduction of the new UV sources gives greater selection of the range of species detected.

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Chemetrics Analyzer II

CHEMETRICS CORPORATION

Analyzer II is a new low cost benchtop system for automating blood chemistry tests and panels. Analyzer II comes in two versions, manually pipetted or fully automated with the customer's choice of options. With automatic pipetting, Analyzer II can perform several hundred tests or 30 panels per 8-hour shift. One of Analyzer II's unique features is its ability to run unattended sequential batches of different types of chemistries. It can be pre-programmed for up to 55 tests and reprogrammed by the user as new tests and procedures become available. State-of-the-art data measurement and statistical routines are available. Analyzer II conducts both single point and kinetic reagent and serum blanking. Five standards may be used. Throughput is up to 60 enzymes or 375 endpoints per hour. The full range of options includes auto-pipettor, programmable memory, patient report collator, Syva curve-fit routines, and interface to printer or computer.

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NEWMAN-HOWELLS

The Iatroscan TH-10 analyser combines a flame ionisation detector with a unique adaptation of TLC. The system is based on thin, re-usable quartz rods coated with silica gel or aluminium. Individual components are detected by passing the rod through a hydrogen flame and the resultant chromatogram is displayed on a two-pen recorder. Output from an analogue integrator in the TH-10 is fed to the second pen.

Circle No. 88 on Reader Enquiry Card.

SHIMADZU

The Shimadzu range of computing integrators for GLC and HPLC is one example of Japanese microprocessor application. The C-RIA is an advanced printer/plotter instrument with full alpha-numerical programming capability, which enables it to measure any peak and do all the calculations any chromatographer may need. The C-RIA can even calibrate itself from analyses of known solutions.

A smaller integrator, called the C-EIB, is also available; essentially it has printer output in place of printer/plotter. Again the quality of performance and construction is very evident and its price is very low.

Shimadzu chromatographic products are distributed in the UK by Dyson Instruments Ltd of Durham, who will supply further information on request.

Circle No. 89 on Reader Enquiry Card.

CARLO ERBA

The Carlo Erba Fractovap 4160 high resolution dedicated gas chromatograph is the result of more than 20 years experience in gas chromatograph manufacture and design. The Fractovap 4160 series offers a remarkably versatile system for the efficient introduction of samples to the column. A completely new, dual injection system is available which includes the Grob on-column injector and the Grob split-splitless detector. This provides an optimised injection system for each type of sample and allows for automated sampling procedures. The system is also ideal for thermolabile compounds and being septum-less the on-column injector completely eliminates all problems connected with ghost peaks and baseline drift associated with septum bleed. It eliminates the deposition of septum particles cut by the syringe as it passes through a septum in the injection area. The Grob on-column injector on the Fractovap 4160 also features a specially adjustable additional temporary cooling system which avoids discrimination between low and high boiling point sample compounds. To go with the sophisticated injection system a dual carrier gas circuit including metal bellow pressure controllers, metal shutoff valves and gauges is provided as standard. Two other configurations are available as options — one provides single pressure regulation for both injectors and the other is designed to allow constant pressure operation for both injectors, permitting constant flow operation for the on-column injector. The Fractovap 4160 is the first gas chromatograph capable of constant flow operation in temperature programmed capillary gas chromatography. To further facilitate easy and efficient operation the Fractovap 4160 has a low thermal mass column oven allowing fast heating and cooling rates during temperature programming operations. Capillary column installation is made simple by an ingenious adjustable geometry support, and inlet/outlet port configurations. Temperature readout is by a multi-access LED display and a system of multicoloured coded lights indicates the status of each system.

Two new temperature programmers are available: The LT 400 is a fully electronic, single ramp programmer using digital thumb-wheel switches for unambiguous setting of parameters; it incorporates a digital stopwatch and LED's to indicate

programme status. This programmer also actuates the automatic cooling function and can perform single or multi-cycle operations and provide remote actuation of external units such as automatic samplers and the autosplitter SL 416 control module. The LT 430 programmer is a microprocessor-controlled unit offering multi-ramp and multi-level facilities. Some applications of glass capillary gas chromatography require more complex configurations in the temperature programme and the design concept of this programmer caters for these situations. The detection system for the Fractovap 4160 is an all-glass, zero dead-volume ionisation detection system. The range of accessories includes an automatic head-space sampler (model HS250) which makes possible fully unattended head-space analysis. Full technical back-up facilities are available from Carlo Erba in Milan, and in the UK, from Erba Science in Swindon.
Circle No. 90 on Reader Enquiry Card.

VARIAN

The Varian 5000 series is a family of powerful microcomputer-based liquid chromatographs that emphasise responsiveness and simplicity. There are six basic models in the 5000 series, ranging from simple isocratic to fully automated gradient systems. Each is designed to provide competitive price and performance. The liquid chromatographs are upward expandable and offer a wide range of options that make it possible to configure the 5000 into a large number of difference LC systems for specific applications. Varian give strong, continuous support, and provide service, training and applications assistance. A brochure detailing the 5000 series can be obtained by circling the enquiry card number below.

Also available from Varian is the 3700 series of dual column gas chromatographs. The 3700 brings together all the latest breakthroughs plus new applications-orientated design concepts. It is a modular series, so that a chromatograph can be chosen for any specific application. All components are upgradable and interchangeable. A catalogue about this fully automatic system is also available.
Circle No. 91 on Reader Enquiry Card.

UNIVERSAL SCIENTIFIC

The Uniscil UFC.120 fraction collector is designed to meet the requirements for a functional, full-size, low cost fraction collector. The instrument will accept 120 15 cm^3 tubes, is $50 \times 18 \times 26$cm high and weighs 3kg. Fractions are collected on a timed basis from 5s to 90 min per fraction by means of an integral electronic timer with a repeatability better than 0.01%. The UFC.120 is rated for use in coldrooms or in laboratories. A unique feature of the instrument is its configuration. The delivery head is mounted on a toothed, endless belt and the head sequentially traverses each of the static collection tubes. This makes the instrument more compact, and because all electronic and mechanical components are mounted above the delivery head, damage by spillage is impossible. The elegant design of the instrument, combined with a minimum of moving parts ensures a high degree of reliability and very low cost.
Circle No. 92 on Reader Enquiry Card.

SPECTRA-PHYSICS

The new SP4100 computing integrator features automatic integration, built-in BASIC programming, an x–y plotting capability, intelligent dialogue, 'foreground-background' operation and a range of pre-programmed data reduction methods. Designed primarily for use with gas or liquid chromatography systems, the SP4100 is microprocessor controlled and features an alpha-numeric keyboard, a full width LED display and a printer/plotter output. The BASIC programming capability allows the user to modify existing data reduction methods, to develop special reports or applications,

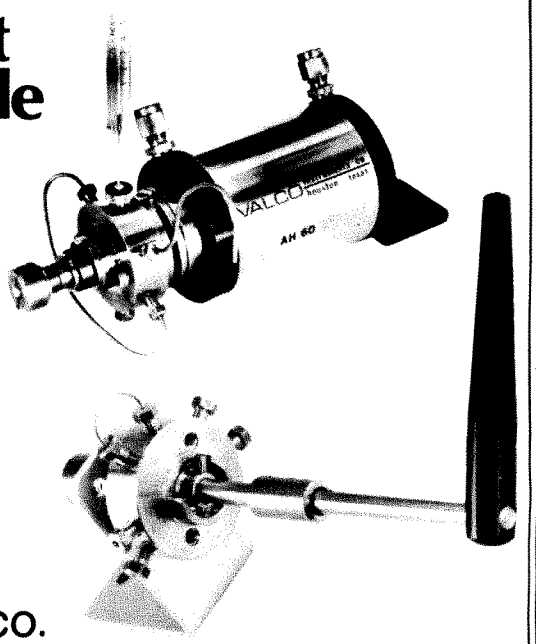
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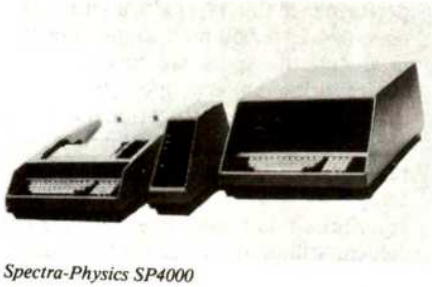
Circle No. 13 on Reader Enquiry Card.

and even to re-programme the standard instrument ROM software. The foreground-background operation allows the system to be integrating chromatograms while simultaneously running BASIC programmes or plotting graphical data.



Spectra-Physics SP8000

Spectra-Physics' SP8000 is a microcomputer-based HPLC system offering the ultimate in performance and flexibility in the field of quantitative analysis. The system is designed to meet the diverse needs of all chromatographers from methods research to repetitive quantitative analysis, and it is available with a variety of detectors, a built-in data system with printer/plotter and automatic injectors and samplers.

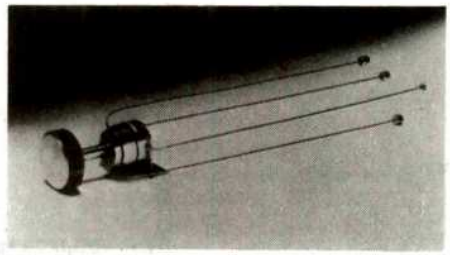


Spectra-Physics SP4000

The microprocessor-based SP4000 is a multi-channel data system for chromatography which has found wide usage in research, methods development, and quality control applications. Designed for flexibility and convenient operation, the system features a visual display in its central processor. The floppy disk option allows bulk storage of data files, special programs and 'raw data' for rapid recall into the CPU. The results of each run are provided by a printer/plotter which combines the chromatogram with a detailed output report. Multiple analyses may also be summarised when statistical or long-term information is desired. The SP4100 data system may now be incorporated into the SP4000 to increase flexibility by providing multi-user BASIC programming and graphics capability.
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TECHMATION

Carle have developed the new RotaSphere high pressure liquid sample valve for analysis of natural gas liquids which is now available from Techmation. The valve is intended for liquid phase sampling of pressurised mixtures up to 1,500 p.s.i., at temperatures to 50°C, and is guaranteed leak-free. Unique design is based on a stainless steel sphere pierced by two ports of equal dimensions. Each port acts as a chamber containing a metred volume of sample. The steel sphere is held by two hemispheres of filled Teflon under great pressure from external half-cells. Rotation of the sphere places the volume of sample in the sample chamber directly in the carrier line. The volume of the stainless steel chamber cannot change with time, ensuring sample volume repeatability over greatly extended periods. RotaSphere is one of the key elements in Carle's series-S and new series-SX analytical GCs for LPG and liquified natural gas analyses.
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Techmation RotaSphere high pressure liquid sample valve

BIO-RAD

New materials, equipment and techniques for chromatography, electrophoresis, cell culture and HPLC are introduced in the 30th issue of *Bio-Radiations*, just out from Bio-Rad Laboratories. Articles include applications data, useful references, and 'how to' information. This issue includes a feature on five ways to take advantage of hydroxylapatite chromatography for separating and concentrating proteins, and — for HPLC — a cheap high quality reverse phase column, and an inexpensive, disposable column that precleans samples to improve HPLC results. A free copy of *Bio-Radiations* 30 can be obtained by circling the enquiry card no. below.
Circle No. 95 on Reader Enquiry Card.

DIONEX

Ion chromatography has been developed by Dionex Corporation to analyse cations and anions in flue gas scrubber solutions. Their Model 14 ion chromatograph can easily analyse the major ionic components of SO_2 flue gas scrubber process streams in approximately 30 minutes. Ions which may be analysed include Mg^{2+}, Ca^{2+}, Na^+, NH_4^+, K^+, Cl^-, SO_3^{2-} and SO_4^{2-}.

The Dionex AutoIon system 12 analyser is a fully automatic system for rapid analyses of ions in solution. It is equipped with a labour-saving 99-sample capacity lab sampler which provides high throughput of ions in complex matrices at trace levels and above. The system 12 analyser is also available with a one or six site on-line loading system for 24-hour process/quality control operation. Automatic data handling accessories can also be ordered for the system. A program controller provides automatic eluent, regenerate and sensitivity changes to allow multiionic quantitative analysis profiles. Chief applications of the system 12 are for inorganic anions such as F^-, Cl^-, NO_2^-, PO_4^{-3}, Br^-, NO_3^-, SO_4^{-2}, organic acids, metal oxides, alkali metals, alkaline earths, ammonia and amines. The system 12 can be used in air pollution, water pollution, elemental analysis, soil analysis, brine analysis, power production and QC/process.



Dionex IC 16

A simple and rapid means of analysing complex mixtures containing both organic acids and inorganic ions at trace levels has been developed by Dionex. The dual system ion chromatograph 16 utilises newly developed ion exchange columns which allow separation and detection of all ions in complex mixtures with pK_a of less than 7. Weak acids and their salts, including organic acids as C_1–C_{10} carboxylic acids, organic phosphonic and sulphonic acids, and carbonic acids, and strong acids or their salts, including ions such as chloride, nitrate and sulphate can be determined in a single analysis. Because the ion chromatograph 16 is two systems in one, anions and cations can be determined simultaneously with a single sample injection. The IC 16 includes auxiliary valves. These allow the use of additional chromatographic detectors such as UV. Pre- or post-columns can be used individually or coupled with this dual system. Column gradients can also be used.
Circle No. 96 on Reader Enquiry Card.

New high temperature chromatography detector



from Analysis Automation

This new PI51-02 detector will operate at temperatures up to 300°C. allowing column temperature up to 280°C.

Retains all functions of standard HNU photoionization in terms of sensitivity, wide dynamic linear range (10^7), broad range of species detected and low noise.

New applications include: pesticides, drugs and polynuclear aromatics.

For full details of the PI51 contact:

analysis automation limited

Cherwell Boathouse, Bardwell Road, Oxford OX2 6SR
Telephone: (0685) 511186/53713 Telex 837509

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THE CHAIN STRAIGHTENERS
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One of the most exciting stories in the history of Science – in the tradition of The Double Helix.

A narrative account of the remarkable burst of scientific discovery and technological innovation that gave science mastery over the 3-dimensional structure of high polymers and the world its most useful group of plastics and synthetic rubbers.

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To be published in November by The Macmillan Press
£9.00 0 333 2529 7

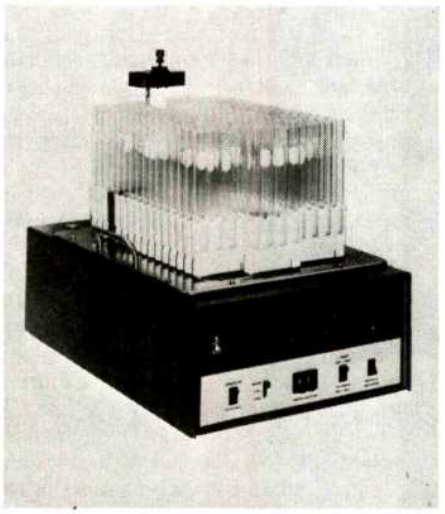
For further details contact The Publicity Dept., The Macmillan Press, 4 Little Essex Street, London WC2R 3LF.

MACMILLAN PRESS

ISCO

The ISCO model 1850 fraction collector features a solvent-resistant stainless steel tray which may be lifted out for group sample processing and storage, or for washing. Test tubes from 12 mm to 18 mm in diameter are held in 35 small self-standing racks, providing a total capacity of up to 210 tubes. Digital displays continuously indicate preset fraction interval (time, drop, or volumetric units); the number of units deposited in each tube; and the number of tubes filled.

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ISCO 1950 fraction collector

TBT

TBT, a subsidiary of L'Air Liquide Ltd, have developed a range of gas chromatographs for the analysis of permanent gases. They feature a high frequency gas discharge detector that displays a very high sensitivity to all gases, and this is coupled to a well developed electronics section which gives very good stability and freedom from base line noise. The combination enables detection limits of 0.1 p.p.m. for hydrogen, oxygen, nitrogen, methane, and so on in various bulk gases. The chromatographs are of modular construction and the modules include various column configurations enabling back flushing, cutting and column switching; and also a microprocessor-based programming unit for automatic operation. These instruments are suitable for laboratory and process use. A free catalogue is available.

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HPLC TECHNOLOGY

HPLC Technology Ltd announce the introduction of a new range of HPLC materials known as Techsil. The range comprises silica, alumina, C_2, C_6, C_8, C_{18}, C_{22}, nitrile amino, phenyl, SAX and SCX phases. Efficiences of 50,000–80,000 theoretical plates per metre can be obtained using normal slurry packing techniques.

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CAMAG

The Camag U-chamber system for high performance thin layer chromatography is now available. 50-mm square plates are used and the spots are placed near the centre using an accurate template. The control of all phases (stationary, mobile, vapour) is so exact that retention data is reproducible to±0.01 R_f units. When the sample is applied to the wet layer after equilibrium has been reached (with the solvent still eluting) the chromatographic information obtained can be transferred directly to column chromatography or HPLC.

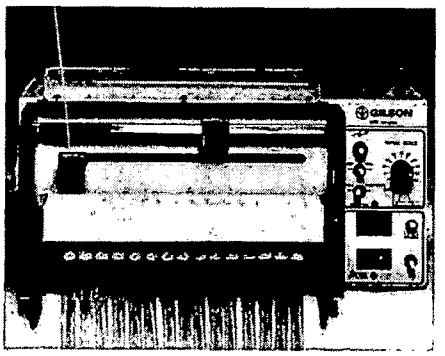
The Camag Vario-KS chamber permits the simultaneous investigation of up to five different solvent systems on one standard 20 × 20 cm TLC plate scored into channels by the user. The Vario-KS is thus invaluable to the chromatographer who is developing TLC systems to separate his samples in a variety of different solvent tanks. The chamber is a closed system allowing complete control over the internal atmosphere. Layer activity can be controlled and hence resolution optimised, and separation behaviour can be pre-determined. Also activity gradients can be set up along the direction of development for bridging differences in compound polarity. The plate is held in place layer downward over the solvent trough and conditioning tray. The solvent reaches the layer by means of a wick and an accessory is

available to enable continuous development.

Baird and Tatlock (London) Ltd are suppliers of Camag equipment in the UK.
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GILSON

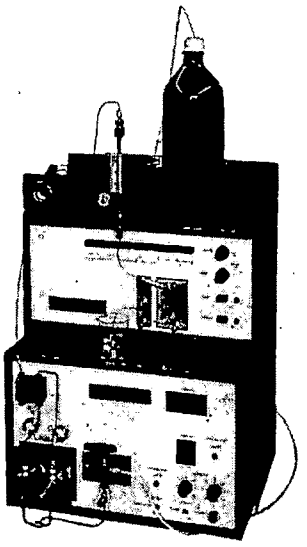
A new peak selective fractionator collecting only peaks has been produced by Gilson Medical Electronics, Inc. The Gilson peak collector connects to UV, visible or fluorescence detectors to perform high flow preparative collection or repeat sample injection for HPLC. This peak selective fractionator collects only peaks, while the solvent between peaks is discarded. Peaks are identified by the threshold level and by increasing or decreasing concentration. Peak identification circuitry controls the mobile indexing head, collecting one peak per tube and up to 15 peaks per sample injection. The model CPR features a threshold level setting, compound peak separation and automatic cycle repeat or shutdown.
Circle No. 101 on Reader Enquiry Card.



Gilson's peak collector

KRATOS

The Schoeffel Instrument Division of Kratos have available a new low cost, compact, high performance liquid chromatograph. Built around the company's LC 250/1 research quality dual piston pump, the complete chromatograph occupies less than 15 inches of bench space, including the detector. The LC 250/1 constant volume pump operates at up to 6,000 p.s.i., and provides reliably accurate flow rates between 0.1 and 9.98 ml min^{-1}. The pump is suitable for analytical, high pressure separations as well as medium flow semipreparative separations. A solvent rack with column and injector brackets is featured in a space-saving design. A compartment below the rack is provided for placement of the detector within only a few inches of the column outlet. This new compact chromatograph includes: LC 250/1 constant volume pump, the new model 7125 syringe loading sample injector (featuring no sample loss, and no need for flushing between injections), the new SF 740 UV detector (which can operate at any wavelength between 200 and 300 nm), and the solvent/column rack. The system is suitable for a variety of applications in the clinical or research lab; and, by the addition of a second LC 250/1 pump plus the company's decilinear gradient programmer, may be upgraded to a full featured gradient system without component modification.



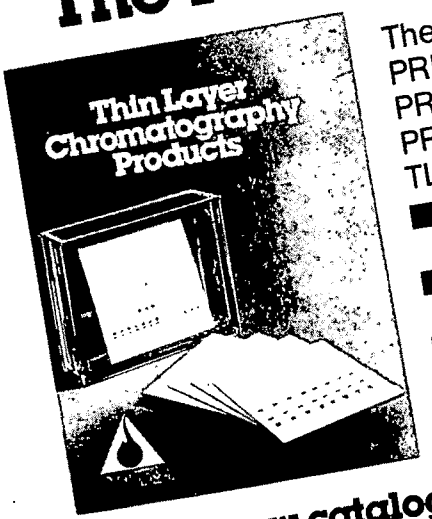
New compact LC from Kratos

In a new newsletter currently available from Schoeffel Instrument Division of Kratos, HPLC applications are described. Called *Liquid and Thin Layer Chromatography Notes*, the newsletter treats two applications in depth. One report deals with the use of stopped-flow excitation wavelength scanning in the fluorescence analysis of catecholamines. In this application, the excitation spectra of various catecholamines are used to provide positive compound identification. The second report is the separation and UV detection of amino acids and peptides at 200 nm. Other features included in the newsletter are a 'Bibliography Update' section which provides several references of interest to the biomedical/biochemical chromatographer; and 'Instrument Corner'. The newsletter is free.
Circle No. 102 on Reader Enquiry Card.

SPECAC

SPECAC have recently increased their range of HPLC injection and switching valves, which in the past have proved to be economical and reliable. Their new valve is constructed of high quality materials and features a low 'dead volume' for micro-injections and the ability to be used in systems operating up to 7,000 p.s.i.
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ANALTECH

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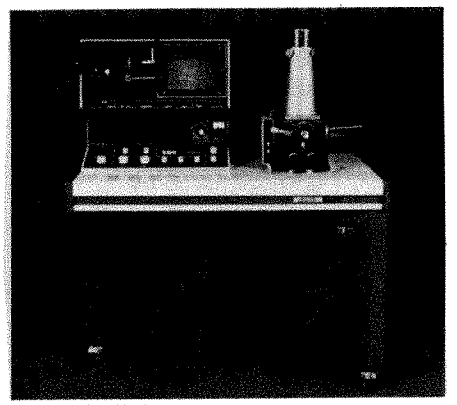
Newly on the market

Hydride generation system. Techmation. Development of a custom-designed hydride generation system has enabled the SMI Spectraspan range of plasma emission spectrometers to be used for the determination of ultra trace levels of As, Sb, Se and other hydride-producing elements difficult to detect by conventional AA techniques. The system is new to plasma emission spectrometry, and provides a 10–100-fold increase in sensitivity over direct nebulisation for As and Se. It can also be used for Hg vapour production for low-level Hg determination. The hydride generating assembly consists of a reaction cell, drying tube, hydrogen delay column, flow meter and valves. The standard Spectrajet III ceramic sample tube is replaced by a special sample introduction tube.
Circle No. 62 on Reader Enquiry Card.

Thermo circulator for UV instrumentation. Perkin-Elmer. The new versatile thermo circulator from Perkin-Elmer is designed to pass thermostatically controlled liquid through spectrophotometer jacketed cell holders or cells to maintain a constant temperature. Typical examples of other applications are spectrophotometer cell holders, refractometers, viscometers, culture flasks, blood gas analysers, chromotography columns and density gradient tubes.
Circle No. 63 on Reader Enquiry Card.

Multichannel oscillographic recorder line. This new generation of Gulton 2, 4, 6 and 8 channel oscillographic recorders provides response up to 140 Hz, high accuracy and exceptionally sharp traces. All models include an eight speed, push-button selectable chart drive. Writing is by thermal styli on heat-sensitive rectilinear grid chart paper. Automatic stylus heat compensation maintains a constant trace width control. Sturdy coaxial styli are standard and long-life ceramic tip styli (with a 2-yr guarantee) are optional. Thirty interchangeable plug-in signal conditioners are available for recording of volts, amps, temperature, strain, a.c. to d.c., frequency to d.c. EKG, watts, log to d.c., current to d.c. and more.
Circle No. 64 on Reader Enquiry Card.

Blood Bank refrigerator. Kelvinator Scientific. The UC-26-RG-BB is a 26 cu ft refrigerator used for the storage of whole blood. Temperature is factory set at 4°C and capacity is 300 blood bags. Features include: solid state digitasl electronic readout of air and/or solution temperature, automatic alarm system with warning light, son alert and 60-min recall timer, door ajar light, audio alarm, push button test and remote alarm connection.
Circle No.65 on Reader Enquiry Card.



The Joel JSM-T200

Scanning electron microscope. Jeol announce a new addition to their range of scanning electron microscopes, the JSM-T200. Not only is the specimen just displayed on the high resolution screen but it is also fully focused automatically. Several other automatic features such as evacuation and image recording are included and all have manual override control. The specimen chamber is large and contains two separate stages — a eucentric one for 20-mm working distance and one to accommodate large specimens up to 5 in. diameter at 48-mm working distance. Better vacuum conditions are achieved by the T200, which has a large diffusion pump with a wide throat situated directly below the specimen chamber.
Circle No. 66 on Reader Enquiry Card.

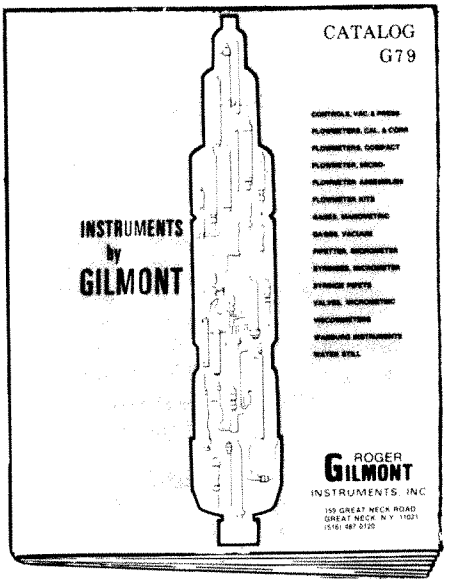
Trace metal analyser. Tecator. A new analyser, Striptec, for precise trace metal determinations is being introduced by Tecator. Striptec gives a very large dynamic measuring range, with no special preparatory steps. The instrument is capable of simultaneous measurements of several metals with high precision at very low concentrations (p.p.b. levels). In combination with Tecator's programmable Digestion Systems for multi-sample preparation, Striptec allows convenient and reproducible conditions for the measurement of metals not only in liquids and inorganic materials, but also in all kinds of food materials.
Circle No. 67 on Reader Enquiry Card.

Glassware washer. Labconco has introduced a glassware washer which thoroughly scrubs every type of laboratory glassware and plasticware. Constructed of strong, corrosion-resistant stainless steel, the Labconco glassware washer features a solid-state, push-button control panel. The glassware washer's six washing cycles, including a distilled water rinse and patented H-arm provide three-way, thorough yet gentle washing action. Designed to fit in almost any location, the Labconco glassware washer is available in an undercounter, free-standing or mobile model, all front loading with a distilled water pump option.
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G79 CATALOG YOURS FREE

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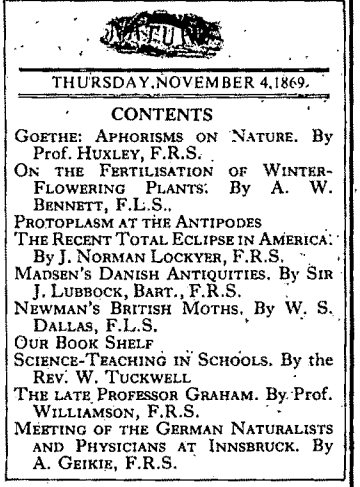
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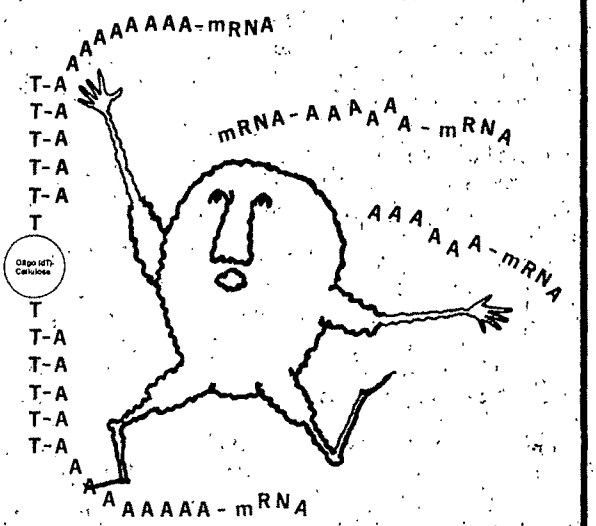
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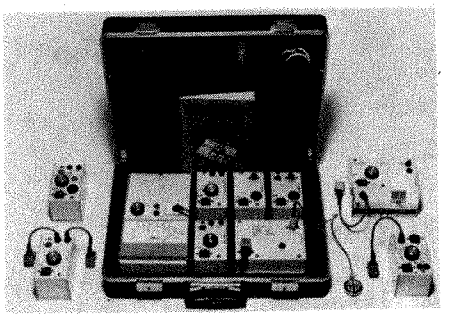
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Liquid scintillation counting. Whatman. Compared with the use of acetate membranes, it has been shown that equal retention is obtained using Whatman glass microfibre filters which at the same time give much higher filtration rates, higher counting efficiency and lower cost. Activity remains on the disc, which can therefore be kept as a reference. Maximum efficiency is achieved with only a small volume of scintillator. For handling large numbers of samples strips of glass microfibre filter can be used. Fractions are collected directly on to the strips and the whole strip is processed. Finally, those areas containing the fractions are cut out and placed in scintillation vials.
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Anti-Ly alloantiserum. Cedarlane Laboratories announce the introduction of a kit designed to test the functional subpopulations of mouse T lymphocytes. The kit contains the following antisera: (1) anti-Lyt 1.2; (2) anti-Lyt 2.2; (3) anti-Thy 1.2. In addition, an ample supply of low toxicity rabbit complement is included with each kit. The product specification sheets included with the kit give details on the dilution and preparation of the reagents for the test, as well as a detailed protocol for cytotoxicity and depletion of a subpopulation of lymphocytes with the antiserum. The kit is designed to treat 2×10^7 cells with each antiserum.
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The Semat briefcase laboratory

Laboratory in a briefcase. Semat. The E1 briefcase laboratory from Semat is completely portable, contained in a tough carrying case, battery powered for field use, and can include up to seven measuring functions provided in a modular format. Parameters which can be monitored are *p*H, conductivity, oxygen temperature, sound, light and colorimetry. The colorimeter module is provided with seven narrow-band filters posessing peak responses ranging from 470 nm (blue) to 700 nm (deep red). The unit is designed to accommodate standard 16-mm test tubes.
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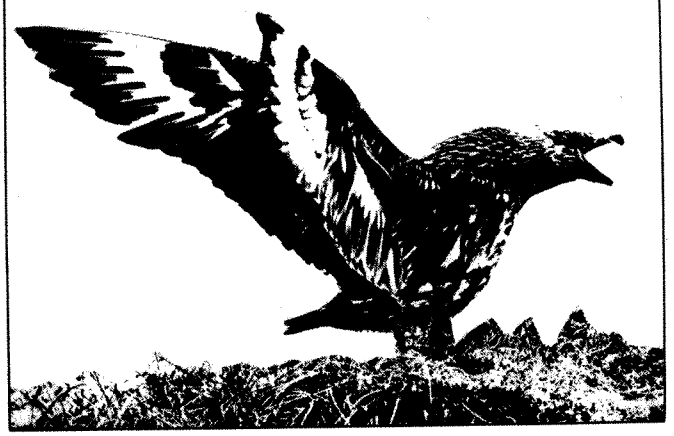
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Liquid chromatography modules. Tracor. A booklet available from Techmation describes the latest modules in Tracor's 900 Series liquid chromatograph. Solvent programming, sample injection and pump system modules which incorporate latest liquid chromatography technology are featured, along with a variety of detectors. Of particular interest are the 970Å variable wavelength UV absorbance detector with automatic scanning option, and the model 965 selective photo-conductivity detector, the first electrolytic detector for liquid chromatography.
Circle No.55 on Reader Enquiry Card.

Antibody plaque counter. Micro measurements announce a new instrument for counting viral/antigen-antibody plaques. A newly developed plaque illuminator system used in conjunction with a high sensitivity scanner and special electronic count circuits enables plaque counts to be made automatically with a high degree of accuracy and speed. Results are expressed as number of plaques per unit area. Using a round frame this area can be made to cover as closely as possible the area occupied by the culture medium. By feeding the results into a pre-programmed calculator extrapolations and statistics can be performed 'on-line'.
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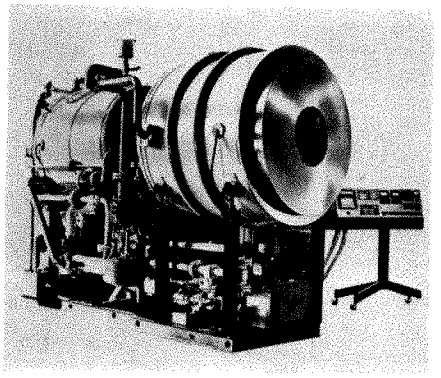
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Recirculating aspirator vacuum pump. Buchler. A recirculating water aspirator vacuum pump that helps conserve water is available from Buchler Instruments. The dual aspirator uses a self-priming turbine pump to recirculate water at a continuous 40 p.s.i. through two high-efficiency aspirators. Only 10% of the water that is normally wasted is used to flush the reservoir and cool the turbine. The aspirators may be used separately for techniques such as vacuum filtration or dialysis, or they may be combined to deliver higher flow-through for vacuum evaporation or rapid degassing. Stainless steel and brass components resist solvent attack. The $\frac{1}{3}$ h.p. heavy duty induction motor is overload protected and has permanently lubricated bearings.
Circle No. 57 on Reader Enquiry Card.

Diamond knife. Du Pont. A new diamond knife introduced by the Du Pont Company has cutting edge lengths of 2–6 mm, and offers maximum performance combined with ease of use. The diamond knife is produced by a new proprietary manufacturing process that achieves greatly improved cutting quality. The diamond knife is designed to provide maximum performance when used in any 'Sorvall' ultramicrotome, particularly the new microprocessor-controlled MT 5000, and all other major ultramicrotomes.
Circle No. 58 on Reader Enquiry Card.

High-performance freeze dryer. Edwards. A new industrial shelf freeze dryer, the LyoMax 2, has been introduced by Edwards High Vacuum. It meets the latest performance requirements of industry in terms of short cycle times combined with accurate repeatability of results, and fine control of process variables, but is designed also for ease of operation and simple maintenance. The machine is very flexible in operation. Maximum effective loading area of over 2 m^2 is provided by four shelves measuring 600 mm × 900 mm. This gives a capacity of up to 14,200 ampoules or 4,250 vials depending on their size, but a novel latching mechanism allows two-and three-shelf configurations to be used.
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The Edwards LyoMax 2

Sterile disposable filter holder. Schleicher & Schuell have announced a new sterile disposable filter holder, the FP 030. Completely sterile and pyrogen free, the FP 030 is available with 0.2 μm, 0.2μm hydrophobic, 0.45-μm or 5-μm pore size Triton-free cellulose acetate Schleicher & Schuell membrane filter. Particular features are the large 5.7-cm^2 filter area, the small imput baffle plate and the 10 bar bursting pressure, giving considerably increased filtration rate, even distribution of the filtrate and optimum stability of the disposable filter.
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High performance electrical/electronic insulation. Chemplast. Chemfluor TFE and FEP spaghetti tubing from Chemplast is designed to provide high quality, high-performance electrical insulation. Made from Teflon fluoropolymer resins, Chemfluor spaghetti tubing offers a unique combination of heat and chemical resistance, insulation properties and flexibility. TFE tubing is capable of continuous service from —240°C to +260°C, has a dielectric constant of 2.1 at all frequencies, is unaffected by chemicals, solvents or weathering, does not become embrittled with age and is non-flammable. FEP tubing has similar properties except for its temperature range which is — 240°C to +200°C.
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Democratic Decision Making

Edited by J. Surrey, Christian Lenzer, C. Phipps and J. Valleix

The outcome of a lively conference at the Council for Europe in Strasbourg, this book examines the challenges facing Europe over the next quarter-century arising from the complex and interrelated problems of energy and environment policy.
The stark choice confronting politicians the report says, is between on the one hand, allowing societies to maintain present life-styles and trends and neglecting the risk of future resource scarcity, and on the other hand of preparing public opinion for unpopular decisions needed to avert future crisis.

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UNIVERSITY OF OXFORD
DEPARTMENT OF FORESTRY
TWO RESEARCH ASSISTANTS

1) To continue research into the water relations of *mycorrhizas*. Research grade 1A or 1B. Practical experience of culturing fungi essential.
2) To study the *feeding ecology of predatory beetles* (in the families Carabidae and Staphylinidae) as related to their pest control potential. Research Grade 1B. Working knowledge of insect population dynamics would be an advantage.

Applicants must have at least an upper second class honours degree in an appropriate subject. Each post is tenable for three years and offers in (Grade 1B) an opportunity to register for a higher degree. Salary scales, which are under review, are currently 1B £3,689 to £6,108 pa and 1A £4,232 to £7,145 pa.

Further particulars of each post may be obtained from the Administrator, Department of Forestry, University of Oxford, South Parks Road, Oxford OX1 3RB, to whom applications, giving curriculum vitae and three references, should be sent by 3rd September 1979. 3007(A)

POST-DOCTORAL POSITION: Enzymologist, with organic chemistry background, for research on the mechanism of the ribulose-P_2 carboxylase/oxygenase reaction. Send vitae and names of references to N.E. Tolbert, Department of Biochemistry, Michigan State University, E. Lansing, MI 48824. W314(A)

UNIVERSITY OF EDINBURGH
DEPARTMENT OF PHYSICS
RESEARCH ASSOCIATES

Research associates are required to work on the following projects in the field of fluid dynamics sponsored respectively by the Science Research Council and the Medical Research Council:

1. Water forces on cylinders. Candidates must hold a degree in science or engineering. Salary £4,333 to £5,199 per annum.

2. Flow through bifurcations and constrictions. Candidates for this post should preferably hold a PhD degree. Salary £4,910 to £5,199 per annum.

Applications should be sent to Dr C A Greated, Physics Department, King's Buildings, University of Edinburgh, Edinburgh EH9 3JZ. Please quote reference 5048. 2950(A)

THE UNIVERSITY COLLEGE OF WALES
Aberystwyth
DEPARTMENT OF BOTANY AND MICROBIOLOGY

Applications are invited for the post of
POSTDOCTORAL RESEARCH ASSISTANT

to undertake work on the metabolism of cytokinins.

Applicants should have an initial degree in Botany, Biology, Biochemistry or Chemistry, with appropriate postgraduate experience.

Salary on the 1A Scale for Research and Analogous Staff £4,333 to £7,521 per annum.

Application forms can be obtained from the Registrar, The University College of Wales, Old College, King Street, Aberystwyth, SY23 2AX, to whom they should be returned no later than Friday, 7 September 1979. 3009(A)

ROYAL HOLLOWAY COLLEGE
(University of London)
RESEARCH ASSISTANT — BIOCHEMISTRY

Research Assistant to work with Dr R.A. Dixon for three years on the enzymology and control of isoflavanoid phytoalexin accumulation in the French bean. Candidates should have a good honours degree in Biochemistry. The successful candidate may register for a higher degree. Salary on scale £4,763 to £5,307 (inclusive).

Applications with curriculum vitae and the names and addresses of 2 referees should be sent to the Personal Officer (N) Royal Holloway College, Egham Hill, Egham, Surrey TW20 0EX. 3003(A)

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RESEARCH TECHNICIANS

The Department of Biological Sciences has vacancies in the Chloroplast Development, Developmental Biology and Microbiology Research Groups. Successful candidates will work under the direction of a senior scientist on a specific research project using the modern experimental techniques and instrumentation of molecular biology.

The posts are for contract periods of one to three years. Applications are invited from graduates and others with appropriate qualifications in biochemistry and/or micro-biology. Salary either on the Technician Grade 2B scale: £2,937 to £3,342 p.a. or Grade 3: £3,122 to £3,553 p.a.

Application should be made by letter giving a full curriculum vitae to the Personnel Office, University of Warick, Coventry CV4 7AL quoting Ref.No.:3/T/79 by 7th September 1979. 3006(A)

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HIGHER SCIENTIFIC OFFICER (BAND II)
LIAISON/INFORMATION OFFICER

Applications are invited for the post of Liaison/Information Officer, the duties of which include overall responsibility for a scientific library, liaison with, and an information service for, the poultry industry, veterinary profession, Agricultural Research Council and Ministry, the Press, journals and educational organisations, and the preparation of publications and exhibits.

Applicants should possess a degree, HNC or equivalent in a science subject, with at least 5 years relevant post qualifying experience. Experience in public relations and editorial work would be an advantage.

Salary in Higher Scientific Officer grade (£4,727 × 8 to £6,211) salary scale currently under review.

Further particulars from the Secretary, Houghton Poultry Research Station, Houghton, Huntingdon, Cambs PE17 2DA. Closing date for applications Friday, 14th September, 1979. 2990(A)

UNIVERSITY OF LIVERPOOL
POST DOCTORAL RESEARCH IN CONNECTIVE TISSUE

The Department of Veterinary Clir Studies is to make an experimental stu over five years, of the changes in biochemical structure of the uterine ce at the time of birth in animals.

Applications are invited from suit qualified candidates, preferably with p doctoral experience in connective ti chemistry, to lead the investigation of interaction of collagen and proteogly in this tissue.

The post is tenable from 1st Octo 1979, for one year in the first instance ar renewable.

The initial salary will be within the ra £4,232 to £5,591 per annum (under revi

Applications, together with the name three referees, should be received not l than 21st September, 1979, by Registrar, The University, PO Box Liverpool L69 3BX, from whom fur particulars may be obtained. Quote RV/752/N. 2969(A

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Owing to continued site expansion, we are looking for an experienced toxicologist to join our Toxicology and Drug Safety Evaluation Department.

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W313(A)

INSTITUT CURIE ORSAY, PARIS

POSTDOCTORAL RESEARCH FELLOWS

Applications are invited for two positions as postdoctoral research fellows to work with Dr E De Maeyer on aspects of the REGULATION OF INTERFERON MRNA SYNTHESIS. It is expected that the successful candidates will have experience in one or more of the following areas: animal virology, cell biology, protein biochemistry, immunology, nucleic acid isolation, molecular hybridisation, cell-free translation.

The appointments will be for two years and the starting salary will be in the range 65,000 ffr to 85,000 ffr p.a. depending on experience (9.73 ffr = £1.00 sterling). Knowledge of the french language is not necessary.

Applications, including curriculum vitae and the names and addresses of two referees should be sent as soon as possible to:

Dr E De Maeyer,
Section De Biologie, Laboratoires 110-111-112,
Institut Curie, Université de Paris Sud,
Campus d'Orsay, 91405 Orsay, Paris, France.

2896(A)

UNIVERSITY OF THE WEST INDIES JAMAICA

Applications are invited for the post of

PROFESSOR/ SENIOR LECTURER IN ORGANIC CHEMISTRY

The appointee will be expected to shape and teach undergraduate courses as well as to develop the graduate programme and will be expected to lead in the further development of research in the Department, in the field of Organic Chemistry. Salary scales: Senior Lecturer: 1979/80 J$15,489 to 19,530 pa, 1980/81 J$17,748 to 21,717 pa, Professor 1979/80 J$20,770 to 25,714 pa, 1980/81 J$22,848 to 28,284 pa (£1 Sterling = J$4.12). Family passages; baggage allowance; FSSU; study and travel grant; unfurnished accommodation will be let by the University at a rental of 10% of salary or a housing allowance of 20% of salary is payable.

Detailed applications (2 copies) with curriculum vitae and naming three referees should be sent as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Applicants resident in the UK should also send one copy to the Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 2963(A)

THE UNIVERSITY OF LANCASTER

DEPARTMENT OF PHYSICS

Ultra-Low Temperature Physics

Applications are invited for the post of

POSTDOCTORAL RESEARCH ASSOCIATE

in the Low Temperature Physics group. The post is financed by SRC for three years (from 1 October 1979) on the Research 1A scale £4,333 to £7,521). The research associate will work with Drs Pickett and Guenault on experiments below 1 mK on superfluid helium-3. A nuclear demagnetisation refrigerator constructed for this research programme is now nearing completion.

Further particulars may be obtained (quoting reference L160/D) from the Establishment Officer, University House, Bailrigg, Lancaster, LA1 4YW to whom applications (five copies) naming two referees, should be sent not later than 14 September 1979. 3001(A)

QUEEN ELIZABETH COLLEGE Kensington (University of London)

RESEARCH ASSISTANT IN PHYSICS DEPARTMENT

Applications are invited from holders of a doctorate degree, or persons with equivalent experience, for a post supported from outside funds. The proposed starting date is 1 October 1979 and the duration is one year in the first instance which may be extended to two further years. The work concerns the "inverse problem" as in the determination of a scatterer from the scattered field and "super-resolution" of images by analytic continuation. The application of particular interest is to laser anemometry.

Applicants may have backgrounds in mathematics or physics and should be familiar with the theory of analytic functions; some computing will also be involved. Salary according to age and experience in range £4,333 to £7,521 pa plus London allowance of £502 pa.

Applications giving the names of two referees should be sent to Professor R E Burge, Physics Department, Queen Elizabeth College, Campden Hill Road, London W8 7AH 2957(A)

THE QUEEN'S UNIVERSITY OF BELFAST

RESEARCH ASSISTANT

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the post of research assistant to join a small team working on the synthesis of potential inhibitors of collagenase and plasminogen activating enzymes funded by the Cancer Research Campaign. Experience in the chemical synthesis of peptides is desirable. The post is for one year initially, but may be renewable for a further 2 years. Commencing salary £3,775 or £4,055 according to age and experience, with Superannuation.

Further details and applications forms, which should be returned by 7th September 1979, may be obtained from the Personnel Officer, The Queen's University of Belfast, Belfast, BT7 1NN, Northern Ireland. 2983(A)

GRIFFITH UNIVERSITY Brisbane, Australia

SCHOOL OF SCIENCE

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A Life Science option is offered by the School of Science and includes an integrated coverage of genetics, microbiology and physiology. Applicants should possess appropriate experience in animal and/or plant physiology, but experience in other molecular aspects of the life sciences would be an advantage. It is anticipated that the main teaching duties of the appointee will be in second and third year courses in physiology. There will be opportunities to teach honours level courses and to pursue research interests and collaborate in interdisciplinary research.

Applications close on 12 October, 1979. It is anticipated that the appointee will commence duties on 1 January 1980, or as soon as possible thereafter.

The appointment will be made in the lower half of the appropriate salary range which is $A21,858 to $25,477 for Senior Lectures, and $A16,291 to $21,401 for Lecturers.

Applicants resident in United Kingdom and Europe may obtain further details from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applicants resident in North America may obtain further details from The School Administrator, School of Science, Griffith University, Nathan, Queensland, Australia 4111. 2960(A)

MEDICAL RESEARCH COUNCIL

BIOCHEMICAL PARASITOLOGY UNIT

MOLTENO INSTITUTE

UNIVERSITY OF CAMBRIDGE

RESEARCH TECHNICIAN

to assist a group studying the biochemistry of parasitic protozoa. The work involves operation and basic maintenance of an analytical ultracentrifuge. Microbiological/Biochemical training and experience in optical/photographic systems essential. HND or equivalent required. Salary range £3,478 to £4,184.

Applications to The Director, Medical Research Council Biochemical Parasitology Unit, The Molteno Institute, University of Cambridge, Downing Street, Cambridge CB2 3EE 2992(A)

THE UNIVERSITY OF HULL
DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for a biochemist or microbiologist to work on a challenging and interresting project financed by the ARC investigating iron metabolism in mycobacteria of veterinary importance. The position is available for three years from 1 October 1979 and is attached to the research group led by Dr C Ratledge. Candidates must have or expect to obtain their PhD within three months of the starting date.

Salary will be on the lower part of the scale £4,333 to £7,521 per annum.

Applications with full details of education, qualifications and research experience, together with the names of two referees should be sent as soon as possible but no later than 10 September to Dr C Ratledge, Department of Biochemistry, University of Hull, HU6 7RX, from whom further details may be obtained.

TECHNICIAN (Grade 3)

Applications are also invited for a Grade 3 Research Technician post to join the above research group for work on the same ARC sponsored project. The post is tenable for three years. Minimum qualifications: ONC or equivalent plus 3-4 years relevant experience.

Salary on the scale £2,931 to £3,336 per annum (under review).

Applications giving details of age, qualifications and experience, together with the names of two scientific referees should reach the Technical Staff Officer, University of Hull, HU6 7RX by 10 September quoting Ref No TBCH/5. 2991(A)

UNIVERSITY DEPARTMENT OF NEUROLOGY
KING'S COLLEGE HOSPITAL MEDICAL SCHOOL

RESEARCH WORKER (Clinical or Non-clinical)

Applications are invited from medically qualified and basic sciences graduates for an investigation of *in vitro* receptor binding sites in human cranial vascular tissue.

Salary up to £5,930 dependent upon qualifications, age and experience. This post is initially tenable for 8 months renewable for a further 2 years.

Application form and job description available from the District Personnel Department, King's College Hospital, Denmark Hill, London SE5 9RS. Telephone 01-274 6222 ext 2254.

Applicants may visit the Department by prior arrangement — Tel: 01-703 5411 ext 245. 2992(A)

LABORATORY SCIENTIFIC OFFICER required for two years for service work in connection with prenatal diagnosis of human neural tube defects. Apply in writing to the Administrative Assistant, Paediatric Research Unit, The Prince Philip Research Laboratories, Guy's Hospital Tower, London Bridge, SE1 9RT. 2976(A)

Please mention
Nature
when replying to these advertisements

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
DEPARTMENT OF ONCOLOGY
SCIENTIFIC OFFICER

Scientific Officer for MRC supported project on the effects of radiotherapy and chemotherapy on mouse tissues. Duties include small animal work, light microscope histology and work with radio-isotopes. Salary up to £3,720 pa inclusive of London Weighting depending on age and qualifications.

Applications with curriculum vitae and names of two referees to Dr E Hamilton, Department of Oncology, The Middlesex Hospital Medical School, Mortimer Street, London W1P 7PN. 2962(A)

UNIVERSITY OF LONDON
INSTITUTE OF PSYCHIATRY
DEPARTMENT OF NEUROLOGY.

Applications are invited for the post of RESEARCH ASSISTANT (Post-Doctoral) in the

The successful applicant will work with Dr B.S. Medrum on the biochemical mechanism of action of anticonvulsant drugs, with particular reference to the metabolism of GABA and related amino acids. Applicants should have relevant experience in enzymology, analytical procedures and isotopic tracer methods. The appointment will be for one year (renewable up to 3 years). The initial maximum salary is £6,932 p.a. + £502 London weighting (from 1st October 1979).

Application forms and further details may be obtained from The Secretary, Institute of Psychiatry, De Crespigny Park, London SE5 8AF (Phone 01 703 5411. Ext. 228) quoting ref: BM/N. 3004(A)

THE HATFIELD POLYTECHNIC

SCHOOL OF NATURAL SCIENCES

POST-DOCTORAL RESEARCH FELLOW IN OPTICAL INSTRUMENTATION

Applications are invited for the post of Post-doctoral Research Fellow with the Applied Physics and Micro-electronics Research Group.

The research programme is concerned with the construction and development of a high-speed laser scattering system for the characterisation and counting of airborne particles.

Candidates should have a PhD or expect to obtain one in the near future, and have experience in the design and construction of optical systems. A knowledge of detector electronics is required and some background in microprocessor practice would be an advantage.

The fellowship is supported by the MOD through the CDE Porton, and the successful candidate will be required to liaise closely with CDE. The appointment will start from 1st October, 1979 and be for a period of three years with a salary in the range ad hoc £5,600 to £6,200.

Further information and application form may be obtained from Dr I K Ludlow or Dr P H Kaye, Physics Group, School of Natural Sciences, The Hatfield Polytechnic, Hatfield, Herts AL10 9AB or telephone Hatfield 68100 Extension 383/509. Closing date: 3 weeks from the appearance of this advertisement. Please quote ref: 242. 2987(A)

EUROPEAN TRAINING PROGRAMME in BRAIN AND BEHAVIOUR RESEARCH

TRAINEESHIPS

Traineeships of three, six or rarely nine months are offered to promising young scientists working in the area of brain and behaviour research in order to broaden their skills and knowledge in a field other than but related to their own. The grants are given to allow them to learn a particular technique abroad for which training is not offered in their own country. The trainee is expected to return to her/his original post upon termination of her/his training so that her/his institute will in turn be able to benefit from newly acquired skills.

All applications have to be accompanied by a letter of recommendation by the sending institute and by a letter of acceptance from the receiving institute. Preference is given to post-docs under the age of 35.

TRAVEL GRANTS

Travel grants are awarded to allow young research workers to visit another laboratory in connection with an on-going research project or to participate in international meetings and symposia. In addition special grants are to be awarded to students to enable them to take part in the yearly meetings of the European Neuroscience Association (ENA) which this year will take place in Rome on September 10-14.

Applications should be accompanied by letters of recommendation from the sending institute and of invitation from the institute to be visited. Applicants should be postgraduate research workers with the exception of applications to attend the yearly ENA meeting. In this particular case preference is given to postgraduate students.

The deadline for completed applications is *March 15 and September 15*. Further information and application forms may be obtained from:

Dr Stephanie Zobrist
European Science Foundation
European Training Programme in
Brain and Behaviour Research
1, quai Lezay Marnésia
F-67000 STRASBOURG
FRANCE
Tel: (88) 35 30 63
Telex: 890440

EUROPEAN SCIENCE FOUNDATION

W306(A)

POSITION AVAILABLE

University of British Columbia

DIVING OFFICER

To be responsible for training, testing and certification of all University divers engaged in academic activities; equipment monitoring; and monitoring of SCUBA diving activities. Must have NAUI Instructor level qualifications, or equivalent, with several years of experience; some academic specialization is preferred. Depending on interest, experience and qualifications the appointee may be invited to participate on a part-time basis in an appropriate capacity in the School of Physical Education. Salary to $25,000 per annum, depending on qualifications and experience.

Applications with complete curriculum vitae and names of three persons from whom letters of reference can be obtained to be submitted not later than October 15, 1979 to Dr R.F. Scagel, Chairman, President's Permanent Committee on SCUBA Diving, c/o Department of Botany, University of British Columbia, 2075 Wesbrook Mall, Vancouver, B.C. V6T 1W5, Canada.

W312(A)

South West Surrey Health District

GUILDFORD

Staff Pharmacist
CLINICAL SERVICES

Applications are invited from suitably qualified pharmacists for this senior post. The successful applicant will be responsible for Clinical Pharmacy Services to the District, which includes a new District General Hospital, the first phase of which is to be opened in January, 1980.

This Staff Pharmacist will join a team of enthusiastic young pharmacists, and will work closely with the Principal Pharmacist, Pharmacokinetics, whose specific responsibility is to provide a Blood Drug Monitoring Service as an aid to therapy. Specialities in the District include Radiotherapy and Oncology, Neurology, Ophthalmology, Clinical Pharmacokinetics and Drug Information. Teaching and research in appropriate fields will be encouraged.

For informal enquiries please contact Miss A M Meier, District Pharmaceutical Officer, Guildford 71122, Extension 535.

Application form and job description may be obtained from the Hospital Administrator, St Luke's Hospital, Warren Road, Guildford to whom they should be returned by 14th September, 1979. 2954(A)

UNIVERSITY OF EDINBURGH

DEPARTMENT OF GEOPHYSICS

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the post of Postdoctoral Research Fellow to work on the Southern Uplands Seismic Profile. The project is collaborative with the Global Seismology Unit of the Institute of Geological Sciences and is designed to investigate the normalization of a reptitive quarry source by means of a permanent local seismic network, LOWNET, so that detailed seismic profiles may be built up with temporary outstations.

The appointment is for 2½ years with a salary of £4,910 to £5,488 p.a. beginning on October 1st 1979. Applications, including a full curriculum vitae and two referees, should be made by 7th Sept., 1979 to:

Dr R.G. Hipkin, Department of Geophysics, J.C.M.B., Mayfield Road, Edinburgh, EH9 3JZ.

Please quote Reference 5051. 3011(A)

UNIVERSITY OF EDINBURGH

RESEARCH TECHNICIAN

Required for the

DEPARTMENT OF MOLECULAR BIOLOGY,

Kings Buildings, for work with Dr P.M.A. Broda on the molecular genetics of degradative plasmids from soil bacteria. Candidates should have training in an appropriate subject to at least degree standard. Laboratory experience would be a strong advantage. The appointment, to start as soon as possible, salary on the scale £3,699 to £4,320 p.a. (under review), and will be until 30 September 1981 in the first instance. Applications, together with curriculum vitae, should include the names of two academic referees. REF: NS317

RESEARCH TECHNICIAN

Required for the

DEPARTMENT OF CHEMISTRY,

Kings Buildings, to assist Dr T.J. Simpson with his studies on the structural and biosynthetic aspects of fungal metabolites. The vacancy is for three years in the first instance and applicants should be qualified in Chemistry or Microbiology to HNC or equivalent standard. Salary on the scale £3,699 to £4,320 p.a. (under review). REF: NS316.

Application forms can be obtained from the Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh, EH1 1LS. Telephone 031-556-2930. 2977(A)

UNIVERSITY OF BRISTOL

SCHOOL OF CHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for this post in the Department of Organic Chemistry, to carry out work on the structures, origins and significance of alkyl porphyrin pigments in sedimentary organic matter and petroleums. This work is part of a wider study directed towards understanding the sedimentary fate or chlorophylls. Instrumentation includes HPLC, mass spectrometry (EI and CI), Fourier Transform ^{1}H and ^{13}C NMR, capillary GLC and computerised GC-MS. Previous experience of the chemistry of porphyrins, chlorophylls or related compounds, or of natural product chemistry would be advantageous, though not essential.

The appointment, funded by the SRC is for one year in the first instance, at a starting salary within the range £4,232 to £4,776 pa (scale subject to revision with effect from 1st October, 1979).

Please send applications, with details and the names of two referees, to Professor G. Eglinton, FRS, Organic Geochemistry Unit, School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS. 2897(A)

SCIENTIFIC COMMITTEE ON OCEANIC RESEARCH

SCOR/IOC Committee on Climate Change and the Ocean (CCCO) seeks applications from internationally experienced oceanographers for a two-year position as

SCIENTIFIC SECRETARY.

Appointee will be stationed in Paris in UNESCO headquarters of Intergovernmental Oceanographic Commission and will work closely with WMO/ICSU Joint Scientific Committee for the World Climate Program as well as with CCCO and national SCOR Committees. Basic Salary dependent on present position, to be supplemented by post allowance, fringe benefits, moving expenses and adequate travel allowance.

Apply to Roger Revelle, Mail Code B-031, University of California, San Diego, La Jolla, CA 92093, USA. Telephone (714) 452-4849.

Appointment to be administered through Scripps Institution of Oceanography, University of California, San Diego. The University is an equal opportunity affermative action employer. 2997(A)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF BIOCHEMISTRY

RESEARCH ASSOCIATE
(Plant Biochemistry)

Applications are invited from postgraduate biochemists to work with Dr D.E. Briggs on 'Aspects of the Metabolism in the Cereal Embryo.' The appointment is for three years. The work will involve following the changes in endogenous components of the embryos during germination, isolating subcellular components of embryos, and following the fate of radioactive substances supplied to embryos. This work is funded by the ARC. The Research Associate, who may register for a PhD if suitably qualified, will be employed on the lower end of the IB salary scale: £3,775 to £5,488 (October scales) plus superannuation.

Applications, including the names and addresses of two referees and a curriculum vitae (2 copies) should be sent to Miss Linda Heyes, Administrative Assistant, Senate Registry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT by 14th September 1979 at the latest. 2994(A)

THE UNIVERSITY OF WESTERN AUSTRALIA
Perth

UNIVERSITY LIBRARIAN

Applications are invited for the position of University Librarian which will become vacant on 1 January, 1980 upon the retirement of the present Librarian, Mr L Jolley.

Candidates must possess appropriate academic and professional qualifications and have had extensive experience of librarianship in a large library. A high degree of administrative ability is a pre-requisite for the appointment. The Librarian is a member of the Professorial Board of the University and is eligible for appointment to its academic committees. The salary will be that of a Professor, currently $A34,119 per annum. Benefits include superannuation similar to FSSU, fares to Perth for appointee and dependent family, removal allowance, study leave and long service leave and housing loan scheme. Further information including conditions of appointment is available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Acting Staffing Officer, University of Western Australia.

Applications in duplicate stating full personal particulars, qualifications, experience and the names and addresses of three referees should reach the Acting Staffing Officer, University of Western Australia, Nedlands, Western Australia 6009, by 22 September, 1979. 2989(A)

THE UNIVERSITY OF SYDNEY

LECTURESHIP IN PHYSICAL CHEMISTRY

Applicants must give promise of excellence in experimental research and in teaching of Physical Chemistry.

The position is expected to be filled by a probationary appointment of three years, capable of leading to tenure but if all the University's requirements for tenure are deemed to be satisfactorily met tenure may be granted at the time of appointment. Appointee should preferably be able to commence duties early in 1980.

Salary range: $A16,291 to $21,401 p.a.

Applications including curriculum vitae, list of publications and names of three referees by *1 November 1979* to the Registrar, University of Sydney, NSW 2006, Australia. Enquiries to Professor W.J. Moore, School of Chemistry. Information also available from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 2999(A)

UNIVERSITY OF BRISTOL

POSTDOCTORAL RESEARCH ASSISTANT

An NIH funded post for work on the isolation, affinity labelling and catalytic mechanism of mammalian glycosidases is available immediately.

Applications are invited from persons holding, or expecting to hold, PhD, degrees in Biochemistry, Organic or Physical Chemistry, or Biology. Applicants should send their curriculum vitae and the names of two referees to Dr M L Sinnott, School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS. 2888(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF GENETICS

TECHNICIAN (GRADE 2B)

required from 1st October 1979, or as soon after as possible. Duties include preparing undergraduate classes and the care and maintenance of equipment, apparatus and biological stocks. The Department is particularly interested in cytogenetics, population and ecological genetics and microbial genetics, and works with a wide range of organisms including plants, mammals, insects, bacteria and viruses. Candidates must possess ONC as a minimum qualification. Salary in range £2,757 to £3,138 pa.

Application forms may be obtained from the Registrar, The University, PO Box 147, Liverpool L69 3BX. Quote ref: RV/746/N. 2968(A)

ELECTRONICS ENGINEERS:

WITH AN INTEREST IN PHYSICS

The Daresbury Laboratory, an establishment of the Science Research Council situated in the Cheshire countryside is constructing the world's largest Van de Graaff accelerator — the Nuclear Structure Facility.

In order to support the complex electronic and computer systems currently being installed to perform data acquisition on the Daresbury Nuclear Structure Facility and to give assistance to the visiting University Teams, the Laboratory has a requirement for Electronics Engineers with experience in digital electronics and computer systems.

The duties will include responsibility for commissioning, installing and maintaining the data acquisition system and there will also be opportunities for design and development work. A knowledge and interest in Nuclear Physics would be an advantage.

Candidates should possess an Honours Degree and some appropriate experience is preferred.

Appointments will be made at both Scientific Officer and Higher Scientific Officer level on salary scales £3,289 - £5,085 and £4,727 - £6,211 respectively. The salary scales quoted are under review.

The Laboratory also offers a generous leave allowance, a non-contributory superannuation scheme and a flexible working hours scheme.

Closing date: 14 September 1979.
Please write or telephone, Warrington 65000 Ext. 467 for an application form quoting reference No DL/695/ to:

The Personnel Officer
DARESBURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD

Telephone Your Advertisement to our offices 01-831 6901 for fast and efficient service.

MYCOLOGIST HAWAII

ASSISTANT PROFESSOR.

Mandatory qualifications: ability to teach courses in general mycology and conduct research related to basic aspects of mycology. Desirable qualifications: experience in electron microscopy. Write for details: Dr D. J.C. Friend, Acting Chairman, Department of Botany, 3190 Maile Way, University of Hawaii, Honolulu, Hawaii 96822, U.S.A.

An Equal Opportunity/Affirmative Action Employer.

Closing Date: September 15, 1979. W308(A)

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF GENETICS

Applications are invited from men a women for a post of Lecturer in the abc Department, to start preferably not la than 1 January 1980.

Applications in any field of Genet invited. Initial salary in range £4,333 £5,199 a year on scale rising to £8,99 year. Particulars from the Registrar a Secretary, the University, Sheffield S 2TN to whom applications (5 copi should be sent by 1 October 1979. Ple quote ref: R354/G. 3008(A

UNIVERSITY OF NATAL

DEPARTMENT OF PHYSICS

DURBAN

Applications are invited from suitably qualified persons, regardless of sex, religion, race, colour or national origin for appointment to the post of

LECTURER IN PHYSICS

The successful candidate will be expected to take part in the teaching, research, and administrative duties of the Department. The Department carries out Research in Solar-Terrestrial Physics, Plasma Physics, Metal Physics and Foundations of Quantum Mechanics. Research experience in one of these fields will be an advantage.

The salary will be in the range

R8 100 - R13 200 per annum

and the commencing notch will depend on experience and qualifications. In addition an annual vacation savings bonus is payable, subject to Treasury regulations.

Overseas applicants are initially appointed on a two-year contract basis.

Application forms, further particulars of the department and information on pension, medical aid, group insurance, staff bursary, housing loan subsidy schemes, long leave conditions and travelling expenses on first appointment are obtainable from the Registrar, University of Natal, King George V Avenue, Durban 4001, South Africa, with whom applications on the prescribed form, must be lodged not later than 23rd September 1979 quoting the reference Adv. D62/79. W310(A)

MEDICAL RESEARCH COUNCIL
TOXICOLOGY UNIT

Scientific staff member (non-clinical, with at least two years of postdoctoral experience) required to continue studies on the mechanism by which chlorinated compounds disturb the regulation of haem and haemoprotein metabolism in the liver.

A suitable candidate will be offered a limited appointment of five years. Salary in the range £4,776 to £6,108 according to age and experience; London Allowance; Superannuation provision and six weeks annual leave.

Applications, complete with curriculum vitae should be sent to Dr F De Matteis, Toxicology Unit, MRC Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF by 15th September, 1979. 2946(A)

MRC Medical Research Council

MEDICAL RESEARCH COUNCIL
TOXICOLOGY UNIT
SCIENTIST

Scientist required to carry out research on the action of cadmium and other metals on mammalian systems. The aim will be to understand the mechanisms of the toxic action of such metals and the studies can range from a molecular to a whole animal approach.

A suitable candidate should have at least two years postdoctoral experience and will be eligible for a limited appointment for five years. Salary according to age and experience in the range of £4,776 to £6,108.

Applications should be sent to Dr W. N. Aldridge, Toxicology Unit, Woodmansterne Road, Carshalton, Surrey SM5 4EF by 15th September, 1979.

2947(A)

MRC
Medical Research Council

UNIVERSITY OF EDINBURGH
DEPARTMENT OF PATHOLOGY
SENIOR LECTURSHIP WITH SPECIAL REFERENCE TO EXPERIMENTAL PATHOLOGY

The University Court invites applications for the post of
SENIOR LECTURER
with teaching and research duties. The Lothian Health Board will award appropriate honorary status.

Candidates should have a higher medical or scientific qualification, considerable experience of some branch of experimental pathology and of teaching. Excellent research facilities are available.

The non-clinical salary scale for Senior Lecturers is £8,182 to £10,097. The clinical salary scale for Senior Lecturers without honorary Consultant status is £7,820 to £9,670 and with honorary Consultant status is £9,528 to £12,084. Membership of the appropriate University Superannuation Scheme is required.

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, EH8 9YL, with whom applications (12 copies) including curiculum vitae and the names and addresses of three referees, should be lodged not later than 28th September 1979.

Please quote ref. no. 513. 2978(A)

NAPIER COLLEGE OF COMMERCE AND TECHNOLOGY
Lothian Regional Council
RESEARCH ASSISTANTS

Applications are invited for Research Assistants to carry out the following projects, all of which are in support of the College's programme of CNAA Degree development.

1. Microprocessor control of the Injection Moulding of Polymers.
2. The design of a Computer Data Base System for Administration.
3. Design practice and procedure as related to the production of artifacts in small manufacturing industries in Scotland.
4. Operation of the Health and Safety at Work Act in the Public Sector.

Candidates should have a good Honours Degree in an appropriate discipline. Other candidates with equivalent qualifications and relevant experience are encouraged to apply. Registration for higher degrees is encouraged.

Salary on Scale £3,267 to £3,993.

Application forms and further particulars from:

THE ACADEMIC REGISTRAR
Napier College of Commerce and Technology, Colinton Road, Edinburgh, EH10 5DT 2974(A)

THE OPEN UNIVERSITY

Faculty of Science

TECHNICIAN – SCIENCE AND TECHNOLOGY MECHANICAL WORKSHOP (£3,984 to £4,758 p.a.) (under review).

A Technician is required to work as part of a team supporting the research and teaching work of the above two Faculties.

The person appointed would be required to have a high standard of competence in the use of centre-lathes, horizontal and vertical milling machines, drilling, surface grinding, fitting, gas and electric welding, sheet-metal working and some woodwork. The work is varied and interesting and consists of development work, one-offs and small batches. The nine disciplines served cover fields from materials science to biology.

Applicants should have an HNC or equivalent, together with a recognised apprenticeship within a relevant field of industrial activity and, at least 15 years' post apprenticeship. Experience in a development workshop either industrial or educational would be an advantage.

Application forms and further particulars are available from The Recruitment Officer (JD1980/1), The Open University, P.O. Box 75, Walton Hall, Milton Keynes, MK7 6AL or telephone Milton Keynes 63404: there is a 24 hour answering service on 63868.

Closing date for applications: 7th September, 1979. 3002(A)

THE QUEEN'S UNIVERSITY OF BELFAST
POSTDOCTORAL RESEARCH ASSISTANT
DEPARTMENT OF CHEMISTRY

Required to work with Dr J J McGarvey on the development of ultrasonic and photochemical relaxation techniques for kinetic studies on labile metal complexes. The post is tenable for one year from 1st January 1980 (or other starting date to be arranged) with the possibility of renewal for a second year.

Commencing salary in the range (under review) £4,233 to £4,776 with USS benefits.

Applications giving a curriculum vitae and the names and addresses of two referees should be sent as soon as possible to the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland.

2971(A)

QUEEN ELIZABETH COLLEGE
Kensington
(University of London)

LECTURER IN MICROBIOLOGY

Applications are invited for the post of Lecturer in Microbiology. Applicants should be general microbiologists with an interest in microbial structure and function, and in biotechnology. The proposed starting date is 1 January 1980. Salary on scale £4,333 to £8,992 pa plus London allowance of £502 pa.

Apply to the College Secretary, Queen Elizabeth College, Campden Hill Road, London W8 7AH, from whom further particulars and an application form may be obtained. Closing date 28 September, 1979. 2958(A)

UNIVERSITY COLLEGE OF SWANSEA
Senior Research Assistant

Applications are invited for the vacancy of **Senior Research Assistant** in the **Department of Chemistry**. Applicants should be Chemists or Biochemists with a PhD., or shortly to submit for such a degree. The successful applicant will work on an MRC sponsored project with Dr J.M. Williams on structural studies of the carbohydrate moieties of glycoproteins of clinical interest.

The appointment will be for one year in the first instance, from 1 October, 1979 with the possibility of renewal for a further two years. The commencing salary will be on a scale up to £4,910 per annum, plus USS/USDPS benefits.

Informal enquiries may be made to Dr J.M. Williams, but application forms (2 copies) and further particulars must be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea, SA2 8PP, to whom they should be returned *as soon as possible*.

2995(A)

UNIVERSITY OF SHEFFIELD
DEPARTMENT OF BIOCHEMISTRY
TECHNICIAN (GRADE 3)

required to assist in a S.R.C. supported project for a period of two and a half years on biochemical and biophysical aspects of cellular energy transformations. Previous laboratory experience is expected and familiarity with spectrophotometric/fluorometric instrumentation would be an advantage.

The successful applicant will be able to learn a wide variety of laboratory techniques and will be encouraged to work with minimal supervision. Commencing salary £2,931 p.a. (under review).

Please write as soon as possible giving the names of two referees to the Administrative Officer (Personnel), (Ref. S1345/N), The University, Sheffield S10 2TN. 3012(A)

UNIVERSITY OF SUSSEX
BIOCHEMISTRY LABORATORY
POST-DOCTORAL RESEARCH FELLOW

Applications are invited for a post-doctoral research fellow (Grade 1A) to collaborate with Professor Sydney Shall on an SRC 3-year project grant to examine the response in intact cells of ADP-ribosylated and phosphorylated chromatin proteins to cytotoxic drugs. Experience in handling chromatin proteins, in immunology or in protein modification would be an advantage. Salary on scale £4,333 to £7,521 per annum (under review) with superannuation benefits.

Further details may be obtained from, and applications should be sent to, Professor Sydney Shall, Biochemistry Laboratory, Biology Building, University of Sussex, Brighton, Sussex BN1 9QG.

Applications should include a curriculum vitae, a description of previous experimental experience and the names of three persons from whom references may be sought. 2953(A)

GUY'S HOSPITAL MEDICAL SCHOOL GRADUATE

required for two years for cytogenetics laboratory engaged in cell culture work and diagnostic chromosome analysis. Experience not essential but applicants should have a good basic understanding of genetics.

Applications to the Administrative Assistant, Paediatric Research Unit, The Prince Philip Research Laboratries, Guy's Tower, London Bridge, SE1 9RT.

2981(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL

DEPARTMENT OF MEDICINE

POST-DOCTORAL IMMUNOLOGIST

required for work on the role of complement in induction of immunological responses. The project is supported by the Medical Research Council until October 1980 but there will be a continuing opportunity in the Unit for a suitable individual. For further details please contact Dr M B Pepys, Immunological Medicine Unit, Department of Medicine, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS or telephone 743 2030 extension 258.

Salary up to £5,077 plus £502 London Allowance.

Application forms are available from and should be returned to the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS quoting reference number 2/220. 2956(A)

OPPORTUNITY FOR RESEARCH EMPLOYMENT IN GREECE

Applications are invited for the position of

DIRECTOR OF THE INSTITUTE OF ORGANIC CHEMISTRY

of the National Hellenic Research Foundation, Athens, Greece.

The candidates should be leading authorities in the field of Organic Chemistry with a substantial research record and must be experienced in the administration of R & D.

The salary of the Directors of Institutes of the N.H.R.F. corresponds to that of full Professors of Greek Universities.

Deadline for applications is December 31, 1979. Detailed curriculum vitae should be sent to Professor A. Kontaratos, Administrating Director, National Hellenic Research Foundation, 48 Vassileos Constantinou Avenue, Athens 501/1, Greece. Director of Personnel. W309(A)

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Microbiologist

Pharmaceuticals & Toiletries

Ashe Laboratories manufacture and market a wide range of pharmaceutical, toiletry, dietary and baby care products. Among our products are brand names such as Amplex, Pro-Plus, Bisks, Sebbix and Maws.

A vacancy has occurred for a microbiologist, based at our main manufacturing site at Littleborough, Lancashire, but responsible for our other sites at Barnet and Leatherhead. A certain amount of travel, on an irregular basis, would therefore be involved.

As well as carrying out microbiological testing of products, the successful candidate will be responsible for the development and monitoring of hygiene policies and of a microbiological monitoring system for the three sites.

The ideal candidate will have a degree (or equivalent) in microbiology and two to three years in industrial or hospital microbiology.

An attractive benefit package of salary plus bonus, five weeks' holiday, and relocation expenses will be offered.

Please write with brief details or telephone for an application form.
P. Stack, Ashe Laboratories Ltd., Kingston Road, Leatherhead, Surrey.
Tel: Leatherhead 76151.

ASHE LABORATORIES LTD

2986(A)

FRESHWATER BIOLOGICAL ASSOCIATION

SENIOR ANALYTICAL CHEMIST

Vacancy at Windermere Laboratory. Duties to include: general co-ordination of analytical programme and development of close liaison with biological staff; routine analysis; development of new methods; oversight of radiochemical laboratory, including training of scientific staff in techniques and safety.

Candidates should hold a first degree or relevant industrial qualification and have considerable practical experience. The pensionable post is graded HSO/SSO and starting salary would be £5,800 or above (approx. 10% additional pay award is due 1st January 1980).

Further details and application forms may be obtained from: The Secretary, Freshwater Biological Association, The Ferry House, Ambleside, Cumbria LA22 0LP. Closing date 14th September 1979. 2979(A)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF PHARMACY

Post-Doctoral Research Appointment

BIO-ORGANIC CHEMIST

Applications are invited for a post-doctoral appointment, supported by the S.R.C., to work in collaboration with Professor B.W. Bycroft on the biosynthesis of Penicillins and related peptide antibiotics.

The candidates should have an interest in, and preferably experience of, biological chemistry. The appointment will be effective from 1st October, 1979 at a salary within the range £4,333 to £4,910 p.a.

Anyone interested should apply, enclosing curriculum vitae and the names of two academic referees to the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD, as soon as possible. 2984(A)

NON-MEDICAL SCIENTIFIC OFFICER (BASIC GRADE)

Applications are invited for the post of non-medical scientific officer to the Sheffield Haemophilia Centre. The Department is actively involved in clinical and research activities relating to haemorrhagic and thrombotic disorders. Preference will be given to candidates with experience of platelet biochemistry and the successful applicant will be primarily responsible for the continued development of this area within the department. The haemophilia centre is included in the University Department of Haematology, Sheffield and offers excellent facilities for those wishing to obtain higher postgraduate qualifications.

Minimal requirements are a 1st or 2nd class honours degree in the biological sciences.

Salary £3,486 to £4,275 per annum.

Further information from Dr F.E. Preston, Consultant Haematologist or Mr R.G. Malia telephone 0742 26484 (Ext 3022)

Applications with curriculum vitae and names and addresses of 2 referees to the District Personnel Officer, B Floor, Hallamshire Hospital, Glossop Road, Sheffield, S10.

Closing Date for applications 3rd September, 1979. 2975(A)

UNIVERSITY OF AARHUS

Denmark

A one year temporary position as adjunkt/lektor (associate professor or assistant professor according to qualifications) with possibility of extention is vacant at the Department of Molecular Biology and Plant Physiology starting January 1, 1980.

The successful applicant will be required to work in the area of symbiotic nitrogen fixation in higher plants as part of a group within the department. The research will primarily deal with the molecular genetics of Rhizobium. Applicants with research experience in bacterial and molecular genetics will be preferred.

The successful applicant must, on equal terms with the other members of the staff, share the teaching responsibilities — from first-year level to supervision of graduate and post-graduate students. Further information can be obtained from professor K.A. Marcker, phone Denmark (06) 12 51 77.

Applications containing curriculum vitae, information on scientific and educational activities, a list of publications plus wherever possible 3 copies of published material, and any additional information relevant for the evaluation of the applicant's scientific and educational qualifications should be sent to the Faculty of Science, University of Aarhus, Ndr. Ringgade 1, DK-8000 Århus C, Denmark, before 7, 1979.

The report of the evaluation committee will be sent to all applicants. W316(A)

THE UNIVERSITY OF SHEFFIELD

DEPARTMENTS OF HAEMATOLOGY, HUMAN METABOLISM AND CLINICAL BIOCHEMISTRY

RESEARCH FELLOW

Applications are invited from men and women for a two year appointment to work in the field of thrombosis research, joining a research group working on clinical and biochemical aspects of platelet function, prostaglandins and the vessel wall. Excellent clinical and laboratory facilities are available. The work would be suitable for an MD or PhD thesis.

Tenable immediately and Salary is up to £6,129 a year subject to age and qualifications. Further information from Dr F.E. Preston or Professor Russell at the Hallamshire Hospital, Glossop Road, Sheffield. (Tel. 0742 26484).

Please quote ref: R351/G. 3000(A)

UNIVERSITY OF LEICESTER

DEPARTMENT OF PHYSICS

RESEARCH ASSOCIATE IN IONOSPHERIC PHYSICS

A research assistant is required to support our research programme into ionospheric disturbances at high latitudes. These investigations are being conducted using the new European Incoherent Scatter Radar (EISCAT), and ample opportunity will be available for the development of experiments using this powerful new research facility.

Applicants should preferably hold a PhD degree in Physics or Mathematics and have a sound knowledge of plasma physics.

The salary will be in the range £4,333 to £5,488 (under review). Further details are available from Dr T B Jones, Physics Department, University of Leicester, Leicester LE1 7RH. 2970(A)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London)

DEPARTMENT OF BIOCHEMISTRY & CHEMISTRY

requires a

TECHNICIAN.

Duties equally divided between assisting with teaching work and research. Experience in tissue culture techniques would be an advantage but this is not essential.

Salary on Whitley Council Scales according to qualifications and experience.

Application forms available from the School Secretary, R.F.H.S.M., 8 Hunter Street, London, WC1N 1BP or telephone 01-837-5385 Ext.10. Closing date: 7 September 1979. 2996(A)

UNIVERSITY OF OXFORD

DYSON PERRINS (ORGANIC CHEMISTRY) LABORATORY

POSTDOCTORAL RESEARCH ASSISTANT

A postdoctoral Assistant is required to join a group engaged in the synthesis of biologically active peptides. Experience is desirable but not essential. The appointment, which is for one year, renewable for a second year, will start as soon as possible. Salary (Grade 1A) in the range £4,776 to £5,850 (under review) according to age and experience, plus superannuation.

Applications, with curriculum vitae and the names of two referees, should be sent as soon as possible to Dr G T Young, The Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY. 2944(A)

AGRICULTURAL RESEARCH COUNCIL

INSTITUTE FOR RESEARCH ON ANIMAL DISEASES

VETERINARIAN

with microbiological experience is required to join a project team investigating the role of virus and bacterial pathogens in the aetiology of outbreaks of diarrhoea in calves and piglets.

The Institute is studying the pathogenic mechanisms involved in E. coli infections and in breeding resistance; it has also a programme on enteritis viruses and the Veterinarian will form a link between these areas.

Candidates must be Members of the Royal College of Veterinary Surgeons.

Salary on the VRO Scale: £5,448 pa to £7,966 pa (currently under review). Starting salary according to qualifications and experience.

Non-contributory pension scheme.

Application forms from the Secretary, Institute for Research on Animal Diseases, Compton, Nr. Newbury, Berks RG16 0NN quoting ref. 412. 2964(A)

POST-DOCTORAL POSITION IN TUMOUR IMMUNOLOGY. NIH salary scale. Send curriculum vitae and interests to: Dr J.S. Haskill, Department of OB/GYN, University of North Carolina, Old Clinic Bldg. 226H, Chapel Hill, NC 27514. W311(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF IMMUNOLOGY

Applications are invited for the post of postdoctoral Senior Research Assistant in the Department of Immunology to work on tumour specific stimulation and suppression of the immune response. Previous experience in tumour immunology or cellular immunology would be advantageous.

Initial salary will be either £4,232 or £4,505 per annum (under review).

Applications together with the names of three referees should be received not later than 20th September, 1979 by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/750/N. 2967(A)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF ZOOLOGY

POSTGRADUATE OR POSTDOCTORAL RESEARCH ASSISISTANT

Applications are invited for this post funded by SRC and available for 2 years. The successful candidate will work on the evolution of green-geckos using various techniques including the extraction and characterisation of keratin monomers. Previous biochemical training and experience, particularly in polyacrylamide gel electrophoresis and iso electric focusing, is desirable.

Commencing salary up to £4,910 per annum.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 14 September 1979. 2973(A)

UNIVERSITY COLLEGE OF GALWAY

JUNIOR LECTURESHIP IN BIOCHEMISTRY

Applications are invited for the above post. Salary scale £4,852 × (10) — £6,706. Closing date for receipt of applications, 7 September 1979. Further information may be obtained from the registrar. 2966(A)

FELLOWSHIPS

SANDOZ RESEARCH INSTITUTE in Vienna, Austria

is one of three international research centres of the Swiss based pharmaceutical company SANDOZ.

Currently, we offer a

POSTDOCTORAL FELLOWSHIP

in our Department of VIROLOGY. This opening is for investigation of molecular and cellular aspects of Herpes virus latency and reactivation. The candidate should hold a PhD or an equivalent degree with a background in animal virology.

Experience in tissue culture methods and electron microscopy techniques would be of advantage.

The successful candidate will find excellent working conditions in well equipped laboratories. The appointment will be for an initial 2-year period. We offer an attractive salary. Travel expenses from and to the country of origin will be paid.

Please send application including curriculum vitae and credentials to



Dr N.Zacherl, Personnel Manager,
SANDOZ Forschungsinstitut,
A-1235 Wien,
Brunner Straße 59.
Austria. W315(E)

POST DOCTORAL RESEARCH FELLOWSHIPS

The National Institute of Allergy and Infectious Diseases is recruiting for two positions which are available immediately:

LIPID BIOCHEMIST to join an ongoing cell biology-biochemistry program concerning cell surface membranes. Research to involve analytical characterization and identification of lipid constituents present in cell surface membranes isolated from protozoan parasites.

BIOCHEMIST/CELL BIOLOGIST with demonstrated ability to analyze and isolate membrane proteins that are in limited quantities in order to determine their function and immunogenicity.

Requisite are PhD in biochemistry, pharmacology, microbiology, cell biology or allied area, and research experience in preparative and analytical —TLC, —GLC, and mass spectroscopy of lipids. previous experience in membrane spin labeling techniques also desirable. No parasitology experience required but offers the candidate an opportunity to enter protozoal research in a field of great importance to tropical health.

These positions will be filled at the Staff Fellow level ($15,120 to $25,000) depending upon qualifications and experience.

Please send resume, reprints and, or preprints of publications, and names of three references by September 21, 1979 to: Postdoctoral Search Committee, Laboratory of Parasitic Diseases, Building 5, Room 114, National Institute of Allergy and Infectious Diseases, NATIONAL INSTITUTES OF HEALTH, Public Health Service, Bethesda, Maryland 20205. An Equal Opportunity Employer. W307(E)

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Appointment at the appropriate level will be for a fixed period of up to years.

Depending on qualifications and experience stipends will be fixe within the following ranges:

Junior Research Fellow	£3,695 — 4,5
Senior Research Fellow	£5,092 — 6,21
Principal Research Fellow	£6,605 — 8,4

Qualifications: Junior Research Fellow. Candidates must have a fir or second class honours degree with at least tw years post-graduate research experience.

Senior Research Fellow. Candidates must have a fir or second class honours degree with at least thre years post-graduate research experience.

Principal Research Fellow. Candidates must als have achieved very high standing in a relevant field c science and have been engaged in fruitful research fc a considerable period and generally accepted as leading worker in his field.

Application forms, quoting reference AX794/151. 708/AXS for retur by 13th September '79 from:

Scientific Personnel Officer, Admiralt Underwater Weapons Establishment, Portland Dorset. Telephone: Portland (0305) 820381 Ext 3433. 2965(E

UNIVERSITY OF KENT AT CANTERBURY

APPOINTMENTS IN SPACE SCIENCES IN THE ELECTRONICS LABORATORY

Applications are invited for the following two research posts tenable from 1 November, 1979 and financed by Science Research Council grants under the direction of Dr J A M McDonnell: (1) A 3-year Post-Doctoral Fellowship for the development of instrumentation for the detection and analysis of cosmic dust on future space missions. Experiments for the joint ESA/NASA Comet Mission, Recoverable Space Shuttle Packages, Lunar Orbiters and Refurbishable Satellite systems are planned. Relevant research experience in physics, astronomy, space research or instrumentation is desirable. Salary Range: £4,232 to £4,776 (under review) (2) A 2-year Post-Doctoral Fellowship for sensor calibration, mission development and data analysis in connection with the International or Solar Polar (Out of Ecliptic) Mission in collaboration with the Max Planck Institute, Heidelberg, and the Space Shuttle Recoverable Long Duration Exposure Facility. Suitable post doctoral experience in space projects is essential for this post. Salary Range: £4,232 to £5,048 (under review). Application forms and particulars may be obtained from the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH to whom completed applications should be returned by 17 September, 1979. Please quote ref. A44/79. 2972(E)

FRIEDRICH MIESCHER INSTITUT:

A Postdoctoral Fellowship

in Biochemistry is available for the a of DNA synthesis in cultur mammalian cells. Experience purification of DNA enzymes, DN binding proteins or *in vitro* DN replication is desired. The fellowsl will run for two to three yea

Candidates should send c.v. a three letters of reference Dr. L. Jimenez de Asua, Friedri Miescher-Institut, PO Box 27 CH-4002, Basel, Switzerland. W292(E)

UNIVERSITY OF SUSSEX

SCHOOL OF BIOLOGICAL SCIENCES

RESEARCH FELLOW IN PLANT PHYSIOLOGY

Required as soon as possible to work one-year ARC financed post-doct fellowship investigating characterisation of vacuoles and tonop from higher plants. Experience membrane isolation and enzymol required. Salary on the scale for Rese Fellow 1A, £4,333 to £7,521 pa (u review).

Curriculum vitae with the names of referees to Dr J L Hall, School of Biolo Sciences, University of Sussex, Brigl BN1 9QG, who can be telephoned fur details on (0273) 606755. 2955(E

FELLOWSHIPS

Continued from previous pages

RESEARCH FELLOWSHIP

A post-doctoral fellow with experience in immunology, somatic cell genetics or DNA cloning technology required for a project investigating the genetic control of development. Specific experiments planned include the genetic manipulation of murine teratocarcinoma stem cells and various stem cell hybrids.

Three year appointment. Salary range £5,800 to £7,100. For further information telephone Dr P. Goodfellow (01.242.0200 ext 265).

Applications with curriculum vitae and the names of two referees should be sent to the Personnel Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, WC2 quoting reference 371/79. 2980(E)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF BOTANY

RESEARCH FELLOWSHIP

Applications are invited from

PHYSICISTS or ELECTRICAL ENGINEERS

to work with Dr R P C Johnson on the photon correlation spectrometry of flow and Brownian motion in sieve tubes.

The successful candidate will be expected to collaborate in the analysis of laser light scattered by particles moving in cells and in models of cells, under the light microscope. Candidates should have considerable computing experience. The work will involve the use of a signal correlator, desk-top computer and the University's Honeywell 66/80. Appointment will be for two years and is funded by SRC. Commencing salary up to £4,910 per annum on the Research and Analogous Range 1A Scale.

Application forms available from The Secretary, The University, Aberdeen, with whom completed applications (2 copies) should be lodged by 14 September, 1979. 2951(E)

STUDENTSHIPS



Northgate Research Studentship at University College, Cork.

Northgate Exploration Limited, through its wholly owned subsidiary, Irish Base Metals Limited, is sponsoring a research student to work under the direction of Professor P.M. Brück, Department of Geology, University College, Cork, on "The Geology of the Blackstairs Unit of the Leinster Granite and its Economic Potential."

The object is to carry out a detailed study of the main pluton, its satellite bodies and the metamorphic aureole. Work will include detailed field mapping, concentrating on structures, delineation of granite varieties, areas of hydrothermal alteration and associated mineralisation, and detailed laboratory petrographic studies.

The studentship will be tenable over a three year period beginning Autumn 1979, amounting to a value of £1,450 in the initial year plus field expenses.

Applicants should, as a minimum requirement, hold or expect to obtain an Upper Second Class Honours degree in Geology. Full details together with the names of two referees, should be submitted by applicants to The Secretary, Department of Geology, University College, Cork, Ireland, as soon as possible. 2998(F)

STUDENTSHIPS continued

THE ANIMAL VIRUS RESEARCH INSTITUTE PIRBRIGHT

ARC RESEARCH STUDENTSHIP 1979

Applications are invited for an Agricultural Research Council Studentship to join a group working on the genetics of foot-and-mouth disease virus. The programme is concerned with the biochemical analysis of mutants and recombinants in order to map the biological properties of the virus on the physical genome, and to provide information on the nature and extent of recombination in the virus. The Studentship will be tenable for up to 3 years from October 1979 and the successful candidate will be expected to read for a higher degree. Applicants should possess a good honours degree in a Molecular Biological subject with an interest in genetics.

Application forms and further particulars may be obtained from the Secretary, The Animal Virus Research Institute, Pirbright, Woking, Surrey, GU240NF. 3005(F)

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FOR SALE:- EDWARDS VACUUM COATER MODEL 306. As new condition with H.T. supply, Plasmaglo accessory and twin filament attachments. Price £2500.00. Telephone 0268-743247 Mr. Ford. 2985(L)

ASSOCIATESHIPS

UNIVERSITY OF CAMBRIDGE

PLANETARY SCIENCES UNIT

DEPARTMENT OF MINERALOGY AND PETROLOGY

POST DOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a post doctoral research assistant to join a group carrying out stable isotope geochemical studies of lunar samples and meteorites. A major proportion of the effort presently concerns the development of new, very sensitive computerised mass spectrometric techniques. Scope exists for an instrumentalist or analyst.

The post, funded by the Science Research Council, is for three years of range 1A of the nationally agreed scale.

Applicatins enclosing a *curriculum vitae* and the names of two referees should be sent to Dr C.T. Pillinger by September 24th 1979. 2982(P)

QUEEN ELIZABETH COLLEGE

Kensington

(University of London)

RESEARCH ASSISTANT

PHYSICS DEPARTMENT

Applications are invited for a r assistantship, supported by th Research Council, available from 1 October 1979. Th the fabrication and computer-plotted opical appointment may be at pos. (aged 24) or at first degree le case registration for a higher deg possible. Salary in range £3,775 pa plus London allowance of £502

Applications, giving the names o referees, to Professor R. E. Burge, Phy Department, Queen Elizabeth Colleg Campden Hill Road, London W8 7AH. 2959(P)

GRANTS

UNIVERSITY OF LONDON

GRANTS FOR RESEARCH

Applications are invited from members o the University and teachers in Schools o the University for grants from the Centra Research Fund to assist specific projects o research and for the provision of specia materials and apparatus. Grants are not made for normal maintenance.

Applications must be received not later than 15 September, 1979. Forms of application and further particulars may be obtained from the Secretary to the Central Research Fund, University of London, Senate House, London WC1E 7HU. 2961(H)

COURSES

A Short Course

NATURE, ORIGIN AND EXPLOITATION OR MARINE MANGANESE NODULES AND METALLIFEROUS SEDIMENTS

To be given by Dr. D.S. Cronan

Department of Geology, Imperial College, at Monash University, Victoria, Australia 27-30 November 1979.

Registration: Australian Dollars 60 for the course, by 15 October. Enquiries to Dr L A Frakes, Department of Earth Sciences, Monash University, Clayton, 3168, Victoria, Australia. 2988(D)

UNIVERSITY OF NOTTINGHAM

FACULTY OF AGRICULTURAL SCIENCE

A.R.C. RESEARCH ASSISTANTSHIP

Applications are invited for the above post, tenable from October 1979, to study the water relations of tillers in wheat. The project will involve an integrated field and controlled environment investigation of the importance of competition for water between tillers and main systems for the growth and survival of tillers.

Applicants should have a good degree in Plant Science or Agricultural Science and will be expected to register for a PhD degree.

The salary is on the scale £3,463 to £3,975. Applications, including a *curriculum vitae* and naming two referees, should be sent as soon as possible to: Dr C.R. Black, Department of Physiolo and Environmental Studies, Universi Nottingham School of Agriculture Bonington, Loughborough L from whom further det obtained. Please quote 79/19.

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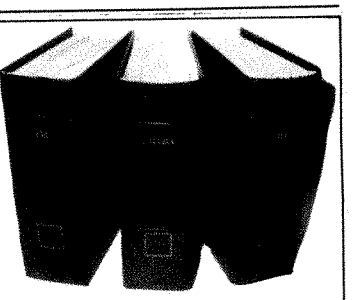
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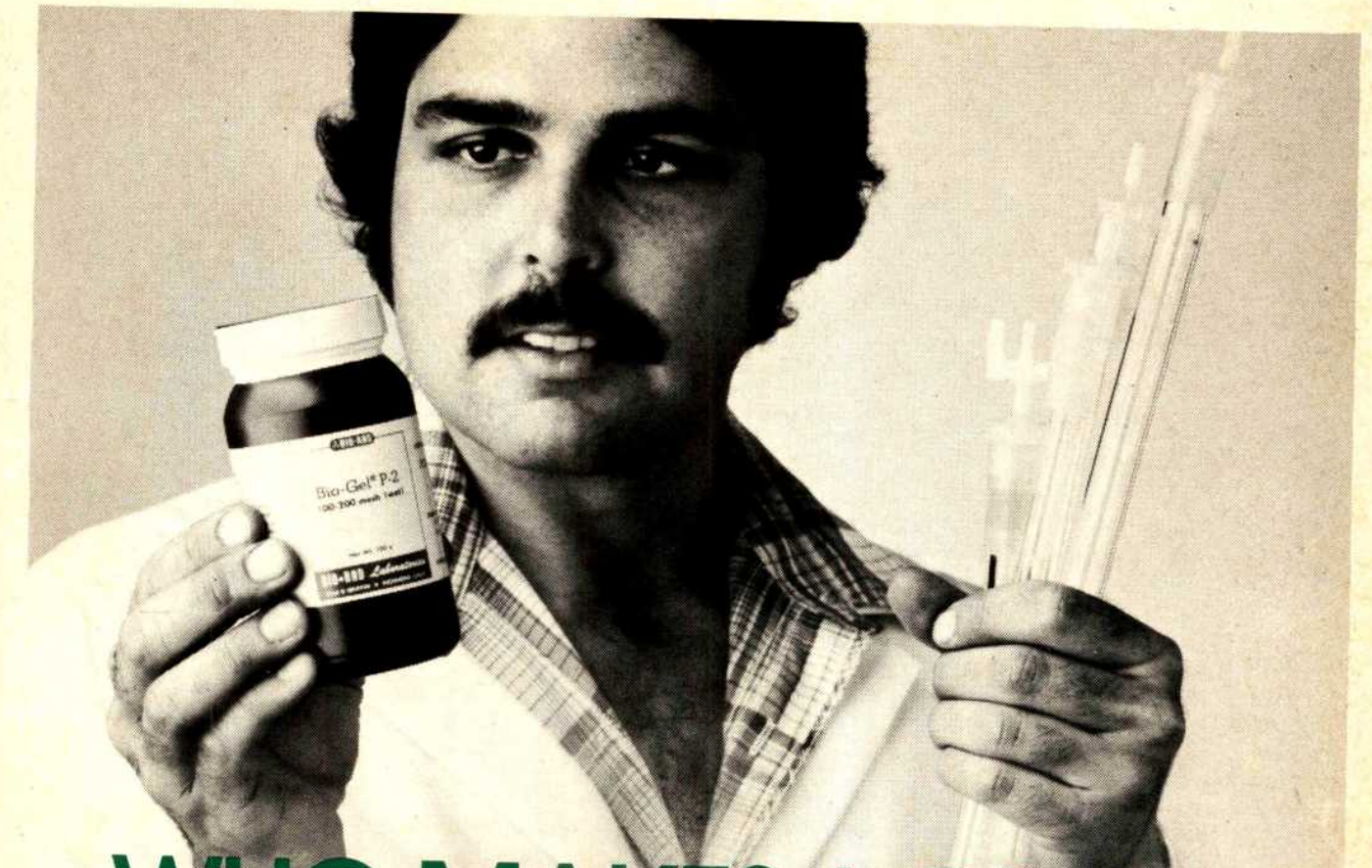
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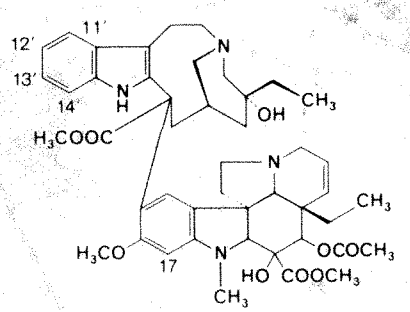
Distribution of labelling in tritium compounds

Modern techniques for the production of tritiated compounds are more sophisticated than those used in the early days of tritium labelling, and produce compounds labelled in specific positions rather than generally labelled. Nevertheless, it is necessary for many tracer applications of tritium compounds to know the *precise* position and configuration of the tritium labels. Traditional chemical methods of doing this are tedious and time consuming and subject to considerable error, and so the routine supply of such information has until recently not been possible.

The Radiochemical Centre, in collaboration with the University of Surrey, has developed over the past eight years the technique of tritium nuclear magnetic resonance (tnmr) spectroscopy for this purpose. This method is much quicker and more accurate than the traditional chemical or biochemical methods for determining distribution of tritium labelling.

It is now used routinely to establish the distribution of tritium labelling produced by the usual methods of tritiation employed at The Radiochemical Centre. We supply accurate details as to the position and configuration of the tritium labels for an increasing number of our labelled compounds.

A number of examples are given below:



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Code TRK.507

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[1β, 2β(n)-^{3}H]TESTOSTERONE
Code TRK.162

1α	5.9%	2α	8.2%
1β	46.0%	2β	39.9%

[6-^{3}H]BENZO[α]PYRENE
Code TRK.501

6	>95%	1	<5%

D-[2-^{3}H]GLUCOSE
Code TRK.361

2(α)	40%	2(β)	60%

L-[3,4(n)-^{3}H]VALINE
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AL-RAWI, J.M.A., BLOXSIDE, J.P., ELVIDGE, J.A., JONES, J.R., CHAMBERS, V.E.M., CHAMBERS, V.M.A. and EVANS, E.A., Steroids vol. 28 (3), p.p. 359 - 375, 1976.

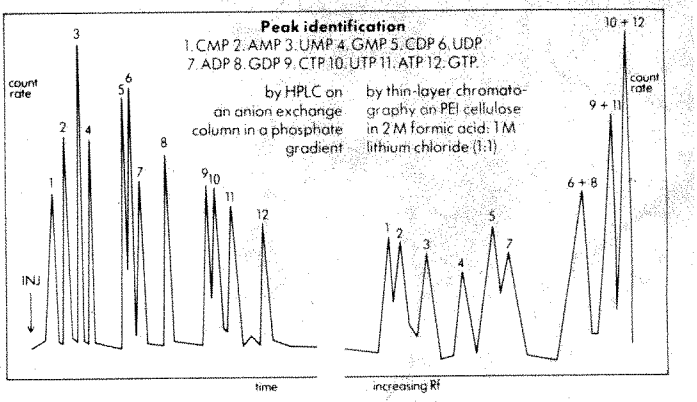
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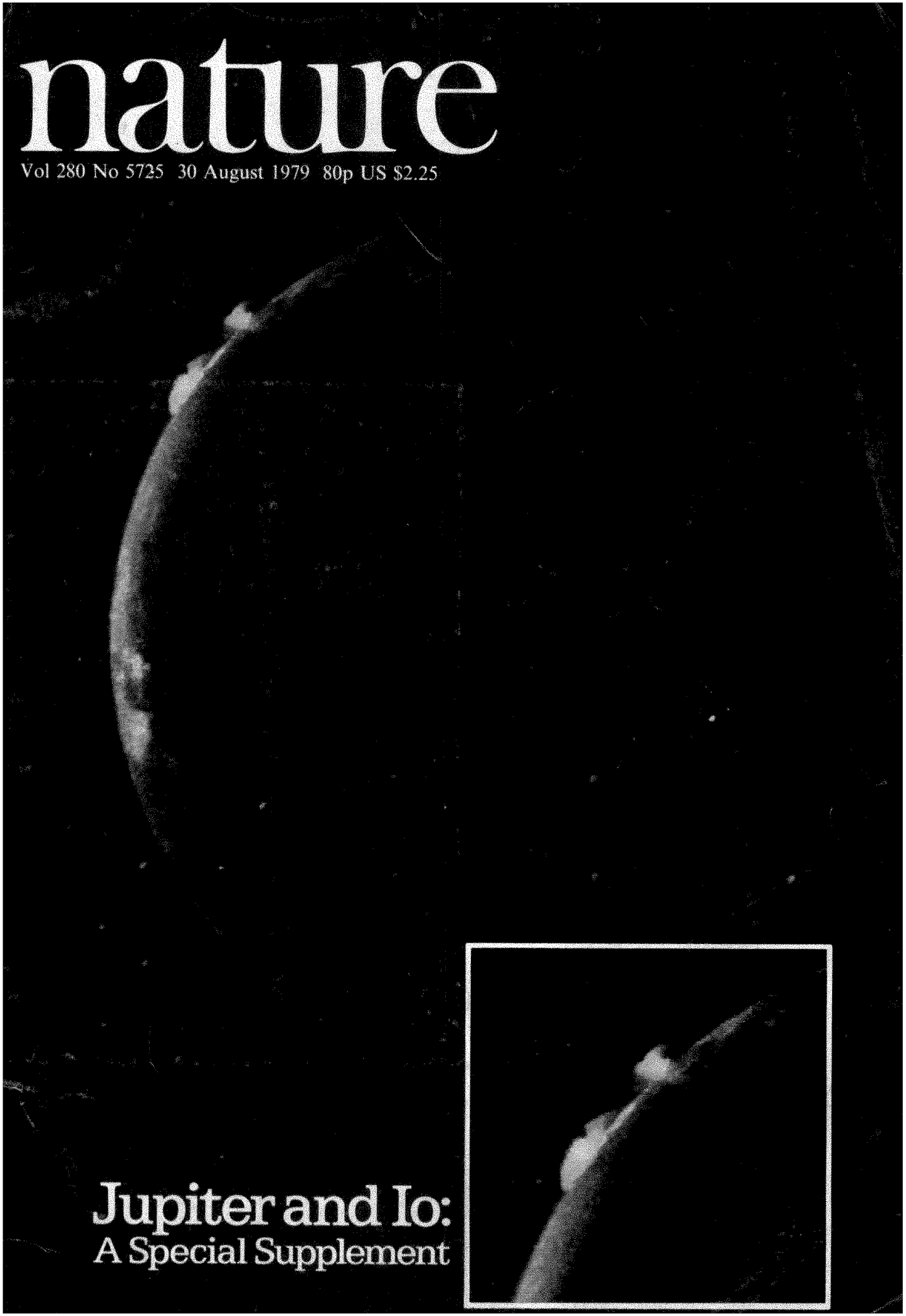
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Vol.280 No.5725 30 August 1979

nature

Volume 280 30 August 1979

Dr Boyson's provocative challenge 709
UNCSTD: hunger and poverty/Iran criticises Group of 77 710
UNCSTD at half time: North stands firm 711
NGOs clash at side-show meeting/Organiser criticised but not bowed 712
India demands "sharp break" from current practices 713
Soviet spacemen return to earth/UK tram lovers book Shuttle space 713
Pioneer may collide with Saturn's ring 714
News in brief 715
Barefoot doctors: symptom not cure 716
Jukes on dietary needs 718

NEWS & VIEWS

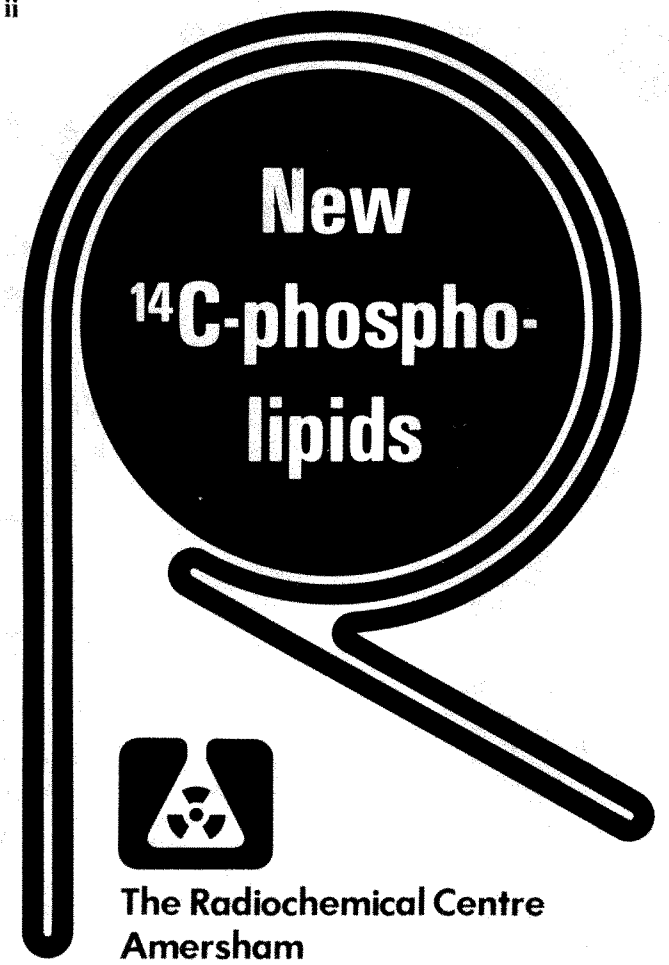
Plant molecular biology comes of age/Bone marrow transplantation: man and mouse/Sperms in 'parthenogenetic' freshwater gastrotrichs/Insect development: the clockface model/Reassessment of α-particle decay of giant resonances in nickel 719

Voyager 1 encounter with Jupiter and Io

Introduction	*G.E. Hunt*	725
Preliminary geological mapping of Io	*H. Masursky, G.G. Schaber, L.A. Soderblom and R.G. Strom*	725
Volcanic features of Io	*M.H. Carr, H. Masursky, R.G. Strom and R.J. Terrile*	729
Volcanic eruption plumes on Io	*R.G. Strom, R.J. Terrile, H. Masursky and C. Hansen*	733
Erosional scarps on Io	*J.F. McCauley, B.A. Smith and L.A. Soderblom*	736
The role of SO_2 in volcanism on Io	*B.A. Smith, E.M. Shoemaker, S.W. Kieffer and A.F. Cook II*	738
Dynamics of volcanic plumes on Io	*A.F. Cook II, E.M. Shoemaker and B.A. Smith*	743
Volcanic resurfacing rates and implications for volatiles on Io	*T.V. Johnson, A.F. Cook II, C. Sagan and L.A. Soderblom*	746
Sulphur flows on Io	*C. Sagan*	750
Photometric evidence on long-term stability of albedo and colour markings on Io	*D. Morrison, D. Pieri, J. Veverka and T.V. Johnson*	753
Identifications and gaseous SO_2 and new upper limits for other gases on Io	*J. Pearl, R. Hanel, V. Kunde, W. Maguire, K. Fox, S. Gupta, C. Ponnamperuma and F. Raulin*	755
The stability of an SO_2 atmosphere on Io	*S. Kumar*	758
Significance of absorption features in Io's IR reflectance spectrum	*F.P. Fanale, R.H. Brown, D.P. Cruikshank and R.N. Clarke*	761
Spectral evidence for sublimates and adsorbates on Io	*D.B. Nash and R.M. Nelson*	763
Spectral evidence for SO_2 frost or adsorbate on Io's surface	*W.D. Smythe, R.M. Nelson and D.B. Nash*	766

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Auroral hiss observed near the Io plasma torus	*D.A. Gurnett, W.S. Kurth and F.L. Scarf*	767
Pre-Voyager velocities, accelerations and shrinkage rates of jovian cloud features	*R.F. Beebe and L.A. Youngblood*	771
Zonal velocity and texture in the jovian atmosphere inferred from Voyager images	*A.P. Ingersoll, R.F. Beebe, S.A. Collins, G.E. Hunt, J.L. Mitchell, P. Muller, B.A. Smith and R.J. Terrile*	773
Jovian cloud structure and velocity fields	*J.L. Mitchell, R.J. Terrile, B.A. Smith, J.-P. Muller, A.P. Ingersoll, G.E. Hunt, S.A. Collins and R.F. Beebe*	776
Voyager observations of small-scale waves in the equatorial region of the jovian atmosphere	*G.E. Hunt and J.-P. Muller*	778
A lower limit on the top of Jupiter's haze layer	*A.F. Cook II, T.C. Duxbury and G.E. Hunt*	780
First results on jovian lightning	*A.F. Cook II, T.C. Duxbury and G.E. Hunt*	794
An interpretation of the Voyager measurement of jovian electron density profiles	*S.K. Atreya, T.M. Donahue and J.H. Waite, Jr*	795
Plasma wave turbulence at Jupiter's bow shock	*F.L. Scarf, D.A. Gurnett, W.S. Kurth and R.L. Poynter*	796
In situ identification of various ionic species in Jupiter's magnetosphere	*J.D. Sullivan and F. Bagenal*	798
Jupiter's magnetic tail	*N.F. Ness, M.H. Acuna, R.P. Lepping, K.W. Behannon, L.F. Burlaga and F.M. Neubauer*	799
Departure from rigid co-rotation of plasma in Jupiter's dayside magnetosphere	*R.L. McNutt Jr, J.W. Belcher, J.D. Sullivan, F. Bagenal and H.S. Bridge*	803
Voyager 1 photopolarimeter experiment and the phase curve and surface microstructure of Ganymede	*K. Pang, C.W. Hord, R.A. West, K.E. Simmons, D.L. Coffeen, J.T. Bergstralh and A.L. Lane*	804

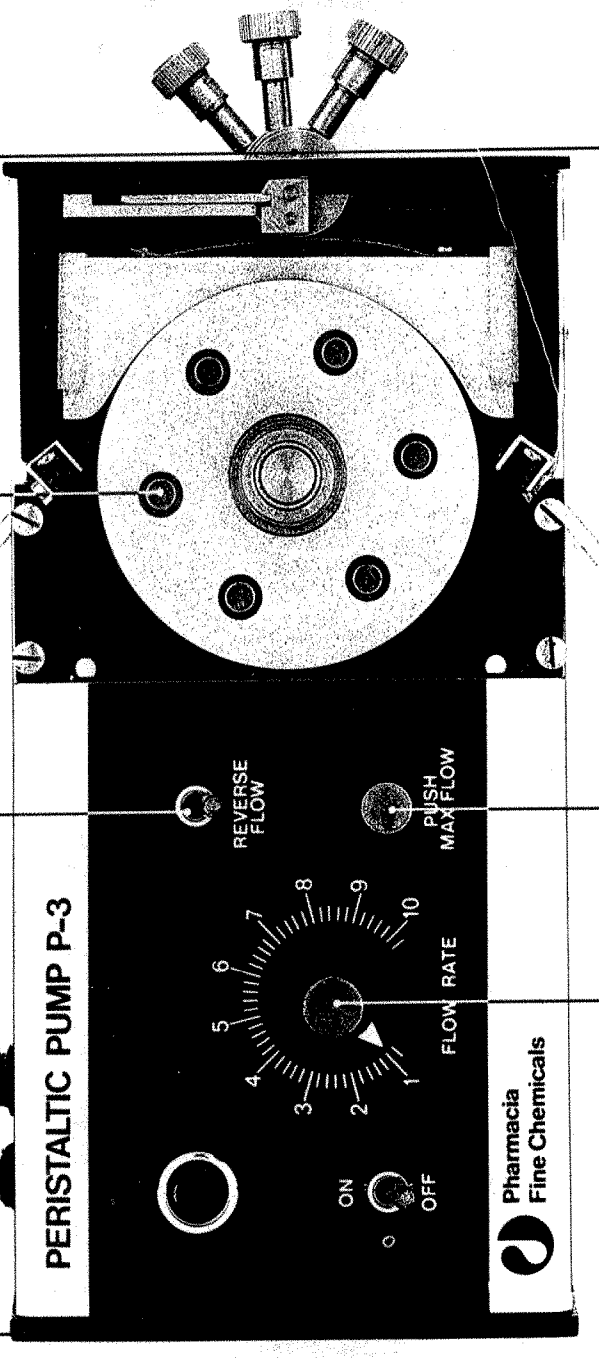
ARTICLES

Voltage-dependent caesium blockade of a cation channel from fragmented sarcoplasmic reticulum	*R. Coronado and C. Miller*	807
In vitro formation of a complex between cytoskeletal proteins of the human erythrocyte	*E. Ungewickell, P.M. Bennett, R. Calvert, V. Ohanian and W.B. Gratzer*	811
Nucleotide sequence of the gene coding for the major protein of hepatitis B virus surface antigen	*P. Valenzuela, P. Gray, M. Quiroga, J. Zaldivar, H.M. Goodman and W.J. Rutter*	815

LETTERS

Discovery of IR bursts from Liller I/MXB1730-333	*P.V. Kulkarni, N.M. Ashok, K.M.V. Apparao and S.M. Chitre*	819
Possible chemical impact of planetary lightning in the atmospheres of Venus and Mars	*W.L. Chameides, J.C.G. Walker and A.F. Nagy*	820
Photoinduced electron transfer across a water-oil boundary as a model for redox reaction separation	*I. Willner, W.E. Ford, J.W. Otvos and M. Calvin*	823
Strong electric field effects on proton transfer between membrane-bound amines and water	*R. Simons*	824
Natural atmospheric ^{14}C variation and the Suess effect	*P.P. Tans, A.F.M. de Jong and W.G. Mook*	826
Life in the calcium chloride environment of Don Juan Pond, Antarctica	*B.Z. Siegel, G. McMurty, S.M. Siegel, J. Chen and P. LaRock*	828
Texture contours can facilitate stereopsis by initiating vergence eye movements	*A.L. Kidd, J.P. Frisby and J.E.W. Mayhew*	829
The herring ear has a unique receptor pattern	*A.N. Popper and C. Platt*	832

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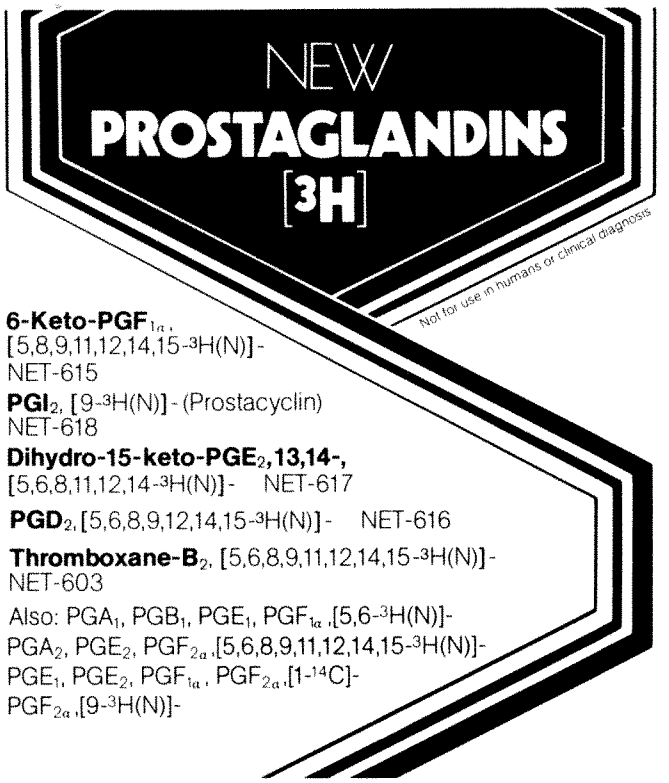
A phosphoglycolate phosphatase-deficient mutant of *Arabidopsis*	*C.R. Somerville and W.L. Ogren*	833
Possible function of juvenile hormone-dependent protein in larval insect diapause	*S. Turunen and G.M. Chippendale*	836
Evidence that murine pre-B cells synthesise μ heavy chains but no light chains	*P. Burrows, M. LeJeune and J.F. Kearney*	838
Selective labelling of the hydrophobic segments of intrinsic membrane proteins with a lipophilic photogenerated carbene	*D.W. Goldman, J.S. Pober, J. White and H. Bayley*	841
A novel coenzyme from bacterial primary alcohol dehydrogenases	*S.A. Salisbury, H.S. Forrest, W.B.T. Cruse and O. Kennard*	843

MATTERS ARISING

Variation with time of a Sun–weather effect	*J.M. Wilcox and P.H. Scherrer*	845
Reply	*R.G. Williams*	846
Anionic copolymerisation of episulphides with elemental sulphur	*A.D. Aliev, Z. Zhumabaev and B.A. Krentsel*	846
Reply	*S. Penczek, R. Slazak and A. Duda*	846
Short-range structure of amorphous alloys	*J.L. Finney*	847
Reply	*R. Wang*	847
Circular hydrogen bonds	*J.L. Finney*	848
Reply	*W. Saenger*	848

BOOK REVIEWS

Technological Decisions and Democracy: European Experiments in Public Participation (Dorothy Nelkin)	*Eric Ashby*	849
Bumblebee Economics (Bernd Heinrich)	*John B. Free*	850
Fluids in The Earth's Crust: Their Significance in Metamorphic, Tectonic and Chemical Transport Processes (W.S. Fyfe, N.J. Price and A.B. Thompson)	*Fred T. Mackenzie and J. Weertman*	851
Industrial Geology (J.L. Knill, editor)	*J.R.V. Brooks*	851
Nucleation and Growth of Thin Films (B. Lewis and J.C. Anderson)	*L. Holland*	852
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nature 30 August 1979

Dr Boyson's provocative challenge

No-one likes cuts in higher education — but if they must come, let them be selective

There was an uneasy feeling around in the British higher education community at the time of the first Conservative budget (in June) that such belt-tightening as was called for (it included the unwelcome increase in VAT) was not the end of the matter. The Government was taking its time brooding about education and science, and was not really expected to show its hand before the Autumn. Now at least one aspect of the forthcoming white paper on education has been made clear, namely that there will be a cut of up to 6% in student intake into universities in 1980. The University Grants Committee, on the recommendation of the Department of Education and Science, has alerted universities not to make 1980 commitments beyond 94% of their 1979 levels.

Dr Rhodes Boyson, the UK Minister in charge of higher and further education, has defended the proposed cuts on the grounds that Britain now has "the most expensive higher education system in the world". This seems hard to justify in the face of recently published figures which in fact show that Britain's spending on education is the third-lowest sum per capita in the Common Market (*Euroforum;* 13 July 79). In 1975 an average of £144 was spent on education per head of population compared with figures of £277 in Denmark, £239 in Holland and £209 in Belgium. Only Ireland and Italy came lower on the list.

It is clear that Dr Rhodes Boyson, minister in charge of higher and further education, is flexing his muscles to do what politicians have traditionally shrunk away from — interfere in the sacred area of a university's autonomy to teach what subjects it wishes. He is reported to have said that he would defend keeping alive all subjects currently being taught, but "in some universities, not all". It is clear that Dr Boyson would seek to impose the least restraint on mathematicians and "hard scientists" whom he regards as in short supply, but he adds ominously that "there is no shortage in certain arts subjects", and he has apparently asked officials at the Department of Education and Science to report on subject areas which have large graduate unemployment. This, of course, makes the assumption that the present-day predilections of employers should control the future of British universities.

However it is important to see these cuts in their detailed perspective — and the number of potential university students change substantially year by year. Recently the percentage of eighteen-year-olds who have been both able and willing to pursue a university course has reamined fairly static. On the other hand, the baby boom of the late 1950s and early 1960s has raised the numbers of those in the eighteen-year-old bracket by a few percent each year. These numbers will continue to grow into the early 1980s, but thereafter will show a progressive decline until by the mid-1990s there will have been a drop of 25% on the peak value in the early 1980s.

It has been a matter of discussion for some time whether universities should be restricted in their intake for the next few years in order to 'tunnel' through the peak, whether they should be allowed to expand up to the early 1980s but then be forced to contract with declining numbers seeking admission, or whether they should be allowed to expand but then encouraged to maintain peak numbers by more vigorous recruitment programmes or by extending opportunities for continuing education, or education at a later stage in life. The last Labour government seemed to be moving towards the last option, whereas the present Conservative government looks as if it is intent on 'tunnelling'. By so doing they will of course begin to close out options on continuing education.

One further piece of perspective must be added. Following the Robbins Report, there was an enormous expansion of university places in the 1960s to meet the Robbins principle that all students who wished to go to university and who were qualified should be allowed to do so. New universities sprung up and hired staff in vast numbers, thus giving the priorities of the mid-1960s enormous emphasis in the university system. There have been continuing discreet mutterings among academics about the quality of some of the departments formed and about the low standards of entry that apply in some places. So although the university community as a whole will throw up its hands in horror at Dr Boyson trespassing on their territory and daring to declare certain preferences, some thoughtful people will be quietly curious to see whether a politican will be able to pull off what has needed to be done for some time but which academics could never bring themselves to do — to recognise that the quality is not uniform throughout the British university system and to make an effort to ensure that if there have to be cuts, they do not fall uniformly everywhere.

UNCSTD: Hunger and poverty



The co-existence of the enormous potential of science and technology with the continuing poverty of the developing countries was an outrageous contradiction" which the conscience of the world should no longer tolerate, Mr Bradford Morse, administrator of the United Nations Development Programme told UNCSTD delegates last week.

"Science and technology can, indeed must be used to build a better world for posterity. Yet at the present time in almost every part of this small planet in which all of our futures are invested there is hunger, malnutrition, disease, illiteracy and despair" he said.

With UNDP frequently mentioned as one of the UN agencies expected to be made responsible for carrying out in practice many of the conference's eventual recommendations and the agency itself having indicated its willingness to do this Mr Morse's words were listened to with particular interest.

"The choice and utilisation of science and technology have fundamental implications for the pattern and direction of social and economic development in each developing country, and thus for the international distribution of economic activities" he said.

"It is therefore in the interests of the world community, while respecting the various needs and objectives of all nations, to support the efforts of developing countries to strengthen their human resources, institutions and technological capabilities so as to promote broadly-based, increased economic activities. This will inevitably strengthen the foundations for self-reliant economic and social progress, and lead gradually but surely to a more equitable basis for the progress of the world economy as a whole."

Mr Morse said that in June the Governing Council of UNDP had adopted a resolution asking him to take the measures needed, in cooperation with the organisations of the UN system, to enable UNDP to respond "promptly and effectively" to decisions taken at UNCSTD and approved by the general assembly, which may be directed to UNDP.

He had also been empowered to convene a special meeting of the governing council if it proved to be necessary to achieve this. "Thus UNDP is ready to act without delay in following-up the decision of this conference and of the general assembly if it should be called upon to do so", he said.

Mr Morse made no reference to the draft programme of action which had been put forward by the Group of 77 countries. But he described the broad outlines of what he felt the conference could achieve. These included "agreement on firm financial commitments to make an immediate practical start, and on financial targets for the substantial resource flows needed at a later stage", as well as the establishment "within a definite time-frame of the arrangements necessary to convert such targets into actual internationally supported resource commitments."

David Dickson

Iran criticises Group of 77

Iran is not impressed. In pre-revolution days the Shah's government was hopeful about the outcome of UNCSTD. The hope was to convince the developed countries to transfer technology on more suitable terms, better commercial rates, relax some of the stringent patent laws and obtain agreement for training the local manpower with all projects involving the transfer of technology. Now, according to Dr Ali Barzegar, head of the Iranian delegation, Iran believes nothing can guarantee the transfer of technology on suitable terms.

What Iran, and indeed, what the Third World needs in general, is a science and technology generating independence and not dependence on the West. "Such science and technology cannot be imported, it can evolve", says Dr Barzegar.

In Iran, Islam plays a great part in the policy, priorities and infrastructure of science and technology. How can these priorities be safeguarded with imported technology? How can that infrastructure be imported? asks Dr Barzegar.

Again, technology in Iran must enhance the cultural values of the society and allow these values to realise their full potential. How can Iran import technology that will enhance the cultural values of Iran? That technology can only be 'home grown', it must be the product of the efforts of the Iranian people.

Transfer of technology has little or no role to play in self-development. Under the Shah, Iran obtained technology from the West at an unparalleled rate. "What did it do for the people of Iran? Turnkey schemes, foreign trained manpower, financial resources, willingness of industrial countries to transfer technology — all this is not sufficient" says Dr Barzegar.

"We had all this in Iran and nothing has happened. In fact there has been a serious debt in terms of pollution, disparity between rich and the poor in Iran, and erosion of our cultural values. So, we can say with conviction that, in the final analysis the third world stands to lose from the transfer of technology."

The belief of the Group of 77 in some type of 'Japanese miracle' is a major fallacy. Even if the Third World societies stood to gain from the transfer of technology it could only be at the expense of their cultural integrity and cultural values. "Is not the Japan of today more close to America, than to its origins?" asks Dr Barzegar.

Ziauddin Sardar

●The Arab Group is presenting a strong protest note to the UN Secretary General, Kurt Waldheim, about the timing of UNCSTD. The conference clashes with Ramadan, the Muslim month of fasting.

Mr Ali Zainul Abedin Farah, a member of the UAF delegation, said that "we like to spend Ramadan with our families, fasting and worshipping". The delegates who have had to attend UNCSTD have been deprived of the blessings of one of the holiest months of the Islamic calandar.

The protest note asks the UN to make sure that such "serious errors" are not repeated in the future.

At their second meeting, the Group also decided that all discussions carried out by the members of the Group should be in Arabic. That means that contributions to plenary sessions and committee meeting from the Arab group will be made in Arabic. The Arab league came prepared for such a resolution as its paper is available only in Arabic.

The Arab Group has made repeated declarations that they stand firmly behind the Group of 77. However, some evidence suggests that there are small factions within the group and that the support for the Group of 77 demands is not unanimous.

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UNCSTD at half time: North stands firm

UNCSTD is only half way through. Yet already it seems clear that whatever emerges from the conference at the end of this week will be less a function of what the developing countries want than of what the developed countries are prepared to give them — and that this is unlikely to be very substantial.

The turning point came last Friday. Up to that point, the main focus of discussion in the working groups had been the proposal put forward by the Group of 77 that the conference should agree to establish a new financing mechanism for science and technology for development, based largely on "automatic" contributions from developed countries calculated according to a pre-agreed formula, and scheduled to reach $2 billion by 1985 and $4 billion by 1990.

The US, which would be the prime contributor to such a fund, had already indicated that they found such a proposal unrealistic. Their opposition was based not so much on the size of the fund, but the way in which contributions would be assessed. Automatic contributions, they claimed, were incompatible with congressional feelings about the aid budget. If anything, extra funds should be strictly on a voluntary basis.

On Wednesday, the Group of 77 asked the developed countries directly whether they were prepared to support an overall increase in funds for science and technology for development. A meeting of ministers from the member countries of the European Economic Community took place on Thursday afternoon to consider the question — but failed to reach a consensus. At one end Britain's Minister for Overseas Development, Mr Neil Marten, argued that the recent cuts in Britain's public spending meant there was no money left for new aid purposes. At the other, the Danes, keen to build a progressive image among certain Third World constituencies, argued that a commitment to providing new funds could and should be made.

The EEC's failure to reach agreement had an unexpected effect. When it was announced, the Tunisian delegation, which is leading the negotiations for the Group of 77 and had been receiving some internal criticism for not being sufficiently tough, walked out of the committee room and, at a hastily arranged press conference, announced that they were leaving unless things moved a bit faster.

The next day, Friday, things did begin to move — but not entirely in the way that the Group of 77 would have wished. The EEC presented an answer to their question, saying merely that the member countries budgets for technological development were growing in real terms, and that additional resources for science and technology "will be available subject to the priorities made in requests from the developing countries". (The US had already said that they were prepared to agree to providing some extra funding).

Soon after the EEC reply, however, Sweden tabled a set of proposals suggesting among other things a very different mechanism than the one proposed by the Group of 77, in the hope that it might form the basis of a compromise proposal on which everyone at the conference might eventually agree.

Central to the package of proposals, which had been prepared by the United Nations Development Programme (see page 710), was the idea that the developed countries should commit themselves to contributing immediately to a more modest fund and, together with the developing countries, work on ways of increasing both contributions and uses of the money at a later date.

This proposal, which many had been expecting, but not quite so soon, put the Group of 77 in a difficult position. On the one hand, they had not been able to convince the developed countries that their own financing demands were reasonable. On the other, it was difficult for them to show that the Sweden/UNDP proposal was unreasonable. Yet it was already known that the US had indicated they would probably support the latter: and this itself was sufficient for the G77 to be sceptical.

The Group's reaction was to play for time. The US and the EEC, which both said they found the Swedish proposals "interesting", suggested that more details should be heard from UNDP administrator Mr Bradford Morse, who was already waiting in the wings. The Tunisian delegation, still under pressure from several of the more militant G77 countries, asked for time to consider the Swedish proposals prior to hearing Mr Morse — who subsequently agreed to reappear on Monday morning (returning to Paris in the mean-time).

The mere fact that the proposal was on the table, however, and that everyone knew it was possibly the only basis for a compromise agreement, meant that the whole initiative had passed from the Group of 77 to the developed countries. Indeed it became clear that some of the more powerful of the Group of 77, who had so far played a relatively back-seat role in the negotiations, did so partly because they were more in favour of the UNDP plan, but were reluctant to be seen splitting G77 solidarity.

By Monday, the whole tone of the conference was different from the previous week. No-one was pretending that the G77's demand on the financial commitments was likely to be accepted, indeed with the first working group, concentrating on methods for building up indigenous infrastructures and the transfer of technology from developed to developing countries, progressing even slower than expected, it was becoming increasingly unlikely that the conference would produce a plan of action at all.

At the same time the central issue for discussion shifted — as the US had planned from the outset that it should — from the financial to the institutional aspects. The G77 had proposed that a new intergovernmental committee be established directly under the United Nations. The US, in contrast, argued strongly that the central co-ordinating mechanism for science and technology issues within the UN system remain under the aegis of the Economic and Social Council (ECOSOC).

On Friday the EEC, partly at the suggestion of the British delegation, who had earlier heard some harsh things spoken about their uncompromising stand on the financial issue, put forward a proposal which it was hoped would meet the demands of both sides. This was that an intergovernmental committee, open to all states who wished to attend, should be established by the General Assembly. But that it should report to the Assembly through ECOSOC (which, however, would not be allowed to do any more than add comments to the report).

At the time of going to press, it was still unclear how this issue was likely to be resolved. The EEC proposal had received support from the G77. But on Monday, the US announced that they were still adamant the committee should be appointed by ECOSOC, leading the G77 to issue a statement claiming that the UNCSTD conference had suffered "a serious setback because of the position of one major country."

The Group of 77 added that they hoped the situation would change, but that "the position at present does not bode well". In reply, the US issued a statement regretting "that some countries appear to have misinterpreted or misunderstood some parts of the US position." How much of this was pure negotiating tactics, diverting attention from more significant issues — like the role and status to be given to the secretariat which will service this committee, and whether it should be based in New York, Geneva, Vienna, or some third world country — remains to be seen.

By Monday, the two sides in the negotiations had come sufficiently close to make most observers optimistic that there would be no sudden breakdown in negotiations, and that there was sufficient interest on both sides to engineer a "successful" outcome to the conference. In the process, however, it was clear that most of the more controversial of the developing countries demands had been slowly whittled away during the negotiating process.

David Dickson

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NGOs clash at side-show meeting

The main idea behind the non-governmental organisation's (NGO) forum, according to its organiser Dr Karim Ahmed, is to bring together the many different types of NGO with an interest in science and technology for development — especially those "bored" lobbying delegates at UNCSTD. What he regards as most important, however, is not how the forum is conducted but what happens after it. "If we do not have a plan to follow the conference, all our efforts will have been a complete failure", he told the opening session last week.

Just what should be achieved and how, however, were points of fierce disagreement throughout the first week. Some NGOs, critical that the forum had no plans for making a direct impact on the main UNCSTD meeting, argued that it should prepare a statement for UNCSTD. But during discussions early on in the week, that idea was dismissed by suggestions from the organisers that the preparation of such a statement would be a waste of time and that the NGOs with consultative status can lobby UNCSTD anyway.

As a result of those discussions — together with criticisms that there was no room within the forum programme for discussion of the two background papers prepared in advance — it was agreed to devote brief daily meetings and one whole day session to trying to formulate a 'plan of action' for the NGOs after the conference. The plan of action should come up with something "at least in terms of mechanisms", said Ahmed. One idea might be a clearing-house or secretariat for managing and channelling funds.

Agreeing on a firm plan, though, is bound to be difficult because of the diversity of the NGO group. There are basically three types of NGO, says Ahmed: those representing the professional interests of scientists, mainly from developed countries, those representing development groups often working at the village level in developing countries, and those which are politically active in issues such as environment and disarmament who want to impress on the Third World the dangers of "non-benign" technology. "What do these three groups have in common? They have rather diverse interests," says Ahmed.

The group mainly representing the professional interests of first world scientists has already broken away from the rest. Before the start of the forum, the Group of 19, which was formed last year and issued a 'declaration' at the Singapore Symposium, announced the setting up of a new body with the aim of implementing the principles emerging from UNCSTD. The new body is to be based on the International Council of Scientific Unions (ICSU) and will seek funds from UN agencies for specific projects. Its precise nature is to be decided on by a preparatory meeting sometime this week.

Many of the other types of NGO view the Group of 19 with suspicion, seeing it as an elitist organisation made up of scientists who ask how their knowledge can be applied to development rather than taking the developing country approach of asking what are the problems first.

The main discussion at the NGO forum, during the first week however, did not centre on the politics of formulating concrete proposals for action. Attempts were made to discuss some of the more pragmatic problems of development such as the role of energy and environment policy and the arms race in development. But even this part of the proceedings has gone badly. Many delegates have criticised the organisation of the conference which does not allow useful debate. If the NGOs are to bring pressure on governments then they should be using the forum to thrash out specific proposals and identify areas for special attention, they argue.

Judy Redfearn

Organiser criticised but not bowed

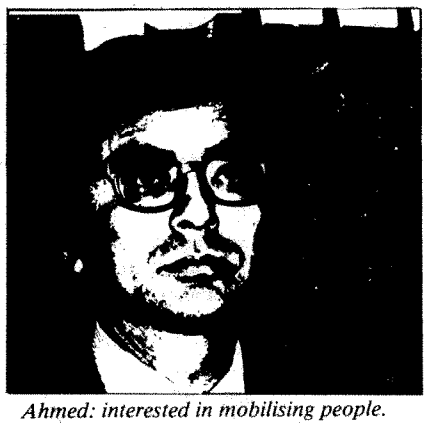
Ziauddin Sardar profiles Dr Karim Ahmed, chairman of the forum for non-governmental organisations

Next to Joao Frank da Costa, the Secretary-General of UNCSTD, Dr Karim Ahmed, the chairman of the NGO forum is probably the most hard working man in Vienna this week. While da Costa has received a considerable amount of attention, little is known about Ahmed who, after all, has just as great a responsibility.

Ahmed, a Pakistani ex-patriate, who became a naturalised American citizen last year, describes himself now as a "typical middle class American". He traces his background to Japan, where his father, originally from Bombay, was an import-export merchant. He married a local girl, and just before the Second World War broke out, took his wife and children to Shanghai where they spent four years. In 1945, the family moved to India, and after the partition to Karachi.

In Karachi, Ahmed's father was responsible for setting up Habib Textile Mills, one of the major textile mills of Pakistan. Ahmed confesses that "the mill does not belong to the family any more". But he is "well off", and the standing of his family in Pakistan is on a par with Adamjee, Dawud and other textile industrialists. In religious affiliation he belongs to the Memons, a pious and strong group of Sunni Muslims.

Ahmed did not follow his father into the textile industry. "My interests were more academic", he says. He graduated from the University of Karachi and then decided to pursue his higher studies in the United States. After obtaining a masters in chemistry from the University of Minnesota, he moved to Harvard where he was awarded a doctorate in biochemistry in 1969.



Ahmed: interested in mobilising people.

During his student years, Ahmed developed a keen interest in radical issues. He worked for years as a volunteer for black organisations, and during the mid-sixties with Ralph Nader's public interest research group.

"I have always been conscious of social aspects of science and technology", he says. His first major research project was a lead-poison screening programme which assessed the potential impact of lead poisoning on the black community. Ahmed says proudly that "it lead to many city ordances". He now spends most of his time investigating the environmental impact of technology as a research director of the Natural Resources Defense Council, a cooperative venture between lawyers and scientists.

Ahmed's involvement with the UN began only two years ago. "I have always been interested in mobilising people" he says. He saw the NGO forum as a challenge and "went out" for its chairmanship.

There has however been considerable criticism of Ahmed's handling of the forum. He accepts the criticism as "symbolic", saying "there are some people who must criticise", and considers that most of it is based on a misunderstanding of the role of the NGO forum. "We are not a counter-conference to UNCSTD", he says. "Neither are we a political group. The purpose of the forum is to allow as many NGOs as possible to meet and discuss the important issues". But he hopes that some of them will organise joint ventures and carry them out after the conference. □

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India demands "sharp break" from current practices

In a strong statement that reflected many of the demands being made by other member countries of the Group of 77, the head of the Indian delegation to UNCSTD strongly criticised the way in which current forms of assistance from the developed world tends to increase the dependence of the developing world and to accentuate disparities in scientific and technological capabilities.

"A sharp break from this pattern so as to lead to a better sharing of the capabilities and to a lessening of global income disparities is called for" Dr D I Lakdawala, deputy chairman of India's Planning Commission, told the conference.

The experiences of two International Development Decades had led to a realisation among developing countries that the present structure of science and technology could not fulfil the types of promises that had been made earlier, such as providing growth with social justice and fulfilling minimum basic needs, he said.

Pointing out that a science and technology plan had been a central feature of Indian economic planning in recent years, Dr Lakdawals said that the Indian experience stressed, in particular, the need for a proper delivery and extension system to ensure that the fruits of science and technology became widely available.

"It also underlines the need for a proper infrastructure and appropriate economic policies and fiscal incentives to encourage application of scientific and technological discoveries on the farms and in the factories. And in this task developing countries can greatly benefit from mutual cooperation," he said.

In the past, a purely sectoral approach to issues such as technology transfer or access to information had often failed to develop endogenous capability in science and technology and to bring a proper share of benefits to developing countries.

It is essential that in future the selection of sectors and programmes and the methods of their execution must fully reflect the preferences of the developing countries concerned. The policy should be to build up full endogenous capabilities so that in the name of immediate benefit, dependence is not perpetuated and self-reliance is fostered."

It was along these lines that the countries of the Group of 77 had constructed the draft programme of action being debated by the conference, to which India had given full support. □

● India's efforts on primary health care — see page 716



Happy home-coming for cosmonauts Ryumin (left) and Lyakhov

Soviet spacemen return to Earth

Long-term orbital missions such as that completed recently by cosmonauts Vladimir Lyakhov and Valerii Ryumin, will be the mainstay of the Soviet manned space programme for the foreseeable future, according to a recent statement of Academician R.Z. Sagdeev, Director of the Institute of Space Studies. Priority will be given, he said, to establishing how long a cosmonaut can remain in orbit without damage to his health — an important preliminary to the establishment of the permantently-manned orbital stations predicted by Tsionkovskii.

Not surprisingly, the programme of the mission has placed considerable emphasis on physical and psychological health. Prophylactic measures ranged from the use of the Chibis pressure-suit to simulate the cardiovascular tone of normal gravity to the visit of pop-singer Valentina Ignateva to the mission control centre to sing to the crew. No medical surprises were expected from the mission; on a previous mission there was considerable excitement when the 120-day deadline was passed, since this is the lifespan of red corpuscles. According to one of the medical team, Dr Anatolii Egorov, this time it was simply a matter of collecting statistical data of the body's reaction to gradually extended periods of weightlessness. Owing to the crew's "exceptionally conscientious" attitude to exercise, their health remained excellent.

The experimental programmes of the mission took the now standard forms — aserophysical and geophysical observations, "Zero-gravity technology" including a new aluminium-spraying experiment, and biological and genetic experiments. Towards the end of the mission, they assembled an orbiting radio-telescope, inaugurating, in *Pravda* words "a new stage in space research, that of extra-atmospheric astronomy".

Ironically, however, while the space-biologists seek ways to extend the length of manned missions, there are hints that certain planners would like to reduce the human involvement. In a radio interview, one of the designers of the radio-telescope, identified only by name and patronymic as Valentin Petrovich, said that in the future all such equipment would ultimately be automated, although "in the first stages of development . . . the role of man is very great". Likewise, Dr Konstantin Feoktistov, a frequent spokesman for the programme stressed that the majority of the crew's time is taken up with communications, routine housekeeping and maintenance, medical checks and the need to keep fit leaving little time to carry out the experiments which are, presumably, the main object of having a space station at all. "It follows that more use should be made of automation", he said, as far as the experiments are concerned, with the crew simply "correcting the programmes, repairing and servicing equipment, and so on".

Vera Rich

UK tram lovers book Shuttle space

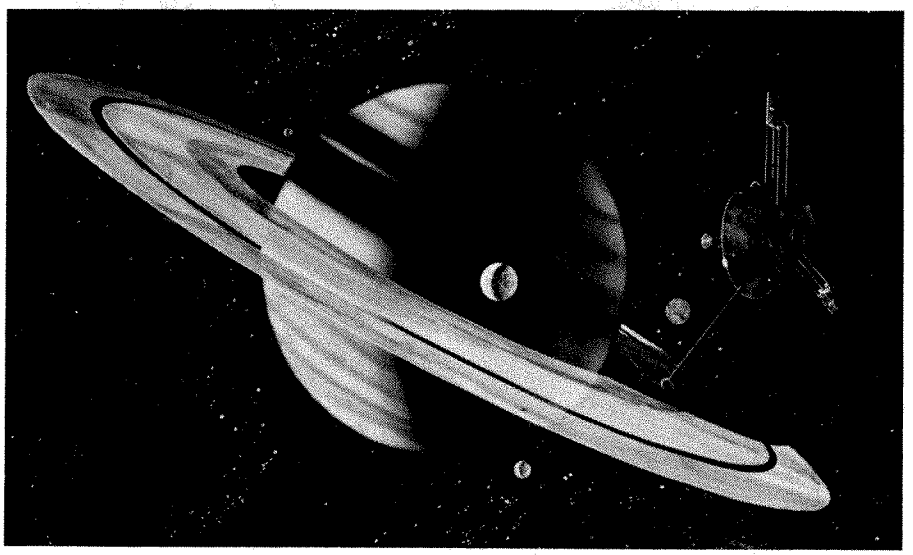
A call from the Tramway Museum in Crich, Derbyshire, has proved unusually fruitful for Professor Eric Laithwaite of Imperial College, London. Enthusiasts there have booked room on board a 1982 Space Shuttle flight and given their 1.5 cubic feet of space to Professor Laithwaite. "It's like a big birthday present", he said.

He will be designing an experiment to observe the behaviour of a precessing gyroscope in zero gravity. This, he believes, may show that inertial mass and gravitational mass were not the same and would demonstrate an effect that could have enormous benefits in developing new propulsion systems.

The museum has booked the space on the Shuttle as a way of publicising itself and of celebrating the centenary of electric traction. □

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Pioneer may collide with Saturn's 'E' ring



By John Douglas, California

AT 14.35 GMT tomorrow (1 September), the US Pioneer 11 spacecraft will cross the plane of Saturn's rings at an extremely shallow angle, and perhaps find out the hard way whether material in the planet's famous rings is spread out farther than appears on photographs taken from Earth. Because of the time taken for its radio signals to reach Earth, mission scientists waiting anxiously at Ames Research Center in California will not know for more than an hour whether the spacecraft has survived its 0.8 second passage through the ring plane; but the outcome will instantly resolve a long-standing debate over mission strategy — and at least a few private wagers.

There are three clearly visible rings surrounding Saturn: from the outermost inwards they are designated A, B and C. In 1969, some four years before the launch of Pioneer 11, Pierre Guerin discovered a fourth ring (D) lying even closer to the planet's surface. Mission scientists had originally voted 12 to 1 to have the spacecraft pass through the 'Guerin Division' between the C and D rings.

The decision, however, caused a conflict with scientists at the Jet Propulsion Laboratory, whose more sophisticated Voyager spacecraft will be flying by Saturn in November 1980 and August 1981. As its name implies, the Pioneer mission is a relatively low-budget scouting expedition, and JPL scientists wanted to make sure that the subsequent Voyagers would be following a safe path through potentially hostile territory. (Such lapses into the terminology of the Old West are understandable among scientists at Ames, where the laboratory facilities have been built on the site of an old stagecoach stop.)

The dispute was finally settled by officials at NASA headquarters in Washington, who decided to forego the advantages of having Pioneer pass through the Guerin Division only 14,400 km above the planet's surface. They estimated there would be too much chance of encountering material within the division. Instead, they targeted the spacecraft to cross the ring plane at the presumably safe distance of 112,000 km, well outside the A ring which extends to only 77,400 km. After passing through the ring plane for the first time, Pioneer will approach within 21,400 km of Saturn's southern hemisphere, then cross the ring plane again at the same height.

Although this plan is still in effect, new data have revealed that even this 'outer option' may not be as safe as originally anticipated. Radar measurements indicate that there may be substantial material extending as far as 343,000 km from Saturn's surface, in a band now being tentatively called the E ring. This material does not appear strongly on ordinary photographs, probably because the E ring is composed of widely scattered large chunks, which can be more easily detected by radar.

When asked whether he thought the Pioneer 11 spacecraft would survive both its passages through the ring plane, chief mission scientist John Wolfe estimated the chances of survival at about 50%. If the spacecraft should hit a sizeable chunk of debris as it passed the rings it could well be destroyed, he said, but more than three-quarters of the experiments intended for the mission would already have been completed. In particular, Ames scientists would have photographs of Saturn with a resolution a factor of 10 better than can be obtained with the best Earth-based telescopes.

If Pioneer 11 does not survive its crossing of the ring plane, the greatest loss to the mission will be the proposed encounter with Saturn's principle moon. Titan, the largest known planetary satellite in the solar system, has a thick atmosphere, and there has been much speculation over whether it may be able to support life. Pioneer 11 is not instrumented adequately to fully answer this question, but it has been designed to make some relevant measurements.

In particular, Pioneer instruments should be able to characterise Titan's atmosphere, by determining its opacity, and the composition and layering of its aerosols. Previous Earth-based observations have revealed that Titan's upper atmosphere contains an orange-coloured layer of smog, which could be due to organic compounds. Pioneer's measurements of particle bombardment will indicate whether methane in Titan's atmosphere is being dissociated in a way likely to form organic polymers. The presence of ammonia will also be sought, since nitrogen is a constituent of the reddish organic polymers thought to exist in the smog layer.

Finally, the spacecraft will measure the heat balance of Titan. The surface of the satellite is thought to be too cold (perhaps —193°C) for life to survive, but it has been suggested that there may be a warm layer in the atmosphere, caused by the greenhouse effect or volcanic action, where the evolution could have occurred.

At the very least, the Pioneer flyby of Saturn should produce some spectacular photographs of considerable scientific interest. Because of the spacecraft's speed, the rings will photograph only as white blurs, although other instruments on board may determine the size of the chunks of rocks and ice which constitute the rings. If Pioneer survives the passage through the ring plane, there should also be a startling visual effect as the rings suddenly reverse in brightness. During its approach, Pioneer will view the rings against the Sun, and they should appear dark, with the gaps bright because of scattering by the small amount of material resident there. After the ring plane passage, the rings will suddenly revert to their normal appearance.

Whatever happens during the crucial encounter with the ring plane, Pioneer will have lived up to its name, for JPL scientists will almost certainly use the findings of this preliminary mission to target the Voyager spacecraft. Meanwhile, Ames scientists have begun to argue that such pathfinder missions will continue to be valuable. Only four months ago, NASA headquarters announced that future planetary missions would be handled entirely by JPL; but since then the top administration of the agency has shifted and the new administration has stated that no laboratory will be automatically excluded from any future missions. It is now reconsidering Ames approach of low-budget exploratory missions as a viable option for future interplanetary exploration, particularly in view of the team's record in successfully controlling costs. The Saturn encounter may well provide the needed impetus for continuing this approach. □

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news in brief

Watches warning: Stricter control of some digital displays for liquid crystal watches has been urged by the UK's National Radiological Protection Board following publication of its first consumer products report. The board began testing devices which could expose the public to radiation in 1976 and its report concentrates on two main devices — ionisation chamber smoke detectors and liquid crystal digital watches that contain gaseous tritium light sources. Most of the smoke detectors, were found to be satisfactory, and where problems were detected, the board reports that these have now been identified and solved. However, in some watches, the tritium light displays — which eliminate the need for batteries — were found to leak "significant" amounts of tritium when subjected to fairly violent tests. In some cases, this reached as much as 10,000 times the recommended limit for radiation emission. The board described this as "clearly unacceptable", although in reality it is unlikely that watches would be subject to the tests' stringent criteria. Nevertheless the board urged that greater care be taken in manufacture and added that in cooperation with the Federal Office of Public Health in Switzerland — where many of the light displays are made — a new set of guidelines was now being drawn up.

Mystery leak: Reports of a leak of radiation apparently emanating from the Israeli Embassy caused the London Fire Brigade's Chemical Incident Squad to go into action last week. When they reached the scene, however, the radiation had stopped. The radiation burst was picked up by monitoring apparatus on a fire appliance at the Old Court Place fire station next to the embassy. After two hours, it stopped, but resumed again for a short time later.

An embassy spokesman said they were "totally mystified" by the incident. There was nothing in the embassy that could be a source of radiation. The only possible culprit, a small X-ray machine used for examining suspect parcels, had been checked, and was found to be in good order. Fire Service headquarters at Lambeth were unable to comment on the type of radiation discovered. A Home Office scientist, however, told *Nature* that the only radiation-sensing devices carried on fire appliances were gamma-ray dosimeters and survey meters.

Helium supplies in danger: The US Department of Energy spends $400 million a year on research into helium related technologies, and in hearings before the Subcommittee on Energy and Power of the Committee on Interstate and Foreign Commerce, helium was touted as the key to a "supertechnology" which could help mitigate the energy shortage through its use — as a liquid — to cool metals to superconductive state.

However, the supply of helium is in danger. Scientists said some 13 billion cubic feet of helium are released into the atmosphere each year as a waste product of natural gas extraction. The National Research Council estimates that at this rate, US supplies of helium (approximately 718 billion cubic feet) will last only another 30 years. The subcommittee wants Congress to pass the Helium Energy Act to enforce the conservation of helium.

Carcinogen data criticised: In an editorial in the Journal of the American Medical Association, the editor, Dr William Barclay, criticises scientific reports of carcinogens and the tests on which they are based. He accuses the media of encouraging "the chicken little phenomenon", heightening public anxiety by a constant barrage of news stories citing substances in general use as carcinogens, based on evidence which "is often tenuous and unconfirmed". Dr Barclay also castigates the methods of testing these substances, on the grounds that dosages used often exceed any that man might be exposed to. He also claims that the results of tests are finally evaluated by "persons of questionable expertise in the field of tumour histopathology".

UK chemical companies get drug warning: Chemical companies throughout Britain were being warned by the Royal Institute of Chemistry last week about some of their products that have been revealed as basic ingredients of a powerful hallucinogenic drug, known as BZ, which is said to be 100 times more potent than LSD. The move follows a campaign by the National Society for Crime Reduction and Social Justice — who are sponsored by the Church of Scientology — who have called for Government restrictions on the sale of the chemical ingredients of BZ, or 3-quinuclidinyl benzilate as it is properly known, and its inclusion within the Misuse of Drugs Act. The society says that the synthesis procedure for BZ is at present freely available from the British Patents Office and can be manufactured for less than £100. The group also wants the Ministry of Defence to release details of its experiments using BZ on soldier volunteers to provide public information about the drugs' lasting effects. They claim that BZ was originally developed in the US and tested as an incapacitating agent by the military and the CIA.

Pugwash council urges SALT ratification: The council of the Pugwash conferences has urged the rapid ratification of the SALT II nuclear weapons treaty by the US and Russia. "This step is essential for the avoidance of nuclear war and for enhanced international security", urges a prepared statement released after the council's recent meeting in Mexico City. Although it admits the treaty does not halt some strategic nuclear weapons now being developed, the council stresses that SALT II opens the door to further lowering of the level of nuclear confrontation and substantial cuts in nuclear weaponry. "In the interests of world peace, we urge the earliest ratification of SALT II and the immediate beginning of negotiations on SALT III", it concludes.



Three tears for the crocodile: The Indian government has launched a massive programme to save the crocodile from extinction, following an investigation by Dr H.R. Bustard of the Food and Agriculture Organisation into the declining population of the three varieties of Indian crocodile. One effect of this decline is a sharp rise in the population of predatary varieties of fish like the catfish which has led to a decreased yield of edible fish.

The Indian Government has therefore undertaken, on a priority basis, a project for crocodile breeding at several centres, including Hyderabad and Madras in the south and Orissa and West Bengal in the east. About a dozen officers are now being trained in the technique of capturing reptiles at the Central Crocodile Breeding and Management Training Institute, Hyderabad. When the hatchlings have grown to a length of about three feet, they are released into a reservoir. The Orissa Government has inaugurated a project for rearing gharial (above), one of the crocodile varieties, at the village of Tikerpada, with two villagers as guards. The nearby Sartkoshia Gorge area, of about 950 sq. km, has been declared a crocodile sanctuary. Another sanctuary is being set up t the Similipal tiger reserve, where again it is hoped to breed crocodiles in captivity.

The need for sanctuaries underlines the critical position facing Indian crocodiles. Quite apart from its skin, used for making shoes and fancy bags, crocodile meat is a valuable commodity, for Indian tribesmen believe it to be a cure for asthma.

from Javid Hasan in Bangalore

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Barefoot doctors: symptom not cure

Anil Agarwal continues his assessment of Janata's effect on science in India

POSSIBLY the greatest achievements of the Janata government lay in its efforts to redirect attention towards rural health care. Soon after the government took over office in April, 1977, Mr Raj Narain, the then Janata Health Minister, announced a new policy for health care. An official report from his Ministry pointed out that despite the fact that India had built up a network of 5,300 primary health centres (PHCs) and 37,000 sub-centres in the rural areas, "when judged by the three simple yardsticks of literacy, life expectancy and infant mortality, our country comes low down in the 'Physical Quality of Life Index'.

Presenting his new policy, Mr Narain explained that the Janata government would for the first time try to "put people's health in people's hands". "My own conclusion," he said, "is that one of the principal reasons behind this (ill-health in villages) is inadequacy of people's participation in the health care programme. They have not shown the necessary enthusiasm for it. They have been almost indifferent."

The new government proposed that each village with a population of 1,000 would select its own community health worker (CHW). This person would be trained for three months in elementary health care, including traditional medical systems, during which he would receive a stipend of Rs200 per month. After his training, the CHW would work in his village and receive from the government Rs50 per month as an honorarium (not a salary) for his services to the community. He would also receive from the government medicines worth Rs50 every month which he would distribute free.

The CHW was thus expected to become, as Indian officialdom put it, a "people's health guard", a representative of the community on health matters, responsible only to his community. Though he would provide first aid and dispense a few medicines — a responsibility important in establishing his credence in the community — he would essentially be an agent for health education, inculcating better nutritional practices and mobilising the community on such issues as environmental sanitation, immunisation and family planning.

As such the new scheme was not based on any new ideas. The previous government had at various times appointed various committees to report on rural health care; and the Srivastava Committee appointed in 1974 had recommended rural health care schemes based on community participation. But few of the past Health Ministers, even the most erudite amongst them, ever showed enough courage to brush aside the objections of the powerful medical establishment. Mr Raj Narain (often described as a "buffoon" by Indian political commentators for his rustic behaviour) rushed ahead with his scheme nevertheless. A senior Health Ministry official told me: "All he knew was that something had to be done to change the existing situation and it had to be done fast. He knew nothing about how it ought to be done. But he was not prepared to listen to the vested interests in the medical establishment either."

In just six months of Mr Narain's tenure, his Ministry launched the world's biggest scheme to train 'barefoot doctors' outside China. In the first phase of the scheme, villages under 777 primary health centres were chosen. The PHC doctors were expected to train community health workers in batches of about twenty. This phase ended in September 1978, and in the next phase some 1056 more PHCs were chosen. By December 1978 some 80,000 CHWs had been trained. The final aim is to train over half a million CHWs by the end of the current five year plan (1978-83) to serve each of the 560,000 villages of India.

Objections from the medical profession

The CHW scheme has attracted considerable criticism: some critics are hostile to its very aims, while the others are sympathetic to its aims, but doubtful about its achievements. The first type of criticism has come mainly from the powerful lobby of doctors. Professional organisations like the Indian Medical Association claim that the scheme will only legalise quackery. They feel that CHWs will do great harm — possibly even increasing drug resistance amongst disease organisms — by handing out medicines in inadequate dosages. In any case, they argue, India has a vast number of unemployed doctors and the government should first plan schemes to employ these.

The pressure to create more government jobs for such doctors is obviously very high, but the professional organisations seldom seem to realise that doctors are not prepared to work in villages — even if they were paid a salary ten times that of the CHW's honorarium. They have never suggested a sensible scheme for rural health care that would be within the ambit of the country's meagre resources. Positions for doctors in many PHCs remain vacant despite the high rate of unemployment.

Those who are sympathetic to the scheme point out several flaws that can be partly attributed to the speed with which it was organised. The training of CHWs has been left to PHC doctors who have in the past seldom shown any interest in rural health problems. These doctors are also expected to teach the CHWs about traditional medical systems, about which they know nothing. CHWs are taught everything from Western medicine to Ayurveda and homoeopathy. But without a proper integration of Western and traditional medicine, the course has become a confusing pot-pourri. Not only does the CHW's training need to be improved, but research must also be conducted to standardise his technology.

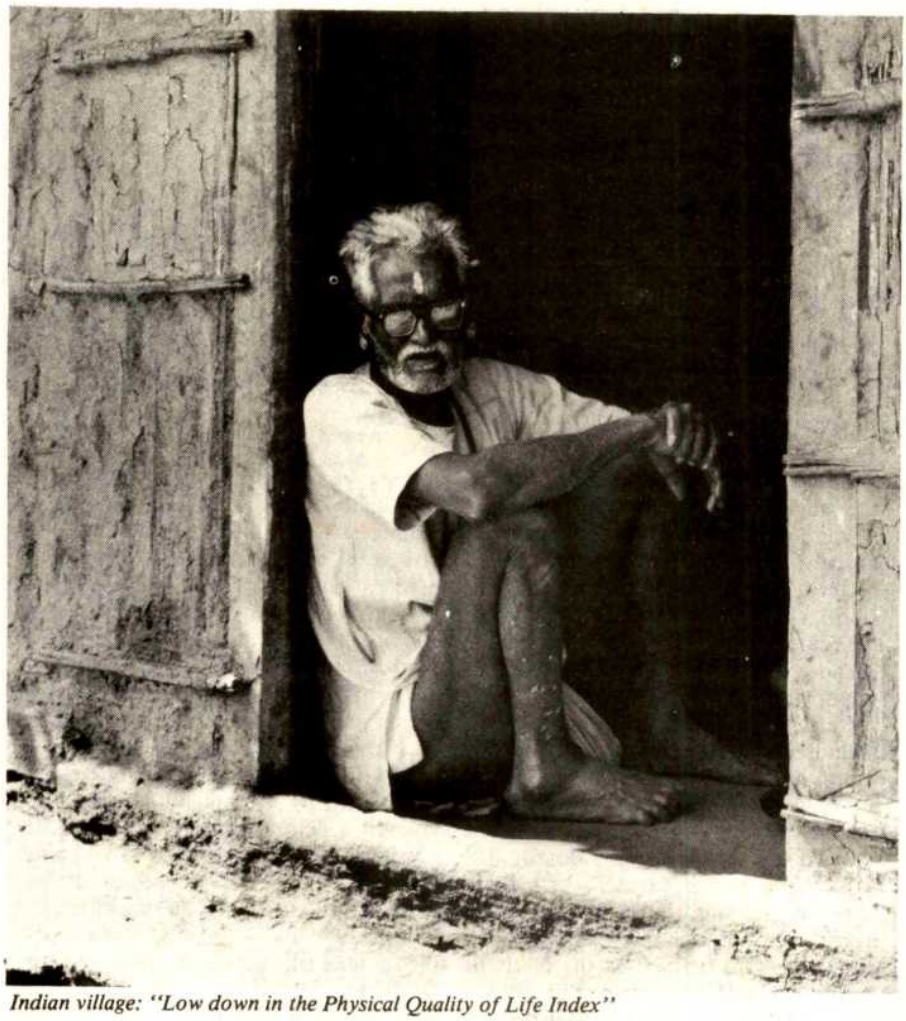
But the more important questions that such sympathetic critics have raised draw attention to the highly stratified socio-economic nature of the Indian villages in which the CHWs have to operate. The idea of CHWs in India has clearly been helped by the success of their counterparts in China. But China has a different, and relatively egalitarian, social structure. Will the Indian CHWs actually serve those who need them most?

Even before the scheme began, Dr Debabar Banerji, Professor of Community Health at the Jawaharlal Nehru University in New Delhi, pointed out that it would benefit mainly the rich farmers who dominate the village councils. Though in theory it was an excellent idea to let the community choose its own health worker, in practice rich farmers would ensure that their own kith and kin were selected. Subsequent studies have confirmed this prediction. The poorest of the poor in Indian villages are the scheduled castes (untouchables). A survey conducted by Dr Ashish Bose of the Institute of Economic Growth, New Delhi, shows that in several PHCs the proportion of CHWs coming from scheduled castes is much lower than the proportion of the scheduled castes in the population as a whole.

Health planners also failed to appreciate at the outset how great an impact the high rate of unemployment in Indian villages would have on the selection process. Though the CHW honorarium was kept deliberately small to attract only motivated people, even this sum of money has aroused the interest of many unemployed young educated people, particularly sons and nephews of the dominant farmers. An extensive survey of 76 PHCs organised by the National Institute of Health and Family Welfare (NIHFW), revealed that as many as 8.5% of CHWs were graduates or post-graduates. Interviews with CHWs revealed that many of them think that the health scheme is basically a job-creation scheme, and that with experience they would probably be employed by the PHCs directly. Such individuals are certain to be disappointed soon, and are likely to move on to more profitable occupations when they can find them. Since the CHW scheme could be endangered if unsuitable candidates are appointed in large numbers, the Ministry of Health has now changed its

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Indian village: "Low down in the Physical Quality of Life Index"

guidelines for the selection of CHWs, to include only candidates who have an occupation, are preferably above the age of 30, and are not highly educated.

The profile of the CHWs actually varies from one area to another depending on its socio-economic characteristics. In extremely underdeveloped hill regions like Meghalaya, where communal tribal structures still exist, the CHWs have been chosen not by village councils but by meetings of the entire village, and here a much larger proportion are women: as women seldom leave their village, they are expected to make good CHWs. In West Bengal, a highly politicised state with a Marxist government, the selection of CHWs has involved political parties. Several CHWs here are reported nevertheless to be particularly conscientious.

Dr Bose's survey of Punjab and Haryana shows that the green revolution prosperity, which has otherwise increased the power of the big landowners, has actually had beneficial effects on the CHW scheme. The first batches of CHWs in this region were certainly dominated by the nominees of the rich farmers who were attracted by the Rs200 per month stipend during training — a large amount even for this prosperous area — but they soon realised that their regular remuneration after training would be only the meagre sum of Rs50 per month. The second batches have, therefore, been marked by a lack of response from the richer families.

This survey has also shown that the proportion of beneficiaries who come from scheduled castes is greater than the proportion of the scheduled castes in the total population. This is because the richer families in these states can afford private physicians and are therefore not interested in the medicines of the ill-trained CHWs. But in the poorer, more stratified, heartland of central India, it is more likely that both the appointments for CHW positions and the benefits of free medicine will go to the more privileged sections of the village community. A Ministry of Health report shows that in the relatively backward state of Uttar Pradesh, village leaders have taken an active interest in the selection of CHWs, often with very acrimonious results which have led to litigation. The NIHFW survey further reveals that over 20% of CHWs selected in this state were extremely young, between the ages of 15 and 19.

Another important question raised by sympathetic critics concerns the CHWs' role: are they supposed to be pill-pushers — some kind of second-grade doctors — or mobilisers of the community, encouraging and organising it to act on its own health problems? The aim of the Indian government was to put people's health in people's hands through this scheme. Most primary health care pioneers like Zafarullah Chowdhury in Bangladesh, David Werner in Mexico, and Carroll Behrhorst in Guatemala, have also come to realise that health is determined by a complex set of socio-economic factors. Pills can do little to alleviate ill-health conditions that essentially arise from malnutrition and poverty. They have, therefore, slowly relegated curative medicine to a low priority and gone on to organise co-operatives to create productive employment, to establish grain banks for food security, and to ensure enforcement of land distribution laws.

Take scabies, for instance, a skin disease which is widely prevalent in India. The main cause of scabies is lack of personal hygiene, which in turn can be ascribed to a scarcity of firewood for heating water. A good health worker will, therefore, not just distribute an emulsion to treat the infection, but also educate people about the importance of cleanliness and mobilise them to organise fuelwood plantations.

Second-grade village technocrats?

But the Indian CHW is mainly a pill-pusher. The NIHFW survey shows that many members of the community, including the CHWs themselves see their main job as distributing pills, and most of the CHWs want to extend their role as minor village doctors by learning to give injections and use the stethoscope. Furthermore, the dominant social groups would certainly raise serious obstacles if any of them were ever to make any attempt to mobilise people. The Indian CHW is, therefore, unlikely to become a radical agent of change. He will, on the contrary, just become an extension of the medical bureaucracy, a kind of second-grade village technocrat, capable only of dumping bleaching powder in village wells to chlorinate and disinfect them instead of organising the community to observe better environmental sanitation. As the new scheme fails to address itself to any of the real and difficult issues that determine ill-health in Indian villages, it is the "same old medicine", according to Dr Banerji.

In fact, by organising such a programme of front line workers only for the villages, and not for the towns, the government is open to a charge of double standards, that it is setting different, and lower, standards of medical care for the rural poor — a charge, curiously enough, made most stridently by professional organisations, which are against the scheme completely.

But if it is accepted that Indian CHWs are going to be just pill-pushers, how good will they be at that job? One indication comes from the National Malaria Eradication Programme (NMEP), which has been using the CHWs extensively to distribute chloroquine tablets and collect blood slides. The NMEP's own malaria workers cover 10-15 villages with a total of

10,000 population. They are rarely able to visit a family more than once a fortnight, and considerable transmission of the disease can take place before they locate a case.

But the CHW is always present in the village. Dr S.M. Pattnayak, Director of NMEP, claims that in parts of Gujarat each CHW has reported 40 malaria cases a year through the blood slides he has collected, whereas the other health worker reported only 30. The CHWs also distribute chloroquine tablets as a presumptive treatment — anyone with fever first gets chloroquine. By launching this massive holding operation to control malaria, NMEP has been able to reduce the incidence from 6.4 million cases in 1976 to 4 million in 1978.

The Ministry of Health officials claim that CHWs have also been particularly useful in providing treatment for minor ailments like scabies, cough, fever and diarrhoea; in distributing eye ointments; in disinfecting drinking water wells by chlorination; and, in some areas, in motivating couples to use family planning methods. They turned out to be very useful in West Bengal and Bihar during the major floods of 1978, claim ministry officials.

Harmful side-effect of free medicine

Professor Banerji says that critics must appreciate even this limited usefulness of CHWs. According to him, the Indian CHW programme, despite its limitations, is "an even more pioneering effort than that of China. The Indian bureaucracy itself has provided an unprecedented opportunity to bypass the medical establishment, break its stranglehold, and reach the people directly. That became possible in China only in the post-revolutionary period. Here, this has happened in the pre-revolutionary period itself." Interestingly, Dr Banerji still remains an important critic of the scheme. His initial criticism had even prompted the Indian government to request the World Health Organisation to keep him off its expert committees.

The future of the CHW scheme, nonetheless, remains open. Dr Bose fears that the CHWs' success as pill-pushers could prove to be their undoing. The more successful they are in distributing free medicines, the greater will be the demand for them. The government will then be expected to supply more free medicines, or else risk disappointment with the CHWs. But not even the richest government — least of all the poor Indian government — can afford to distribute free medicines to its entire population. CHWs will then have to get the community to contribute at least partially towards their own health care. But will they? The NIHFW survey shows that nearly two-thirds of the CHWs believe that the village councils will not be prepared to do so. The annual cost to the government of just maintaining the current meagre honoraria and supply of medicines will be substantial once the scheme is complete, amounting to over Rs650 million.

There will always be powerful lobbies to see even this expenditure reduced. In fact, if pressure on the central government to allow states greater autonomy in financial allocations succeeds, the CHW scheme could suddenly find itself in trouble, for many state governments remain lukewarm towards it. A recent report in the *Times of India* quoted a senior Punjab government official as saying that his state would soon stop the CHW scheme. The statement was later denied, but this incident does show that financial decentralisation, though a good development in itself, could pose a threat. The end of the CHW scheme would indeed be unfortunate. As 'critic' Dr. Bose put it: "where is the alternative to it?" □

Politics of popping pills



THOMAS H. JUKES

After each vitamin was discovered, the obvious task lay ahead of finding out how much of it was needed per person per day. These evaluations have continued through the years, and have included consideration of individual variation in needs. One would not expect the requirement to exceed greatly the amount of the vitamin obtainable from a diet containing a wide variety of typical foods. My early rule-of-thumb, in the 1940s, was that the daily requirement of a vitamin for a human being was roughly in the range of the amount needed per kilo of diet by young animals for maximum growth. For example, this amount is about 1.5 mg for thiamin and 2.5 mg for riboflavin. A percentage may be added to take care of a higher need by some people because of normal biological variability in populations.

Refined methods of measurement, including studies of blood levels, tissue saturation and excretion rates in human subjects are used by the Committee on Recommended Dietary Allowances of the Food and Nutrition Board, US National Research Council, in making recommendations. In it Report, issued every four years, the recommended allowances are defined as the levels of intake adequate to meet the known nutritional needs of practically all healthy persons. The allowances therefore exceed the needs of most people. The Report is useful and valuable as a condensed treatise on nutrition.

In recent years, self-dosage with vitamin pills has become a way of life for many American consumers. The practice is believed by some to produce 'super-health'. The head of the US National Cancer Institute was quoted in *Medical Tribune* recently as saying that he took 'a multiple vitamin preparation' to prevent cancer. No scientific justification was offered for this act of faith. Indeed, certain sceptics, including myself, think that the main effect of such dosage is to enrich urine with vitamins, and to line the pockets of vitamin sellers with dollars. The money involved has led to vitamin pills becoming a political issue. Legislation was specificically enacted by Congress in 1975 to prevent the FDA from restraining the sales of over-the-counter high potency vitamin preparations.

High vitamin allowances were criticised recently in a book by Dr David Reuben, a physician whose biography lists his professional background exclusively as psychiatry. He described the National Academy of Sciences as 'just a nifty little private business, organized and owned by food manufacturers and vitamin sellers. It hires professors of nutrition to decide how much vitamins and minerals they think we should be gobbling and then pays them big salaries'. On 20 July, the Academy, unamused, filed a libel suit against Reuben and his publisher, Simon and Schuster. One wonders how Reuben came to be accepted by Simon and Schuster as an authority on the origin of recommended allowances for vitamins.

If indeed the Food and Nutrition Board had proposed overly *high* allowances, this would certainly have benefited vitamin manufacturers, who are currently enjoying the bonanza that has followed the high-vitamin-C boom. But the Board has been repeatedly accused by the 'health food' industry and other megavitamin enthusiasts of setting the allowances too *low* as a niggardly attempt to hold back the benefits that are alleged to result from high dosage with vitamins. How sad that the conscientious efforts of scientists should be denigrated by venal and incompetent critics! Gloomily, I await the fulfilment of the axiom that any publicity is better than no publicity, and the effect thereof on the sales of Reuben's output.

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news and views

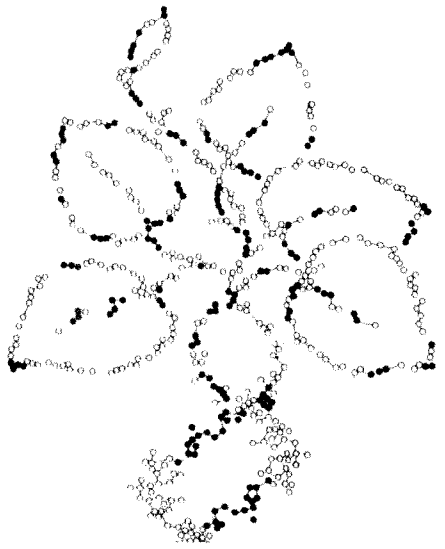
Plant molecular biology comes of age

from Wayne M. Becker

PLANT molecular biology is rapidly coming of age, or at least such was the consensus of 160 plant scientists from several dozen countries who attented a recent workshop on Genome Organisation and Expression in Plants*. The workshop underscored the number of highly qualified young investigators now active in this field, the technical elegance of much of the work, and the rapid progress being made in several areas of genomic analysis and expression in plants.

Probably the single most noticeable technical advance since the previous meeting of this group at Strasbourg in 1976 was the prominence of restriction analysis and cloning techniques. This was illustrated early in the conference by the elegant work of J. Bedbrook (Plant Breeding Institute, Cambridge) and colleagues on the cloning of DNAs characterised as highly repeated (heterochromatin from rye chromosome telomeres), repeated (rDNA from cereals) and unique (gene for the small subunit of ribulose-1,5-bisphosphate carboxylase). Additional applications of recombinant DNA technology were evident throughout the sessions, especially in the area of chloroplast DNA analysis.

The topic of nuclear genome organisation was introduced by W.F. Thompson (Stanford University) with a lucid discussion of sequence repetition and interspersion. To illustrate the spectrum of possible patterns, he considered in detail the genomes of two related species, mung bean (long period interspersion, with almost half of the single-copy DNA as sequences of 6,700 nucleotides or longer) and pea (short period interspersion, with repeated sequences scattered throughout the genome at intervals of about 200 nucleotides). In the same session, R. Flavell (Plant Breeding Institute, Cambridge) described detailed sequence analyses of several cereal genomes and suggested that much of the DNA of complex plant genomes originated by amplification and reamplification of compound units consisting of a unique or low-frequency



repeat sequence flanked by a common high-frequency repeat sequence.

Nuclear transcription was a topic ably addressed by J. Jendrisak and T.J. Guilfoyle (University of Minnesota). Jendrisak described the progressive appearance during germination of polymerase IIB at the expense of IIA, involving the cleavage of the 220,000 molecular weight subunit of IIA to the 180,000 subunit of IIB, and in wheat at least, the modification of a smaller subunit as well (from 25,000 to 27,000 molecular weight). Guilfoyle reported that the well-documented enhancement by auxin of nuclear transcription in soybean hypocotyls is due in part to a higher rate of synthesis of RNA polymerase I, but mainly to an increase in the propagation rate of this nucleolar enzyme. With respect to the actual expression of structural genes, R.B. Goldberg (University of California, Los Angeles) was able, by analysis of R_0t hybridisation kinetics, to distinguish between 'abundant' mRNA (70% of total, but only 500–1500 different kinds) and 'rare' mRNA (which contains the vast majority of different kinds of mRNAs, but in minute quantities). For the rare mRNAs, he estimated 60,000 species in the whole plant, and 25,000–30,000 in a given cell type, of which perhaps 6,000–10,000 are tissue-specific.

Hormonal regulation of gene expression figured in the report of L.S. Dure (University of Georgia), whose catalogue of mRNA subsets specific to various stages of cotton cotyledon embryogenesis and germination includes a novel set functional only during abscisic acid (ABA) arrest and therefore possibly ABA-induced. For gibberellic acid, T.H.D. Ho (University of Illinois) provided further support for a dual effect of the hormone on α-amylase induction in barley aleurone (transcriptional regulation during the first 12 hours, a possible translational effect thereafter). In the case of auxin, L.N. Vanderhoef (University of Illinois) insisted that the gene expression and wall loosening hypotheses are not imcompatible, and tried again to arrange their early marriage. Taking advantage of the absence of fellow auxinologists who might have protested, he went on to declare the wall acidification model 'in trouble' and to propose that the primary mode of auxin at this level may be on Mg^{2+} uptake, with H^+ extrusion as a side effect.

As a prototype for the competent application of molecular techniques to a research programme already in progress, the work of K. Hahlbrock and colleagues (University of Freiburg) on the light-induced enzymes of flavonoid biosynthesis in cultured parsley cells was very encouraging. By purifying the enzymes, raising antibodies and assaying for mRNA activity both *in vitro* and *in vivo*, they could show rapid increases in mRNA activity due to light. Determination of enzyme half-lives allowed the light-induced enzyme appearance *in vivo* to be linked quantitatively to an enhanced mRNA availability.

The biosynthesis and genetic regulation of seed proteins was a topic that attracted special attention because of the implications for genetic engineering of crop plants, a possibility most convincingly advocated by T.C. Hall (University of Wisconsin), but tempered in the minds of many by an increasing awareness of the formidable genetic complexity of the

*A NATO/FEBS Workshop on Genome Organisation and Expression in Plants was held on 11-21 July in Edinburgh, and organised by Dr. C. J. Leaver, Department of Botany, University of Edinburgh.

W.M. Becker is in the Department of Botany, University of Wisconsin - Madison.

storage proteins in most species. Good agreement on the general features of zein synthesis and mRNA properties emerged from the papers by B.A. Larkins (Purdue University), A. Viotti (Laboratorio Biosint. Veg., Milano), and B. Burr (Brookhaven National Laboratory), but controversy arose regarding the true level of heterogeneity of the maize storage proteins, the extent to which this represents expression of different genetic loci, and the number of copies of each zein gene per haploid genome. Probably the most impressive general progress on storage protein analysis in dicots was that reported by Hall on the G1 proteins of *Phaseolus vulgaris*. With the G1 mRNAs isolated and translated previously, they have now prepared and cloned cDNA for use as a probe in detecting genomic G1 DNA segments from a 'library' of cloned bean DNA. Hall also stressed the possible practical implications of differences in both time of onset and rate of protein accumulation and in the differences in total proportion of G1 protein between varieties (range: 18–70%).

Nitrogen fixation is another topic of great potential practical importance that is now increasingly amenable to molecular analysis. Working with *Klebsiella pneumoniae*, R.A. Dixon (University of Sussex) has been able to identify 15 *nif* genes, order them into 7 transcription units, and identify gene products for most of them. D.P.S. Verma (McGill University, Montreal) discussed proteins specific to nodulated plants, including leghaemoglobin (now established as a plant gene product, with about 40 gene copies per haploid genome) and a group of other nodule-specific proteins, including nodulin, a 35,000-dalton protein present in all nodules, but with no assigned functions as yet.

Ti plasmids

The Ti-plasmid of *Agrobacterium* emerged as a strong candidate for the 'most progress since Strasbourg' award. Attention now focuses on T-DNA, described by J. Schell (Max-Planck-Institut, Köln and Rijksuniversiteit, Gent) as a continuous segment of the Ti-plasmid with a molecular weight of 10-15 × 10^6, since this is the DNA actually transferred to plant cells. T-DNA contains the genes for infection and for opine synthesis and consists of at least three functional units, only the central segment being conserved in all types of Ti-plasmids. Schell also discussed possible mechanisms for integration and replication of T-DNA and speculated on the Ti-plasmid as a vector for the introduction of selected genes into plant genomes. W.B. Gurley (University of Wisconsin) reported the presence of four major sites of T-DNA transcription in octopine-type tumour cells, one mapping in the region common to all Ti-plasmids. Whether T-DNA actually codes for structural gene products is a question rendered more amenable to approach by the isolation and preparation of antibody against both octopine synthase and nopaline synthase, as described by J.D. Kemp (University of Wisconsin). Rounding out the Ti-plasmid story, M.D. Chilton (University of Washington, Seattle) proposed a model for genomic integration of T-DNA that depends upon the known homology between the fixed righthand boundary fragment of the T-DNA and the normal plant genome.

Chloroplasts and mitochondria

Organisation and expression of the chloroplast genome was a topic that attracted much attention and reflected in microcosm both the spectrum of techniques and approaches widely in evidence throughout the meeting and the progress during the 3 years since the Strasbourg conference. Using restriction mapping, hybridisation, and linked transcription-translation, L. Bogorad and colleagues (Harvard University) were able to map the following on the 85 × 10^6 circular DNA of the *Zea mays* chloroplast: two sets of ribosomal genes (as inverted repeats), one gene for the larger subunit of ribulose bisphosphate carboxylase (colinear with the mRNA; no intervening sequences), and photogene 32, which codes for a 34,500 molecular weight polypeptide that is processed down to a 32,000 molecular weight thylakoid membrane protein. To this map, J. Weil (University Pasteur, Strasbourg) was able to add (in spinach) a minimum of 21 genes coding for tRNAs for 14 amino acids, T.A. Dyer (Plant Breeding Institute, Cambridge) ordered the genes for the RNAs of the large ribosomal subunit as 23S–4.5S–5S (5′ to 3′), and reported sequencing of the region for 4.5S, 5S, and the 3′ end of 23S rRNA. A scheme for rRNA synthesis in chloroplasts was proposed, involving the processing of a 2.7 × 10^6 rRNA precursor to 16S, 23S, and 4.5S rRNA, and the separate synthesis of 5S rRNA, possibly as a slightly larger precursor.

R.J. Ellis (University of Warwick) introduced the topic of transport and assembly of chloroplast proteins by tracing the fate of the carboxylase small subunit from its site of synthesis on cytoplasmic ribosomes through post-translational uptake and processing, possibly involving receptors in the chloroplast envelope that recognise some aspect of its tertiary structure. G.W. Schmidt (Rockefeller University) identified several cytoplasmically-synthesised chloroplast membrane proteins (CPII polypeptides 15 and 16) which are similarly synthesised as larger precursors, then transported into chloroplasts by a post-translational mechanism involving cleavage to their final length. The term 'transit peptide' was suggested to distinguish sequences involved in post-translational transport from the signal peptide responsible for cotranslation transport according to the now-classic Blobel model.

In a concluding session on the mitochondrial genome, C.J. Leaver (University of Edinburgh), presented results linking cytoplasmic male sterility in maize to the synthesis of a unique mitochondrial polypeptide with a molecular weight of 13,000 (or 17,500) for the T (or C) form of male sterility. In the case of T cytoplasm, nuclear restorer genes suppress synthesis of the variant polypeptide to an extent that correlates well with the sensitivity of the line to the toxin of *Helminthosporium maydis* race T, the fungus which attacks plants with T cytoplasm.

Clearly emphasised at the Workshop beyond the actual data and techniques was the need felt by plant molecular biologists for an international assembly of this sort at regular intervals. The triennial pattern set by Strasbourg (1976) and Edinburgh (1979) points toward another meeting in 1982, with some preference already expressed for the western shores of the Atlantic. □

Bone-marrow transplantation: man and mouse

by Miranda Robertson

APLASTIC anaemia, severe combined immunodefiency and acute myelogenous or lymphoblastic leukaemia, particularly in adults, are all fatal diseases for which bone-marrow transplation is the somewhat desperate remedy. Bone-marrow transplantation, in most of the centres where it is carried out, kills about half of those patients in whom the graft is successful. Death in these cases is caused by graft-*versus*-host disease — the immunological reaction of the grafted lymphocytes against the patient's tissues. In a smaller proportion of patients, the graft may simply fail. It was clear from clinical results reported at a recent meeting on bone-marrow transplantation* that while failures of engraftment are now fewer, the incidence of chronic graft-*versus*-host disease has increased. The meeting brought together clinicians, who work with random-bred men, and laboratory immunologists who work with inbred mice, in the hope that the clinicians might identify outstanding problems to which the research immunologists might supply the solutions.

In the case of aplastic anaemia and severe combined immunodeficiency (SCID), one fundamental problem is that clinicians do not fully understand the disease they are attempting to treat. This is reflected in some anomalies in the results of bone-marrow transplantation in such patients. For example, two of seven

*The International Titisee Conference on Critical Issues of Bone-marrow Transplantation was held in Titisee on May 10-12, 1979 and sponsored by Dr Karl Thomae GmbH.

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surviving patients out of thirteen given transplants for aplastic anaemia at the Hammersmith Hospital eventually recovered full autologous bone-marrow function (E.C. Gordon-Smith, Hammersmith Hospital) which suggests that the function of at least some successful transplants in such patients may be to tide them over until they spontaneously recover, which some of them do on steroids alone. Exactly how high the proportion of spontaneous recoveries may be is not clear: of a series of 168 at the Fred Hutchinson Cancer Center in Seattle, only four spontaneously recovered. Whether in these cases the anaemia is caused by a toxic substance which clears only very slowly, or whether it is a temporary autoimmine problem is not known.

In SCID, on the other hand, it is quite common for successfully transplanted bone marrow to replace everything but B cells (R. O'Reilley, Sloan-Kettering, New York), which suggests a regulatory defect induced by the host. It is unclear at what stage bone-marrow differentiation fails in SCID (R. Parkman, Children's Hospital Medical Center, Boston) and this is hardly surprising since in spite of recent advances in mouse immunology, the ontogeny of the immune system is not understood.

A central problem is of course tissue matching, for which there is no entirely satisfactory test. A mismatch may lead either to the rejection of the graft by the host, where the host is immunocompetent and complete suppression is not achieved, or to graft-*versus*-host disease. B. Torok-Storb (Fred Hutchinson Cancer Center, Seattle) reported that the inhibition of donor cell proliferation by the patient's cells in allogeneic cultures could help to predict graft failure, while D. Schendel (Institute of Immunology, Munich) reported positive cytotoxicity tests between the cells of related donors who showed no serological evidence of mismatch.

An even more subtle source of graft rejection which is not detected by either of these tests is the ill-understood mismatch that will lead heterozygous F_1 mice to reject parental bone marrow though they will accept skin grafts. G. Cudkowicz (State University of Buffalo School of Medicine) reported that cells from the F_1 mice which did not lyse parental-strain targets could nonetheless be shown to bind to them *in vitro*, and suggested that this might correlate with their cytotoxic potential. Whether such subtle effects play any significant part in marrow rejection in human patients is unclear.

Although on the whole the chances of success for bone-marrow transplants across MHC mismatches are low, there have been reports of some remarkable successes (O'Reilley & R. Storb, Fred Hutchinson Cancer Center, Seattle). This may be because some MHC antigens are more important than others: W. Elkins (University of Pennsylvania) finds that to minimise graft-*versus*-host disease in mice it is more important to match for I-region antigens (the mouse equivalent of the human DR antigens) than for K or D antigens (the mouse equivalent of the human A and B antigens). The other possibility is that serological mismatches may not always be a reliable indicator of a strong T cell reponse in an individual case.

Given that perfect tissue matching will almost never be possible, and that mismatching of minor transplantation antigens will always cause graft-*versus*-host disease except in monozygotic twins, can anything be done to remove the cells that will react against the host before the bone marrow is transplanted? Much effort has gone into establishing the identity of the cells in question, and S. Theirfelder (Institute of Haematology, GSF, Munich) and W. Müller-Ruchholz (Kiel University) have both contributed to their identification as T cells. J. Sprent (University of Pennsylvania) reported that anti-theta serum will prevent graft-*versus*-host disease across minor histocompatibility barriers in mice, which suggests that the T cell that mediates graft-*versus*-host disease must be present as a mature alloreactive cell before exposure to the host antigens. The practical implication of this is that graft-*versus*-host disease could be largely prevented by the routine use of effective anti-theta antisera to remove such alloreactive cells before transplantation.

It has recently become fashionable to speculate that graft-*versus*-host disease may be the manifestation of a temporary failure of the donor lymphocytes to regulate one another's activities in a normal manner. There were several reports of abnormal immunoglobulin production during the early stages of graft take. Storb has seen peaks of nonspecific antibody in patients who do not respond normally to specific antigen; Parkman finds abnormally high levels of IgE; and L.J. Dooren (University of Leiden) has found that initially high levels of immunoglobulin subside when donor T cells become apparent. Parkman reported some preliminary evidence supporting the view that acute and chronic graft-*versus*-host disease are two different diseases, the acute form being a failure of suppression (Reinherz *et al. New Engl. J. Med.* **300**, 1061; 1979). Three patients with acute graft-*versus*-host disease had no measurable suppressor cells and the appearance of these cells coincided with the resolution of the disease. Patients with chronic graft-*versus*-host disease were more heterogeneous: two had no suppressor cells and high levels of polyclonal immunoglobulin; four others had two or three times the normal level of suppressor cells. It seems likely that neither diseases such as aplastic anaemia and SCID, nor the anomalous behaviour of lymphocytes derived from grafted bone marrow will be understood until more is known about the ontogeny and normal regulation of the immune system. Immune ontogeny also bears crucially on the immune competence of patients with allogeneic bone-marrow transplants. The key to immune competence, at least for viral infections but probably for all T cell functions, is that T cells recognise antigen only in association with self MHC antigens. Experiments with thymus transplants in mice have shown that T cells learn this specificity to self-plus-antigen in the thymus; R.M. Zinkernagel (Scripps Clinic) has now extended these experiments however to show that while T cells are incapable of responding to antigens in association with MHC antigens they have not encountered during thymic maturation, there is an equally necessary post-thymic maturation step. Thus if a congenitally athymic (nude) mouse of haplotype k is supplied with a heterozygous k × d thymus, the host T lymphocytes are able to kill virus-infected cells of haplotype k but not haplotype d, even though both haplotypes are present in the thymus. However, if such a mouse is then thymectomised (to prevent further T cell maturation) and provided with spleen cells from a donor of haplotype d, its T cells become competent to kill infected cells of both k and d haplotypes. This shows that there is a second stage of T cell maturation which depends on the haplotype of the lymphohaemopoietic cells. For bone-marrow transplantation to restore full immunocompetence, therefore, donor and recipient must share at least one of either the A or the B HLA antigens, and one set of DR antigens. These conditions are nearly always fulfilled because of the need to avoid rejection or graft-*versus*-host disease, but Storb and O'Reilly both reported surprising exceptions. For example, O'Reilly claimed to have restored full immune function with fetal liver and thymus grafts in spite of the fact that the patient's T cells were histoincompatible with her macrophages and B cells. Theoretically it should not be possible for such histoincompatible cells to cooperate, and claims of transplant patients who flourish with a mixture of their own and their donors' lymphocytes are regarded by many with great scepticism. There are two possible explanations for such cases: either there are just enough matched cells in each subset to give immune competence, though not enough to be detected by the methods used; or there happens to be sufficient cross-reaction between the incompatible cells to enable them to cooperate. It is known that such cross-reactions exist.

Puzzles of this sort are inevitable since we do not yet understand lymphocyte interactions in inbred mice, let alone in men. But it is clear that, grim as the prospect is for anyone ill enough to undergo bone-marrow transplantation, it is not as grim as it used to be. Even so, the recent improvement in the prospects, in particular at some centres, owes more to patient and intelligent empiricism than to fundamental insights derived from basic research. □

Miranda Robertson is an Associate Editor of Nature.

Sperms in 'parthenogenetic' freshwater gastrotrichs

from Geoffrey Fryer

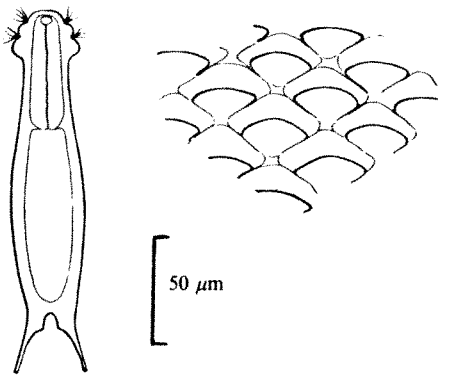
PARTHENOGENESIS has been adopted as a means of reproduction by several groups of freshwater animals, among which the Cladocera and Rotifera are the best known. Such reproduction has several advantages, one of them being that, in climates where active life is profitable for only part of a year, populations can build up very rapidly if every constituent individual is a potential mother. The rapid increase of some planktonic populations of Cladocera in spring in temperate latitudes is an example. Such parthenogenesis is generally facultative and is followed by a period of sexual reproduction which results in the formation of 'resting' eggs that often overwinter or tide the population over other adversities such as a period of desiccation. By such means the ecological advantages of parthenogenesis and the genetic advantages of sexual reproduction are effectively combined in what is often referred to as cyclical parthenogenesis. In some populations of, for example, the cladoceran *Daphnia*, parthenogenesis has come to dominate the reproductive process and sexual reproduction takes place only at long intervals. The logical outcome of such a trend is to become completely parthenogenetic and some populations of planktonic *Daphnia* have conceivably achieved this status. Among the Rotifera, a whole group, the Bdelloidea, is believed to contain only obligate parthenogens, males being unknown, and the same is true of several species of freshwater ostracods. Such reproduction by means of which only female offspring are produced by a process not involving fertilisation is known as thelytoky. Thelytokous animals present problems to the taxonomist, who finds it difficult or impossible to apply the species concept to them, while their existence is something of an embarrassment to the student of evolution.

Another group of animals well represented in freshwater, and in that environment generally believed to include only obligate parthenogens, is the Gastrotricha. In the sea, however, some hermaphroditic forms are known. These microscopic animals — they seldom exceed 200 μm in length — sometimes regarded as comprising a distinct phylum and sometimes a class of the Aschelminthes, are common in a variety of habitats, usually associated with plants or mud surfaces over which they move by means of ventral cilia. Both quick-hatching (subitaneous) and resting eggs are produced but neither kind was believed to be fertilised, though in *Chaetonotus* two types of subitaneous egg have been reported and the organism would not reproduce in culture unless two or more individuals were present, which hints at some sexual process. Now, following a tentative suggestion by Hummon that sperm heads may have been seen in *Lepidodermella squamata*, Weiss and Levy (*Science* **205**, 302; 1979) have positively identified sperms in individuals of this species. These were grouped in packets, either singly or bilaterally, lateral to the intestine and seem not to be associated with any limiting membrane or ducts. Each packet contains only a few sperms — 16 is suggested as a possible maximum, a number that would correspond to the meiotic products of four spermatocytes. Individual sperms have also been found far removed from the packets but no signs of motility have been observed. Of the numerous individuals examined the proportion in which sperms were present never exceeded 8%. As ova are frequently present in sperm-carrying individuals these animals are not males but hermaphrodites.

In cultures, changes in the proportion of sperm-bearing individuals have been observed over short periods of time and the conclusion has been drawn that, as long assumed, parthenogenesis is indeed the usual means of reproduction but that the animals are really facultative hermaphrodites. It seems that hermaphrodites may appear only occasionally in nature, which may explain why they have been overlooked, and one now wonders whether, like the males of cladocerans and rotifers, they appear in response to certain conditions, and whether a kind of cyclical parthenogenesis occurs in some freshwater gastrotrichs.

Much remains to be learned about the sperms of *Lepidodermella*. It is not even known whether they actually fertilise the eggs, and although this has to be tentatively assumed it need not necessarily be the case. All kinds of peculiar cytological phenomena involving reproduction are displayed by small freshwater organisms — giant sperms in ostracods, resting eggs of *Daphnia* which, contrary to the general rule, can be produced without fertilisation, and so on — so caution is necessary. The small number of sperms produced and their lack of motility suggests internal fertilisation but it is not known whether self or cross-fertilisation occurs.

Provided cross-fertilisation is involved, the occurrence of a sexual phase, even if as a rare event, explains why one does not find a host of divergent 'strains' — a puzzle still presented by the apparently completely parthenogenetic bdelloid rotifers. It also makes it easier to understand speciation in freshwater gastrotrichs. There is now no need to invoke asexual speciation, at least in *Lepidodermella*, and this leaves the bdelloid rotifers — more than 200 species of them — as perhaps the only group in which this process seems likely to have occurred.



Lepidodermella squamata and details of its surface scales. (After Brunson).

Thus the discovery of sperms in *Lepidodermella*, besides upsetting what is now seen to be a too easy generalisation, raises all sorts of interrelated problems of interest to the cytologist, ecologist, taxonomist and student of evolution, as well as to those who just like to understand how such minute creatures conduct their business. □

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Squaring the circle

from Peter Lawrence

VERNON French's PhD thesis, an attempt to unravel the mysteries of insect leg regeneration, met with more success than he expected. His results revealed properties of insect legs that were similar to vertebrate limbs and in collaboration with Peter and Susan Bryant he launched the 'polar coordinate model' (*Science* **193**, 969; 1976). In essence, this model attempts to explain formally why certain pieces of legs, insect imaginal disks and vertebrate limbs regenerate (remake the missing bits) and other pieces duplicate (make an extra set of the parts already present in the piece). For good measure the model also tackled a longstanding puzzle — the formation of supernumerary limbs after grafting. When a right leg is grafted onto a left stump two left appendages grow out from the graft/host junction, one on each side of the grafted right leg (Fig. 1).

The model draws liberally on the observations of Bohn who grafted pieces of cockroach legs together which had been cut at different levels in graft and host. Intercalary regeneration occurred at the host/graft junction and replaced the parts normally lying between. The model also

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draws on gradient theory which has long been the preferred way of trying to understand how cells know their position. The essential point of gradient theory is that cells read the value of some continuous scalar variable, the cellular polarity being determined by the local maximum slope of that variable.

French, Bryant and Bryant proposed that this positional information is specified both circumferentially and proximodistally in the limb. When two pieces are placed together intercalary regeneration occurs to fill in the missing values, and this can occur both around the limb circumference and along its length. Experiments showed that the intercalary regeneration in the circumference always occurred by the shortest route — thus if $\frac{1}{6}$th of the limb circumference is removed the limb regenerates; if $\frac{2}{6}$ths is removed the remaining $\frac{4}{6}$th duplicates.

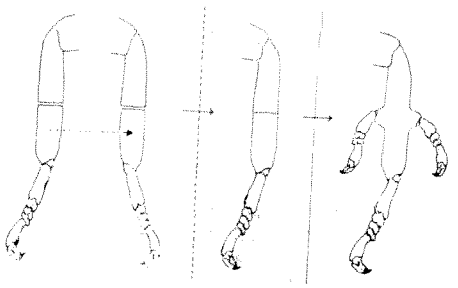
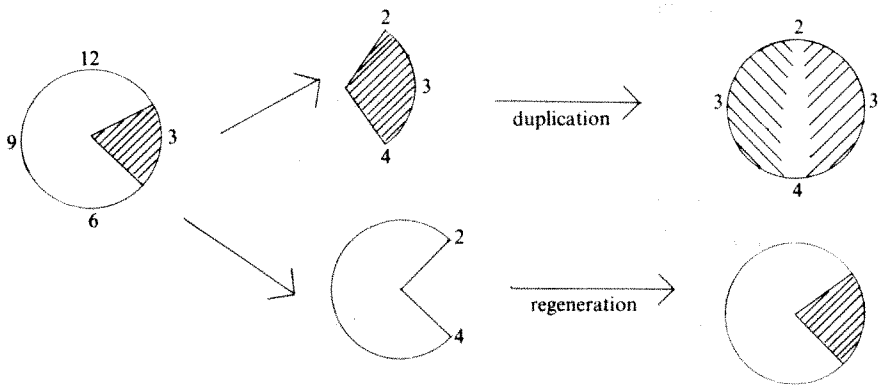


Fig. 1 (From Bohn *Wilhelm Roux Arch.* **156**, 449; 1965).

circle is clockwise or anticlockwise. French, Bryant and Bryant therefore proposed the 'complete circle rule' — that is, whenever a complete circle is formed proximodistal growth will occur to generate another appendage.

The flamboyant launching of this model (in both *Science*, and *Scientific American* **237**, 66; 1977) has led to a flurry of experiment by others. It was soon found briefly after wounding, but that soon after the cells became reallocated to different groups, each of which is then restricted to make structures in only one compartment. But when we specifically try to relate compartments — precisely defined parts of the pattern — to the polar coordinate model, we are somewhat lost.

Nevertheless a model that predicts much of the behaviour of pieces of disks and grafted limbs of both insects and vertebrates cannot be bad. We should now declare a moratorium on cutting and splicing experiments and stop tinkering with the model itself. I wish I knew what we should be doing, but one thing is clear; what started as a song and dance is in danger of becoming a song and dance routine. □



Reinhardt and coworkers (*Devl Biol* **60**, 238; 1977) showed that the first step in this process was the healing together of the cut edges. This is then followed by the intercalation of missing values. It should be noted that after duplication the structure is mirror symmetrical and a further cut at right angles to the mirror plane would produce cut edges all at one level (3). After healing there should be no discrepancy and therefore no intercalation. Recently Littlefield and Bryant have tested this with the bilaterally symmetrical genital disk of *Drosophila* (*Devl Biol* **70**, 127; 1979). They find, as expected, that cuts at right angles to the mirror plane produce a stable piece that heals but does not intercalate.

Another attraction of this model is that it formally explains the growth of supernumerary limbs after grafting, and whether they are left or right handed. This does require one extra rule. After grafting a distal piece of one limb onto a stump on the other side, circumferential intercalation is expected around most of the junction between host and graft — because of the varying disparity at the interface. However proximodistal intercalation is not expected (as the graft and host are cut at the same level). At two points on the circumference, intercalation will lead to the formation of a complete circle — a complete 'clockface' of numbers. It is at these points that the extra legs appear; whether they are left or right handed depending on whether the that the complete circle rule does not always apply as incomplete limbs can and do regenerate (Slack & Savage *Nature* **271**, 760; 1978). Theoreticians started to worry about how polar coordinates might be specified and whether two gradients at right angles might do just as well. In short, the model has been treated to a grilling in a field where models often get more attention than experimental facts. Of course, when formal models are found wanting a little tinkering can take account of the anomaly and the model can emerge unscathed with a shining new accessory.

As usual in developmental biology our understanding is fragmented and parochial, and our models reflect that. For example, the polar coordinate model is not concerned with the cell lineage of the regenerate or duplicate — whether the cells come from host or graft. Imaginal disks are known to be divided into two fundamental regions, 'compartments', each descending from a separate group of cells (Garcia-Bellido, Lawrence & Morata, *Sci. Am.* **241**, 90; 1979). There are indications that limbs of cockroaches are also subdivided into two compartments. Unpublished experiments of Vernon French suggest that during intercalation the contribution of cells is limited to those structures in one compartment. Other experiments on imaginal disks (Szabad *et al. J. Embryol. exp. Morph.* **49**, 229; 1979) suggest that compartment restrictions break down

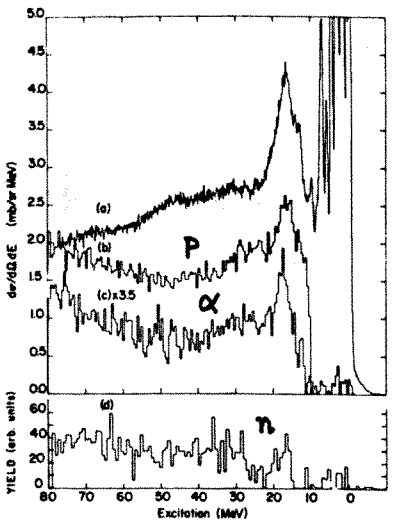
Alpha-particle decay of giant resonances in nickel reassessed

from P.E. Hodgson

THE recent work on the dominance of the alpha-particle decay mode in the decay of the giant quadrupole (E2) resonance in the nickel isotopes (see *News & Views* **280**, 15; 1979) has stimulated several experimental and theoretical studies. Among these is a measurement of the probabilities of alpha-particle and proton decay by a more direct method, and this gives a different result that is more in line with experimental studies of other nuclei, and with theoretical work.

The new measurements were made by a group at the University of Maryland (Collins, Chang, Tabor, Wagner & Wu *Phys. Rev. Lett.* **42**, 1440; 1979). They excited the giant quadrupole resonance by the inelastic scattering of alpha-particles, and determined the probabilities of emission of protons, alpha-particles and neutrons in coincidence with the inelastically scattered alpha-particles. Some of their results are shown in the figure. The top curve is the inelastic alpha-particle spectrum from the reaction $^{58}Ni(\alpha,\alpha')^{58}Ni$ at 140 MeV and at an angle of 16° to the incident beam. The giant quadrupole resonance is visible as a broad peak at an excitation energy of about 16 MeV in the target nucleus. The lower curves are the inelastic alpha-particle and a neutron, respectively. The accuracy was improved by integrating several coincidence spectra over angle, and then

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The top curve is the total spectrum, and the three lower curves are of the alpha-particles in coincidence with protons, alpha-particles and neutrons respectively. The energy scale refers to the excitation energy in the ^{58}Ni nucleus.

subtracting the background continuum. The giant quadruple resonance is visible in all the coincidence spectra, showing that the resonance decays by proton, alpha-particle and neutron emission.

These spectra were integrated to give the branching ratios for the decay of the giant quadrupole resonance, giving 59± 12% proton decay and 12±4% alpha-particle decay. The difficulty of detecting the neutrons prevents the accurate determination of the neutron branching ratio. These results may be compared with the branching ratios from the statistical decay of a 2$^-$ state in ^{58}Ni at an excitation energy of 16 MeV. These were calculated using the Hauser-Feshbach theory, giving 51% proton decay and 6% alpha-particle decay. These are very similar to the observed values, suggesting that the giant quadrupole resonance decays statistically. To confirm this possibility it would be necessary to make similar measurements on a range of nuclei with different particle emission thresholds and penetrabilities, and to compare the results with statistical theory.

An investigation of the decay of the giant quadrupole resonance in ^{40}Ca by Youngblood *et al.* (*Phys.Rev.***C15**, 246; 1977) gave 70±15% proton decay and 21±10% alpha-particle decay, quite similar to the values for ^{58}Ni. For this nucleus the statistical theory gives 74% and 21% respectively, in accord with the experimental results. The relative proportion of alpha decays increases towards the lighter nuclei, and the measurements of Knöpfle *et al.* (*Phys.Lett.***74B**, 191; 1978) gives essentially 100% alpha decay for ^{16}O.

The new results for ^{58}Ni are thus in line with the trend expected from measurements of the decay of the giant quadrupole resonance in lighter nuclei. Detailed shell model calculations of the decay of the resonance in ^{16}O and ^{40}Ca reproduce this trend, thus confirming the experimental results.

It is thus no longer necessary to assume a particular alpha-particle structure to account for the decay of the giant quadrupole resonance in the nickel isotopes. The earlier results that indicated a high probability of alpha-particle decay are thus probably attributable to unrecognised difficulties in the method used by the Maryland group. □

In a recent comment (*News & Views* **280**, 15; 1979) P.E. Hodgson drew attention to an experiment at the National Bureau of Standards in Washington DC (Wolynec, Dodge & Hayward *Phys. Rev. Lett.* **42**, 27; 1979) which appears to show that alpha particle emission is the dominant decay mode of the isoscalar giant quadrupole resonance in the nickel isotopes. This surprising result, derived from an analysis of electron and bremsstrahlung excitation function data is, in the light of recent work at three other laboratories, more controversial than implied by Wolynec *et al.* Work in this laboratory (McGeorge *et al. Int. Conf. on Nuclear Physics with Electromagnetic Interactions*, Mainz, 1979) using a similar experimental procedure but a different approach to the analysis, and alpha scattering coincidence experiments at Maryland (Collins *et al. Bull. Am. Phys. Soc.* **23**, 506; 1979; *Phys. Rev. Lett.* **42**, 1440; 1979) and Heidelberg (Knöpfle *Int. Conf. on Nuclear Physics with Electromagnetic Interactions*, Mainz 1979) have not succeeded in reproducing the NBS result. We should like to point out that a number of unsubstantiated assumptions were made in the NBS analysis and that these taken with other considerations cast considerable doubt on the derived result.

Hodgson points out that the analysis depends on the accuracy of the E1 and E2 virtual photon spectra calculated from theory; there is no experimental test of the E2 spectra (Hayward *Int. Conf. on Nuclear Physics with Electromagnetic Interactions*, Mainz 1979) and tests of the E1 spectra (for example Martins, Wolynec & Moscati *Phys. Rev.* **C16**, 613; 1977) do not seem to be better than 5%. The accuracy of the E1 spectrum is important because the E2 strength is essentially derived from the small difference between the calculated E1 contribution to the electro-alpha cross section and the experimental measurements.

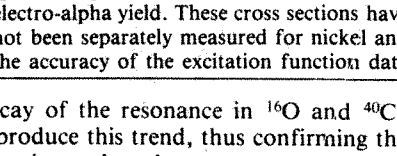
The analysis also depends on knowledge of the monoenergetic photoalpha cross sections which are folded with the E1 virtual photon spectra to predict the E1 contribution to the electro-alpha yield. These cross sections have not been separately measured for nickel and the accuracy of the excitation function data obtained so far does not allow determination of both the multipole contributions and the photo-alpha cross sections. It is therefore necessary to make some assumptions; in the NBS analysis an asymmetric Lorentz shape is assumed for the photo-alpha cross section. The resulting E1 contribution to the electro-alpha cross section increases less rapidly with energy than the experimental data and the difference could also be due to a high energy tail on the photo-alpha cross section.

We have performed a statistical model calculation of the photo-alpha cross sections starting with the photo-neutron cross sections measured by Berman (Lawrence Livermore Laboratory Report No. UCRL 78482, 1976) and find a larger high energy tail than the Lorentz shape of the NBS analysis. We then find that the electro-alpha data can be fitted adequately by including an E2 contribution which exhausts only 10% of the energy weighted sum rule. This E2 contribution is consistent with the alpha particle scattering coincidence experiments but much less than that found by Wolynec *et al.*

Folding our calculated photo-alpha cross sections with the bremsstrahlung spectrum leads to 'radiator-in' yields 20-30% larger than those published by NBS but in agreement with recent measurements in our laboratory. In such experiments it is possible that enlargement of the beam spot size due to multiple scattering in the radiator can lead to counting losses unless a sufficiently large detector is used in the focal plane of the alpha particle spectrometer. In our measurements these losses are kept below 1%. Our electro-alpha results for which these losses are much smaller agree reasonably well with the NBS data.

The main point we wish to emphasise is that E2 strengths derived from electro-excitation function data depend sensitively on the virtual photon spectra and on the photo-alpha cross sections used in the analysis. It is therefore necessary to treat the results with considerable caution.

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A hundred years ago

The British Association at Sheffield

The reports of the sectional proceedings in the local papers seem to us unusually meagre, though the space devoted to the Association both in provincial and in London papers becomes each year increasingly great. The *Times* alone this year has had several leaders on the Association generally, as well as on the principal addresses, and it is gratifying to notice the decided improvement not only in the knowledge displayed in these articles, but also in the attitude of the leading paper towards science. In a somewhat hysterical article on Prof. Allman's address, the *Observer* of last Sunday seems to forget that science has all sorts of followers, and that the real workers rarely come before the public. Notwithstanding the apparently complete ignorance of the writer in the *Observer* as to what science really is, we cannot help agreeing with much that he says as to the present condition of the Association, and the urgent need of reform in its method of work, if it is not rapidly to degenerate into a "scientific garden party".

From *Nature* **20** 28 August, 406; 1879

Voyager 1 encounter with Jupiter and Io

The Voyager 1 encounter with the jovian system in March 1979 generated a wealth of new information about Jupiter's atmosphere, satellites, and magnetosphere. One of the most spectacular discoveries was the evidence of active volcanism on the satellite Io. Eight active volcanoes were discovered, indicating a level of activity higher than found on any other body in our Solar System.

This special issue of Nature presents a set of Voyager papers devoted to many of these discoveries. Principally, they cover the first detailed analyses of the jovian meterology and the properties of Io. The Voyager papers are supplemented by Earth-based studies which relate directly to the spacecraft observations.

The results from the Voyager mission reported in this issue of Nature represent a significant increase in our knowledge of the jovian system. Frequently in the discussions, comparisons are made between features and processes in the jovian atmosphere, magnetosphere, and on Io with their terrestrial counterparts indicating the importance of comparative planetology. The success of the Voyager mission to Jupiter, indicated by these papers, will provide a new perspective for planetary studies.

Garry E. Hunt

Preliminary geological mapping of Io

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Terrain units and their global distribution on Io are summarised. A map of the complex region of Io is also presented.

VOYAGER 1 obtained moderate resolution (1–5 km per television line pair) images of about one-third of Io and images at lower resolution (5–20 km per television line pair) of an additional third of the surface[1]. The low resolution images were obtained using several colour filters (0.35–0.6 μm) which discriminate a myriad of coloured surface units. The moderate resolution pictures are single-colour black and white and they show geomorphic features formed dominantly by volcanic processes[2]. The absence of impact craters implies an extreme youthfulness for the surface that is consistent with the discovery of active volcanism on Io[1,3–5]. This article presents a preliminary summary of terrain units and their global distribution on Io. The global map also provides a context for the accompanying articles that discuss topical aspects of Io's geology, including volcanic plumes[4], the nature of volcanism implied from surface expression of landforms[2], curious layered terrains that are apparently eroded[6], and a model for the volcanic eruptive processes[7].

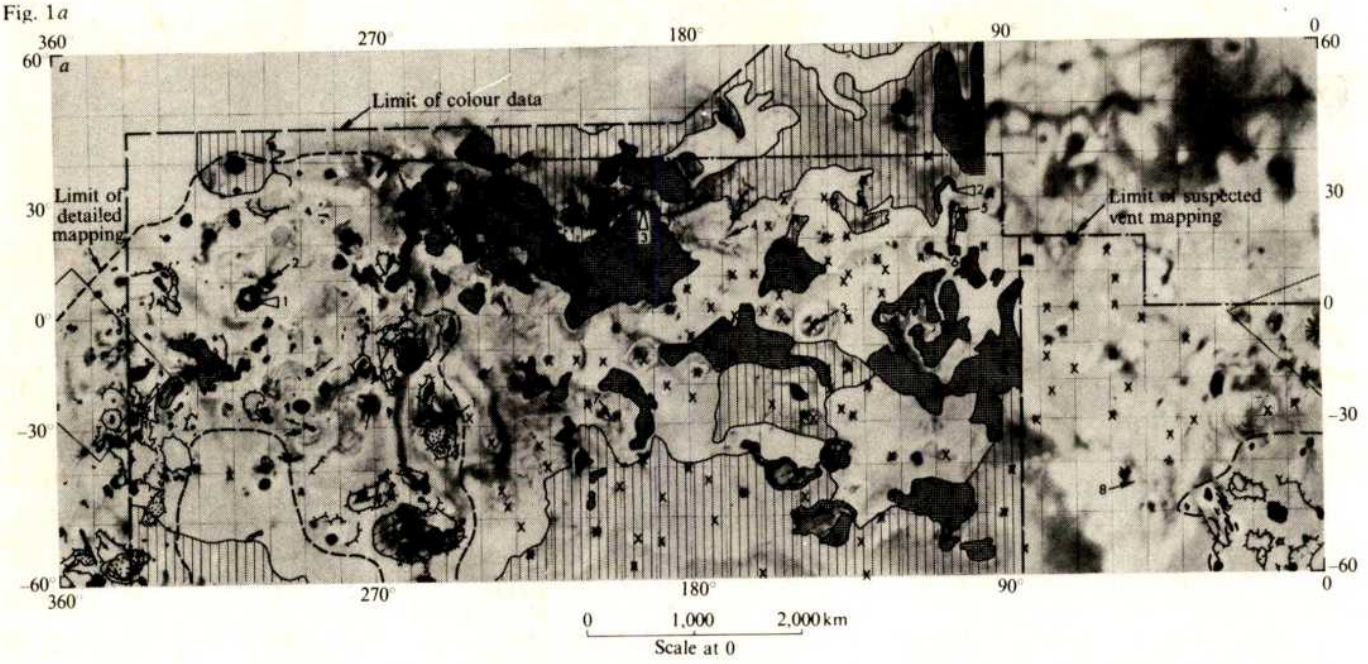
This paper also presents the first attempt to map units in a complex region of Io that shows interlayered flows, volcanic collapse pits, scarps, faults, and layered terrains. The maps were compiled using photogeological mapping techniques developed for mapping active terrestrial volcanic fields; the relative chronology of surface units was determined from overlap and transection relations between surface units. A conventional planetary mapping technique that uses measurements of superimposed impact crater populations to establish relative chronologies for surface units could not be used because no impact craters have been identified on Io's very young surface. The detailed maps presented here (*Plate 5a*, p. 786) show that moderate resolutions obtained by Voyager 1 were sufficient to show such geological relations clearly.

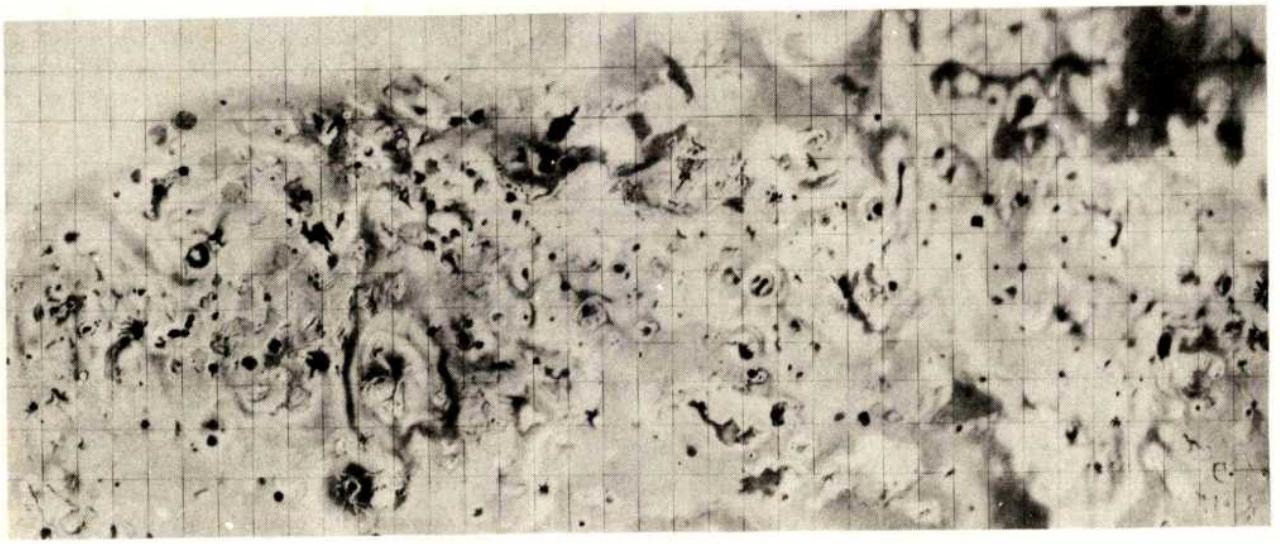
Global map units

The most widespread terrain unit on the surface of Io is comprised of plains deposits that lie between the volcanic vents. In Fig. 1 these plains are divided into four general categories based on colour data where available (none between 0° and 80° longitude); the colour differences are also shown on the cover. The first plains unit consists of white to bluish-white regions of very bright material which may be surface deposits of SO_2 frost[8–11] or the orthorhombic crystal form of sulphur[12]. The second plains unit includes materials of intermediate albedo that show a variety of red, orange, and yellow hues; various forms of sulphur may be present on the surface in these areas as sublimates or sulphurous alteration[1,12–14]. The third plains unit includes brownish hues (black and brown) that are limited

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Fig. 1*a*





primarily to the polar areas. The fourth plains unit is very dark brown to black occurring as large diffuse deposits around a few vents.

These intervent plains generally appear smooth at moderate resolution but are in places characterised by extremely irregular margins with scarps of a few hundred metres or less (Fig. 1). These scarps, although globally distributed, show up well in the south polar region (*Plate 3*) and here show evidence of disrupted surfaces. Details of possible erosional processes are discussed briefly here and more fully by McCauley *et al.*[6]. Linear parallel sets of scarps that form graben, and linear features with no relief, have been mapped as normal faults. Protruding through the plains are isolated mountainous terrain (*Plates 3 and 4*, pp. 784, 785), most of which are estimated to be a few kilometres high based on limb profiles and shadow measurements near the terminator; the highest noted are <10 km.

The map of the planet (Fig. 1) shows the areal distribution of more than 300 suspected vents identified on moderate and low resolution photographs; the distribution of more than 150 vents identified on moderate resolution frames. Most vents mapped from the moderate resolution photographs resemble terrestrial calderas or pit craters. The floors (usually red and/or black) range from extremely shallow to ~1 km in depth. The 'calderas' in Fig. 1 have subcircular outlines, complex terraces, scalloped margins and radiating flows; 'collapse pits' have no discernible rims or surrounding deposits. Vents on the low resolution section of the map (×) are identified solely by their red or black circular forms or adjacent radiating patterns that closely resemble calderas and flows recognised in the moderate to high resolution photographs. The average distance between vents (>15 km diameter) in the moderate resolution coverage of Fig. 1 is ~200 km for both the equatorial and polar regions. The spacing between the inferred vents on Fig. 1 is ~300 km. The average vent size in the moderate resolution mapping areas is ~40 km; maximum vent sizes are ~250 km and ~100 km in the equatorial and polar regions respectively.

In the moderate resolution section of Fig. 1, volcanic flows with complex digitate patterns radiate outward from many vents; flows around other vents are broad and lobate to irregular in plan, with mottled, possibly coarse-textured surfaces[2]. These flows are most common in the equatorial region; flows are rare in the south polar region (not shown on Fig. 1). Some calderas and pit craters are surrounded by bright and dark haloes (*Plates 1, 2* and *4*, pp. 782, 783, 785) (closely resembling dark halo craters on the Moon) and may be volcaniclastic deposits (volcanic ejecta comprised of silicate and/or sulphur compounds). Some elongate fissure vents were the sites of active plumes during the observation period[4]. High resolution images show complexly intersecting flows with low relief and volcanoclastic blankets in some areas. 43% in the moderate resolution areas from +30° lat

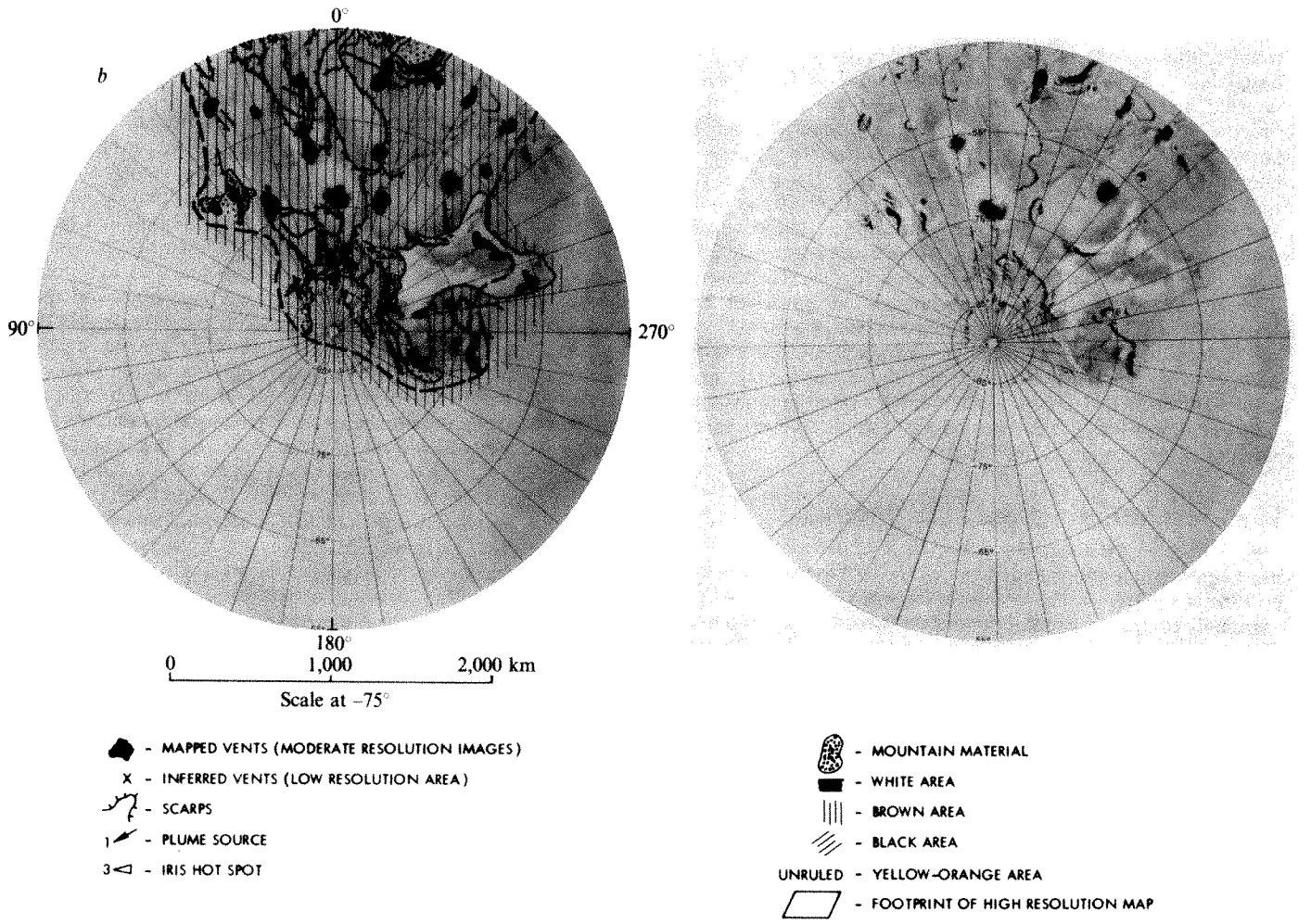


Fig. 1 Distribution of mapped and suspected volcanic vents in the *a*, equatorial; and *b*, south polar areas of Io; the base is an uncontrolled preliminary pictorial airbrush map of Io (mercator projetion–equatorial; polar stereographic project-south pole by P. Bridges). Scale is at 0° latitude.

have mappable flow deposits while 23% of vents between −30° to −60° lat have such deposits. This percentage decreases rapidly towards the south pole to a maximum of 10%. 10–18% of the vents in the equatorial moderate resolution area have radiating deposits indicating possible raised topography; none of the vents mapped in the south polar region of Fig. 1 have such deposits. These percentages may be biased by the small sample area represented by the moderate resolution coverage.

Distribution and regional associations of global units

From Fig. 1 we can make observations regarding the distribution and interrelationships of colour units, terrain units, and surface landforms. All but one of the active volcanic vents, discussed in detail elsewhere[4] are seen to lie within ±30° of the equator. Because half of the surface area of the body lies north and south of these parallels this seems to be a real and important observation. A statistical analysis was made to determine whether inactive centres are also distributed in any latitudinal fashion. The distribution of volcanic vents (calderas, collapse pits and radial flow centres) mapped in the moderate to high resolution area covered by Fig. 1 (60°S–40° N; 250°–360°) was examined using 68 equal areas and modelling the expected number of areas containing 0, 1, 2, 3, 4 vents in the usual way. The χ^2 test indicated a probability $P = 0.6$ that the computed^2 value would be exceeded by chance; the vents seem to be quite randomly distributed.

On the Mercator and stereographic maps (Fig. 1), regional fractures are notably lacking, only the local faults and scarps occur. We interpret the randomly distributed vents and lack of visible regional fractures to indicate that the vents have formed over widely distributed hot spots that are not controlled by strongly patterned convection cells that would lead to plates with detectable margins. This interpretation is consistent with the model of Peale and others that Io has a thin (<20 km), tidally heated crust[15].

Isolated mountainous terrains in Fig. 1 are usually associated with calderas, collapse pits or active centres. Notable examples of mountains bordering calderas occur at 260° W 10° S (*Plate 4a*, p. 785), 255° W 55° S, 350° W 55° S and 40° W 77° S. The largest plume[4] located at 250° W, 30° S is associated with two large tabular mountain units. The implications of these associations are discussed later.

Another important observation from Fig. 1 is the global distribution of colour units. As known from ground-based observations, the polar regions were slightly darker than the equatorial region[16,17]. These units, classified as (dark) brown (to black plains) materials in Fig. 1 are found dominantly north and south of approximately 30–40° latitude. Where these materials bound the bright white areas and yellow-orange regions there is no discernible relief. The plains in the equatorial regions are either white or mixtures of reds and oranges. From the pattern of the distribution of the white areas shown in Fig. 1, we suggest that the entire region may have been a continuous blanket of white material and that the active centres, notably Plumes 3, 4,

5, 6 and 7 have the deposited materials which have buried or removed the whitish materials. Also, it is known from ground-based data[18], that the hemisphere centred near 300° longitude is unusual compared with the rest of the planet. Figure 1 shows that there are few whitish areas in that region. Note also that the largest Plume (1) as well as a plume known to display an enormous UV enhancement (Plume 2), are located in this region[4]. We suggest, as in the case of the smaller plumes to the east, that these plumes have removed or buried the whitish materials. Another observation is that the complex surface flows occur dominantly in the orange and yellow regions. The absence of these complex flow units in the whitish materials as well as in the south polar darker units suggests two possibilities: (1) that the surface flows are, like the plumes, dominantly active in the equatorial region, or (2) that the whitish and dark units represent surface deposits (either condensates or enormous uniform flows) which have buried the complex flows characteristics of the orange-yellow materials.

High resolution volcanic relationships

Figure 1 shows the footprint of a high resolution image which has been studied and mapped in detail. This image (*Plate 5a*, p. 786) is situated in the orange-yellow plains and shows the extremely complex nature of the surface flows found in this unit. This image and accompanying map provide dramatic additional evidence of Io's complex volcanic nature. Numerous pit craters, shield caldera vents with diverse associated flows, pyroclastic and fumerolic deposits, and small volcanic cones have been mapped in this area. Two overlapping circular shield features are characterised by their high albedo summit calderas and sharply truncated outer margins. The circular marginal scarps appear to have been eroded; concentric faults associated with the features' central calderas may have controlled scarp formation and erosion. Dark and bright markings adjacent to small vents may be analogous to tuffs, sublimates or fumerolic deposits; they resemble deposits associated with currently erupting centres elsewhere on Io. Irregular markings on the flow surfaces may reflect variations in texture, chemical composition of the flows, physical state (for example, allotropes of S) or mantling surficial deposits[1,2,12-14].

The direction in which material flowed (indicated by arrows on *Plate 5a*, p. 787) gives at least some qualitative indication of local slope; the calderas on the summits of low shield-like constructs are thus associated with radiating, narrow, finger-like flows. There is little evidence of local, topographic slope for the pit crater deposits; eruptive materials appear to flow away from the crater in only one or two main directions. Materials from many volcanic vents overlap; thus a relative stratigraphy and chronology of eruptive episodes can probably be established; at least locally.

Possible geological models for the crust of Io

Our understanding of the geological character of Io's crust and the processes that dominate depends on the bulk composition of the major plains units exposed on the surface. On one extreme these plains may be thick accumulations of volatiles, that is, SO_2 and S_2 as discussed by Smith *et al.*[7]. On the other extreme, the plains might be predominantly silicate with minor amounts of sulphur and sulphur compounds producing the coloration. In that case the volcanism might be similar in style and character to the range of volcanic processes known in the inner Solar System.

Sulphur-plains model

In the first alternative, that is, thick accumulations of sulphur and sulphur compounds forming the plains, Smith *et al.*[7] point out that assuming a thermal gradient consistent with the tidal heating model[15] at a depth of a few hundred metres sulphur dioxide becomes liquid, and at a depth of about a kilometre sulphur itself becomes liquid. In this case topographic forms with relief or more than about a kilometre cannot be composed predominantly of such volatiles as they would rapidly flow and sink back to a lower topographic relief. Thus, the mountains found scattered throughout the equatorial region and south polar region cannot belong to this volatile shell, but must be protuberances of silicates, presumably part of a silicate lithosphere. As Io's density (3.5 g cm^{-3}) is very close to that of the Moon, it is likely that the interior of Io is dominantly silicate. The tidal heating model of Peale *et al.*[15] suggests that the silicate crust could be of the order of 20 km thick, below that point liquid silicate would exist. Rough calculations by Smith *et al.* in starting with chrondritic abundance suggest that a S_2/SO_2 outer shell is likely to be $\lesssim 10$ km thick[7]. They also point out that below 4 or 5 km sulphur begins to vaporise and would probably be forced into solution or into the pores of the silicate mantle. Assuming that the dominant material making up the plains is sulphur and sulphur compounds, the depth to the solid silicate surface cannot apparently be more than several kilometres and then the mountains would be protrusions of the lithosphere through the liquid/solid sulphur-rich layers. Additionally, the numerous gas vents which form whitish effusions from the crust are usually associated with scarps of substantial relief[6] (several hundred metres or more). McCauley *et al.*[6] suggest that the liquid SO_2 zone is exposed on scarps of several hundred metres. Thus the liquid SO_2 aquifer has access to the surface and spring sapping, geysering, explosive erosion and simple gas effusion can explain the erosion along the scarps as well as the numerous occurrences of the whitish gas plumes.

Mountainous protuberances often ring volcanic centres, and this strongly suggests in the case of the two-layer model (that is, a silicate crust with oceans of volatiles with a solid crust of volatiles) that the apparent volcanic activity in the sulphur-rich layer may be excited by and localised by volcanic processes in the subjacent silicate crust. We suggest that the rings of mountains may in fact represent volcanic centres, perhaps calderas themselves in the silicate crust over magma chambers and that the collapse depressions are simply localised by 'sub-oceanic' volcanism ('oceanic' applies to the oceans of sulphur). Because of the density contrast between sulphur and silicates (nearly a factor of 2), similar to the density contrast between silicates and water by an order of magnitude, silicates that erupted from the protuberances would simply run down into the floors of the sulphur oceans. Volcanic constructs on the floors of the sulphur oceans could excite sulphurous activity above them and perhaps build up to heights of several kilometres required to protrude above the sulphur layer. The fact that most of the silicate protuberances are only 2–4 km above the plains places constraints on both the density contrast as well as overall depths of the silicate magma chambers. If the silicate crust were many kilometres deep, then the construction might be expected to be substantially higher. The implied confinement of the silicate magma chambers to a fairly narrow depth region is consistent with the Peale *et al.*[15] heating model that the outer shell of only 10 km of Io's silicate mantle may be solid.

Silicate volcanism model

The appearance of a wide variety of volcanic forms that are usually associated with chemically differentiated volcanic rocks implies that the eruptive rocks may be normal silicate magmas enriched in sulphur. The forms vary from those associated with mafic rocks, such as basalts (*Plate 2a*, p. 783), to intermediate rocks that form lobate flows (*Plate 2b*, p. 783) or low domes (*Plate 5a*, p. 786), to silicic rocks and ash flow tuffs (*Plate 4a*, p. 785) of latite or rhyolitic composition. Also the mountainous terrain (*Plate 4a*, p. 785) that is 4–8 km high must be of silicate composition as sulphur would flow like a glacier under these stresses. The thin silicic crust would still hold, but sulphur segregation would have taken place only to the extent that the eruptive deposits are sulphur rich, highly coloured, and local accumulations of sulphur deposits might have occurred. More intense study of the Voyager data may allow a choice to be made about the composition (all sulphur or sulphur enriched silicates).

Time scales and surface ages

The absence of impact craters prompted Johnson *et al.*[5] to estimate that the minimum resurfacing rate on Io is $>10^{-2}$ cm yr^{-1}. Resurfacing, meaning removal of craters, can be caused by various surface processes including volcanic flows[2,12], erosion[6] and deposition from eruptive plumes[4]. Johnson *et al.* estimate that deposition from active plumes is 10^{-4} cm yr^{-1}. If this were an average rate for the planet then the entire surface would be coated by ~1 mm in, on average, 1,000 yr. Thus the multi-coloured surface flows are likely to be much younger than this. The processes that are erasing craters would also erase small calderas, scarps, and graben. From the resurfacing rate required to destroy craters ($>10^{-2}$ cm yr^{-1})[4] we can estimate that small calderas and scarp features, having relief of a few hundred metres to a kilometre, are <1 Myr in age; how much less we cannot be certain. The only candidates for ancient landforms are the silicate mountains. Although there are no impact craters on these mountains it is possible that such craters may have been erased by continually renewed mantles of sulphur and sulphur compounds; these mountains could conceivably be billions of years old. The surface colour patterns and landforms in the plains must be less than 10^3 and 10^6 yr old respectively.

Conclusions

First, the distribution of mappable volcanic vents (all types) is statistically random. This hypothesis is consistent with a model assuming very thin crust, high degree of internal melting and a wide distribution of 'mantle hot spots' or convection cells; second a wide variety of volcanic vent types and a similar diversity of deposits exist which indicate a large variation in eruptive mechanisms, rates of effusion and possibly composition of silicate melts and/or volatile components such as sulphur; third the large expanses of layered deposit plains on Io seem to be eroded remnants of large scale volatile or volatile-rich ash flow deposits erupted from calderas, the relics of which may be the rugged, layered and elevated mountain materials.

The images of Io obtained by Voyager 1 are sufficient quality to provide important geological information on this unique body in our Solar System. Geological mapping on high resolution and controlled low resolution maps should provide detailed information on the mechanisms and chronology of eruptive events.

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Volcanic features of Io

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Volcanic activity is apparently higher on Io than on any other body in the Solar System. Its volcanic landforms can be compared with features on Earth to indicate the type of volcanism present on Io.

AN exciting event of the Voyager mission was the detection of a diffuse, umbrella-shaped plume extending 280 km above the bright limb of Io[1]. This discovery was followed by detection of at least seven other plumes[2] and several areas of anomalously high surface temperatures[3], indicating rates of volcanic activity very much higher than on Earth, or on any other body so far observed in the Solar System. The surface of Io shows complementary indications of high eruption rates. The apparent absence of impact craters larger than 1 km in diameter indicates that the satellite is rapidly resurfaced and few, if any, surfaces older than 10^6–10^7 yr survive[2,4]. Numerous caldera-like depressions, commonly surrounded by flow fields, suggest that volcanism is the prime resurfacing process. So numerous are the calderas that nearly all are within 300 km of another. We describe here the various volcanic landforms, compare them with similar features on the Earth, and make inferences concerning the type of volcanism on Io.

An important aspect of ionian volcanism is the apparent part played by sulphur. Ionised sulphur has been detected in the vicinity of Io's orbit from telescopic observations[5] and by the UV spectrometer on board Voyager[6]. Sulphur is also a likely cause of the colour variations observed at the surface[7]. Smith *et al.*[2] suggested that sulphur or sulphur compounds (H_2S, SO_2) may drive the plumes. Later Johnson *et al.*[4] and Smith *et al.*[8] argued that the less volatile gases, SO_2 and/or S_x, are more likely candidates than H_2S. This conclusion is reinforced by the discovery of SO_2 ice on the surface[9–12] and the detection of SO_2 gas near one of the active plumes by the Voyager IR interferometer[13]. Most of the sulphur in the plumes probably returns to the surface on near ballistic trajectories. A small fraction, however, must escape to form Io's torus either by some thermal mechanism, aided by the volcanism, or by magnetospheric sweeping[4]. Because of the apparent importance of sulphur in driving the volcanic plumes, we give special attention to the possible role of sulphur in creating the volcanic landforms.

Although Io was systematically photographed as the Voyager spacecraft approached Jupiter, only a fraction of the satellite's surface was covered at the highest resolution. The best coverage is between 250° W and 30° W south of approximately 10° N. Most of this area was photographed with resolutions down to 0.5 km per pixel. Outside this region, discrimination of surface detail is difficult and interpretation is largely based on comparison with the better photographed area. The general descriptions given here may therefore not apply to the entire satellite. In particular, some of the brighter regions of Io, around 50° W, were not well-photographed and may differ in physiography as well as in albedo.

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Description

Calderas: According to Williams[14], "calderas are large volcanic depressions more or less circular or cirque-like in form, the diameters of which are many times that of the included vent or vents" and most are produced by engulfment. Calderas occur in every region of Io so far photographed. They are generally recognisable by their strong resemblance to terrestrial and martian calderas. In many cases, however, no relief can be detected and a caldera is inferred simply from the presence of a dark circular feature. The calderas are remarkably numerous, with more than five larger than 20 km in diameter for every 10^6 km^2, or over 200 larger than 20 km on the entire satellite. By comparison, the Earth, with 3.5 times the land area, has approximately 15 calderas larger than 20 km in diameter[15]. Over 5% of the ionian surface seems to be part of a caldera, either dormant or active. Where relief is discernible, the calderas are recognised as rimless depressions with steep, inward-facing scarps and relatively flat floors. Rubbly rims that characterise impact craters are absent. Although most are nearly circular, they range widely in shape; some have scalloped walls suggesting collapse about different centres, others have rectilinear outlines, and others have elongate, slot-like shapes. While most seem to have little internal relief, this may be deceptive since the viewing conditions for most of the available pictures are not ideal for discerning topography. In the highest resolution pictures, low escarpments and irregular ridges can be seen on some of the floors. Some, such as the 25 km diameter caldera at 52° S, 33° W, have floors at different levels like many terrestrial and martian calderas. Differences in elevation of the floor may also be indicated by sharply defined dark areas which are probably caused by puddling of lava.

One of the most striking aspects of the Io calderas is the associated albedo patterns (*Plate 3*, p. 784). The floors of most are very dark and the low reflectivity of many is accentuated by bright haloes around the crater. The combination of dark floors and light haloes gives Io its characteristic spotted appearance in the far encounter pictures. Patterns are discernible on many of the dark floors. For example, a faint concentric banding can be seen in the 35-km diameter caldera at 23° N, 227° W and the 200-km diameter, irregular dark feature at 5° N, 305° W (*Plate 1*, p. 782) contains a 60-km diameter bright 'island' and numerous bright spots within the dark area. Although most calderas are darker than their surroundings, some are brighter as at 28° S, 338° W and at 62° S, 20° W, and some have an albedo almost identical to their surroundings as at 74° S, 340° W and 50° S, 12° W. Many calderas have complex albedo patterns in which sharply demarcated, irregular dark areas contrast strongly with the rest of the floor. The dark areas commonly terminate abruptly against the caldera walls. Diffuse dark and light markings are also common.

Very few of the Io calderas seem to be associated with an edifice of significant vertical relief. The low relief seems to be real and not simply an artefact of illumination and viewing conditions since even calderas seen near the terminator show little evidence of an edifice. Some vertical relief must exist, however, as flows seem to radiate away from most of the calderas. The ionian calderas are thus similar to such large terrestrial calderas as Valles, New Mexico, and the Aso and Aira calderas of Japan which have little vertical relief and are associated with vents that produced large volumes of ash. They are distinctly different from the Hawaiian calderas and those of the Tharsis Montes on Mars, which are located at the summit of large edifices built mainly of lava.

Flow patterns: The pattern of flows around the calderas is difficult to discern because the topography of the flows can rarely be distinguished and the albedo patterns are complex. Both dark and light flow-like markings seem to be superimposed on a surface which itself has distinct markings. Furthermore, the presumed flows in some cases seem to have altered the albedo of the former surface. The plumes cause further complication by masking the surface markings with diffuse dark and light overlays. However, certain patterns can still be discerned.

In many cases well-defined dark, linear markings, with lobate margins radiate from a central dark caldera (*Plate 2a*, p. 783). These are presumed to be dark flows because of their association with a caldera and the strong resemblance to the pattern of terrestrial lava flows. Examples are at 35° S–353° W, 85° S–340° W, 37° S–5° W, 15° S–322° W, and 52° S–330° W. In all these cases the flows are long and narrow and the array is markedly radial. There seems to be a succession of flows, the more recent flows being darker. The pattern is very similar to that on the flanks of large terrestrial shield volcanoes such as Mauna Loa (Hawaii) and Fernandina (Galapagos). One respect in which ionian flows appear distinctly different is that the terrain near parts of some flows is very bright. The brightenings commonly trace the edge of the flow and are clearly related to it. The general effect is as though the dark flows were backlighted.

Other dark markings around the calderas may be very broad yet still show the lobate margins characteristic of flows. Examples are at 23° N–277° W and 11° N–272° W (*Plate 1*). These flows resemble the long narrow ones but are broader, perhaps because the slopes over which they have flowed are relatively shallow.

Several well defined bright, lobate markings which originate at a caldera are also observed. These are interpeted as light flows. One originates at a caldera at 7° S–286° W (*Plate 2*) and appears to have flowed south-west 250 km partly flooding two other calderas. Many other calderas are surrounded by bright radial markings with sharply defined edges. These are also probably volcanic flows. Examples are at 8° S–342° W and at 29° S–328° W.

In the most common pattern of markings around the caldera, dark and light markings, while still elongated radially, interweave so that individual units cannot be traced for any great distance (*Plate 5*, p. 786). Whether we are observing light flows on a dark background, dark flows on a light background, or both, is unclear, but the presence of both light and dark flows seem likely in many cases. Such intricate albedo patterns are very common in the region between 0° and 40° S and 280° W and 20° W. They are far less common south of 40° S at the same longitudes.

Another recurrent albedo pattern is one in which a sharply demarcated bright region surrounds a caldera, as though the surface were bleached. The bright region generally has a very sharp crenulated margin, distinctly different from the bright haloes that surround many features. A good example is at 2° N–2° W (*Plate 2b*). The origin of these markings is not known. Many calderas, therefore, are surrounded by albedo patterns that suggest the presence of both dark and bright flows and some are surrounded by a well defined bright region that apparently has no flow structure. In general, no relief can be seen on the albedo features.

Positive relief features: While most calderas do not seem to be within sharply defined edifices, a variety of positive relief features are recognised. Most are puzzling and difficult to relate to terrestrial landforms. Among the more comprehensible, because of their resemblance to low volcanic cones, are two pancake-like constructions at 23° S and 26° S at longitude 345° W (*Plate 5*, p. 786). They are nearly circular, approximately 150 km across, and surrounded by a low escarpment. Each has a bright-floored small crater in the middle. The albedo of the main edifice is uniform and close to that of the surroundings. The pancakes appear to transect and thus be younger than fields of flows from nearby calderas. Similar features occur at 1° N–316° W and at 3° N–326° W. Other positive features surrounded by a low escarpment and with small central craters differ in being markedly non-circular and have ridges on the surface that trend roughly radial to the central crater.

A second type of positive feature is typified by a butterfly shaped structure at 58° S–350 °W (right-hand corner, *Plate 3*, p. 784). At the head is a caldera very similar to the other ionian calderas. The wings are formed by several broad sheets up to 300 km across, each surrounded by a low escarpment. The sheets partly overlap one another and seems to be superimposed

on a dark tail apparently comprised of flows. The sheets themselves have no distinctive albedo pattern and are very similar in brightness to the surrounding plains. Similar sheet-like features are associated with calderas at 18° S–350° W, 42° S–358° W, 10° S–295° W, and 32° S–330° W. Several tongue-like sheets, particularly near the south pole, resemble those around the butterfly but cannot be traced to any specific caldera.

Rugged mountains with high relief, best seen close to the terminator, constitute a third class of positive feature (*Plate 3*). The highest resolution view of one of these mountains at 68° S–35° W shows that the slopes of the mountain have a distinct texture formed of at least two intersecting sets of parallel lineaments. Accurate elevation measurements have yet to be made, but the relief seems large. This particular mountain has a caldera at the base of the south flank. Although the morphology of the mountain itself is not distinctively volcanic, a volcanic origin is suggested by a bright halo that completely surrounds the mountainous area. Not all the mountains have bright haloes. Some are surrounded by sheet-like deposits similar to those described in the previous paragraph.

Table 1 Location of volcanic features*

Type	Voyager Picno	Lat	Long
Long, narrow dark flows	0073J1+000	35° S	353° W
	0147J1+000	85° S	340° W
	0184J1+000	37° S	5° W
	0043J1+000	15° S	322° W
	0199J1+000	52° S	330° W
Broad dark flows	0027J1+000	23° N	277° W
	0027J1+000	11° N	272° W
Light flows	0041J1+000	8° S	342° W
	0135J1+000	29° S	328° W
Bright calderas	0073J1+000	28° S	338° W
	0073J1+000	62° S	20° W
Caldera with concentric markings	0027J1+000	23° N	277° W
Calderas with albedo of surroundings	0077J1+000	74° S	340° W
	0077J1+000	50° S	12° W
Calderas with sheet flows	0073J1+000	18° S	350° W
	0073J1+000	42° S	358° W
	0051J1+000	10° S	295° W
	0137J1+000	32° S	330° W
Pancake-like constructions	0093J1+000	1° N	316° W
	0131J1+000	3° N	326° W
Tectonic uplifts	0119J1+000	43° S	265° W
	0109J1+000	52° S	280° W

* Dark calderas are too numerous to list. For location of active plumes see ref. 10. Errors in location may be as large as 15°.

In a fourth type of positive feature, an originally smooth and level surface appears to have been upbowed and cracked along radial faults (*Plate 4*, p. 785). A smooth surface, broken only by the radial graben, slopes gently away from the centre which is generally occupied by an irregular depression connected to the radial graben. The largest is at the centre of the heart-shaped structure at 30° S–250° W, and is the source of the largest plume on Io[1]. Others are at 43° S–265° W and 52° S–280° W.

Diffuse markings: Diffuse dark and light markings are common in all the regions so far examined and are generally associated with some topographic feature. Near-vertical views of active plumes commonly show a radial dark and light pattern at the plume centre which merges outwards into a bright halo, which in some cases shows a concentric banding[16]. Although the active plume itself must contribute to the appearance from above, most of the diffuse markings are probably on the surface. Many similar markings not now associated with plumes may have resulted from previous activity.

Many calderas have diffuse bright markings. Most commonly the bright patterns around the caldera are asymmetric although some, such as those at 66° S–10° W and 74° S–0° W, are surrounded by a fairly uniform halo. In some places dark streaks, similar to those at plume centres, extend away from the caldera. Generally the diffuse halo is transected by dark deposits on the caldera floor but some caldera floors lack dark markings and instead have diffuse bright markings which appear to originate at the walls. Bright haloes are also associated with many of the dark flow-like features that radiate from some calderas (*Plate 2*). In some cases the bright markings provide a backdrop to the entire flow field, in other cases just one or two of the flows are highlighted. Small, diffuse brightenings also occur in places along the low escarpments that bound the sheet-like features associated with some calderas. As indicated above, bright haloes also occur around some mountainous features.

Diffuse dark features are less common. Several calderas have roughly circular diffuse dark markings that overlap both the wall and the surroundings. Others have roughly radial dark markings very similar to those at plume centres. Other dark markings are very dense and irregular in shape.

One striking aspect of volcanic features on Io is their extreme youth. Johnson *et al.*[4] estimates that Io is being resurfaced from plume activity alone at an average rate of 5×10^{-3} to 3×10^{-1} cm yr^{-1}, yet albedo patterns are clearly visible over much of the surface. A deposit of a few millimetres is sufficient to mask most albedo differences except where there is significant micro-relief. The calculated resurfacing rates, therefore, imply that the albedo features are at most only a few tens of years old and may be only a few months old. Topographic features may survive longer but the lack of impact craters larger than 1 km implies that 100 m of relief is removed in $< 10^6$ yr (ref. 4). Relative ages can be inferred from transection relations between different flows and between flows and calderas. In general, albedo contrasts seem to decrease with increasing age as dark areas become brighter and bright areas darken. The impression is consistent with ageing mechanisms such as steady burial sulphur rich compounds (SO_2 frost, elemental sulphur) or tephra, or physical and chemical alteration of the rock forming materials.

Discussion

In the following we will examine what the high rates of volcanic activity and apparent abundance of sulphur on and around Io imply about the type of volcanism on Io. We assume that the snapshot view of Voyager is typical of much of Io's geological past, and that it has sustained a high level of volcanic activity for much of its history. The assumption is consistent with tidal dissipation providing the required energies[17].

Are the flow-like features seen at the surface mostly silicate lavas or can they be explained solely by sulphur or sulphur-rich compounds? There is evidence to support both these hypotheses and to suggest that both silicate and sulphur rich flows may exist.

Sulphur lava could in principle be juvenile, that is, derived by separation of a sulphur-rich melt from a primitive magma. Such flows are however, unlikely. The solubility of sulphur in mafic magmas has been studied in detail[17]. The solubility depends mainly on the Fe content of the magma and the S_2 and O_2 fugacities. Depending on the value of these variables, 0.1–1% sulphur can dissolve in the silicate melt. At higher values of sulphur a sulphide melt separates out so that the silicate and sulphide melts coexist as immiscible liquids. Once this state is reached addition of more sulphur has little effect on the silicate phase relations, except insofar that Fe is partitioned between the sulphide and silicate liquids. The sulphide melt also acts as a sink for metals such as Cu, Ni, Co and Cr. Separation of sulphide globules from juvenile basalt melts was reported by Skinner and Peck[19,20] in samples from the Alae lava lake, Hawaii, and sulphide globules have been analysed from deep sea basalt[21–24]. Such sulphide melts, however, are unlikely to be the source of sulphur-rich lavas at the surface of Io. In a closed magma chamber, the sulphides tend to sink and form sulphide-rich layers such as are common at the base of many layered intrusions. During the early planet-wide melting postulated by Peale *et al.*[17] such sulphide melts may have separated out to form a core. If the magma rises to the surface, however, it tends to

degas and lose much of its sulphur, which condenses on the surface or in voids within the near surface rocks. Most terrestrial basalts thus lack sulphide globules. Exceptions are those erupted on the ocean floor, where degassing is prevented by the high confining pressure.

Accumulation of sulphur at the surface of Io probably resulted from pervasive outgassing of the interior. The results would be similar whether the outgassing occurred early as a result of runaway melting of the interior as suggested by Peale *et al.*[17] or whether the outgassing occurred more slowly as a result of progressive melting. Many of the lighter volatiles such as water would be lost to space whereas the heavier volatiles, such as sulphur would mostly condense in the near surface regions[4]. The net result would be interbedded lavas and sulpur-rich deposits near the surface. If subsequent volcanism was of sulphur alone then the sulphur would ultimately segregate into a near surface layer. If, however, both silicate and sulphur volcanism continued, as we suggest, then the near surface stack of interbedded lavas and sulphur-rich deposits would be maintained. Near surface volcanism would result in progressive burial of the interbedded sequence until temperatures are reached such that the sulphur melts or volatilises. Peale *et al.*[17] estimate that silicate melting temperatures are reached at depths of 18 km. This implies that sulphur is above its melting temperature below depths of 3–4 km if the crust is primarily silicate. In the upper part of the crust therefore, sulphur would be relatively mobile, being continually transported upwards from the base of the interbedded sequence to be redeposited in the cooler upper parts or on the surface. The silicates may also be recycled. Continued eruption of silicates onto the surface or into the near surface rocks could result in burial of the silicates to depths where they too would be melted and remobilised. The silicates may thus also be recycled, although on a time scale considerably longer than the sulphur. Such a model is consistent with many of the volcanic features seen on the surface.

Many of the flows on Io's surface may be composed of sulphur. Eruptions of molten sulphur have been described from the Siretoko–Iosan volcano in Japan[25] and a small sulphur flow occurs on the flanks of Mauna Loa, Hawaii[26]. During the 1936 Siretoko eruptions, chocolate brown sulphur erupted from a vent on the side of the volcano and fed a brown flow, 20–25 m wide, 1.4 km long, and up to 5 m thick. The flow travelled down a valley that was filled with previous sulphur flows to a depth of several tens of metres and sulphur sublimates cover a considerable area around the vent and adjacent to the flow. The Hawaiian flow is only 5 by 10 m across and up to 45 cm thick, but it may have been partly covered by a younger flow[26]. Both the Siretoko and Mauna Loa sulphur flows have been ascribed to remobilisation of fumarolic sulphur as ground temperatures in the fumarolic areas were raised above the sulphur melting point.

In view of the apparent sulphur-rich environment on Io, and extensive evidence of fumarolic activity, an analogous origin for flows on Io is reasonable. Some of the flows are over 300 km long. Whether sulphur could continue to flow over such distances would depend partly on whether a crust of low thermal conductivity forms over the flowing surface and whether lava tubes form as with basaltic lava flows. Although Watanabe[25] described various surface textures such as pahoehoe and aa on the sulphur, he does not discuss the properties of the lava crust. Flowing basalt generally develops a crust which is extremely vesicular as a result of outgassing of the magma during cooling. The crust generally has a very low thermal conductivity which retards loss of heat from the flow. In many cases the flows crust over completely and the lava flows on a tube below the surface. As a result flow can be sustained over great distances. It is not known whether a sulphur flow in Io would vesiculate in a similar manner. Flow over long distances is, however, aided by two other factors. First, the temperature of sulphur flows is very low compared to the silicate melts. Since heat loss varies as the fourth power of the absolute temperature the rate of loss of heat from a sulphur flow would be two orders of magnitude less than from a silicate flow, all other factors being equal. Second, the peculiar viscous properties of sulphur may help sustain flow, at 200 °C the viscosity of liquid sulphur is 2.15×10^4 cP. On cooling the viscosity decreases such that at 150 °C the viscosity is only 7 cP. If the flows are sulphur then the differences in albedo between the flows may result simply from the different sulphur allotropes[7]. Although the sulphur flows at Siretoko revert rapidly to the yellow monoclinic form, sulphur flows on Io, because of rapid freezing to 130 K, could exist metastably in other states with a variety of colours and albedos[7].

An alternative explanation of the flows is to attribute them to normal lavas, probably basaltic in composition. The variations in albedo would then result from differences in the amounts of volcanic sublimates on the surface or from differential chemical alteration of the flow surfaces. Such deposits or alterations are not uncommon on terrestrial lava flows. The flow itself, including its surface texture, is little affected but its reflectivity and colour may be dramatically affected by alteration and deposition.

The relief of the ionian surface supports the hypothesis that some silicate volcanism has occurred. Several rugged mountains with relief in excess of 10 km are probably composed of silicates. More importantly, however, with respect to the type of volcanism, is the relief within the calderas. Some have steep inward facing scarps that appear to be as much as 2 km high. This implies a significant yield strength[7]. While silicates certainly have the necessary yield strengths, it is not known whether sulphur could support cliffs of such magnitude at 130 K. If not, then the amounts of silicates erupted must be at least comparable to the amounts of sulphur.

The diffuse light markings are less controversial. They are probably sublimates, principally sulphur of SO_2, and could result from either silicate or sulphur volcanism. The origin of the diffuse dark markings is less clear. One possibility is that they are formed by volcanic ash. The ash could be produced simply by erosion of the vents by gas jets that form the observed plumes. Alternatively, the ash may form by rapid degassing of near surface silicate lavas charged with S or SO_2. The anhydrous nature of Io's surface[27,28] suggests water is not involved. Unless the silicate lavas were juvenile such degassing would require that lava assimilate sulphur at intermediate depths and discharge the gas near the surface as both the pressure and the sulphur fugacity fall.

From these rather qualitative arguments we conclude that while there are strong arguments for sulphur flows on the surface of Io, silicate volcanism of the type familiar to us on Earth may also occur. The possibility of both silicate and sulphur volcanism raises some questions concerning the loss of heat from the planet. Repeated eruptions of large amounts of sulphur onto the surface would have an effect similar to increasing the thermal conductivity of the rocks which lie above the zone of sulphur melting. If tidal energy is dissipated deep within the body, that is the heat sources are all below the surface layer not within it, then the effect of increasing the thermal conductivity is to decrease the near surface temperature gradient and so increase the depth to melting of both sulphur and silicates. Large amounts of near surface sulphur would therefore tend to suppress silicate volcanism by forcing silicate magma sources to greater depths. The larger the amount of sulphur transported to the surface the greater the effect.

Johnson *et al.*[4] estimate that Io is being resurfaced from plumes at a rate of 5×10^{-3} to 3×10^{-1} cm yr^{-1}. Assuming the plumes are due entirely to sulphur vaporised at 717 K then brought to the surface and condensed at 130 K, then the rate of loss of heat by vaporisation and cooling of sulphur alone is 1.7–100 erg cm^{-2} s^{-1} at these resurfacing rates. This compares with 500 erg cm^{-2} s^{-1} required to remove the tidal dissipation energy given to Peal *et al.*[17]. Plume activity even at the upper limit of the rates given by Johnson *et al.*[4] thus leaves ample energy to drive silicate volcanism.

The lack of large volcanic edifices may result both from isostasy and the lack of pressure to pump magma to a significant elevation above the general ionian surface. High rates of volcanic

activity imply melting at relatively shallow depths and the large size of many of the ionian calderas suggests magma reservoirs with lateral dimensions greater than their depth of burial. Both these factors should result in efficient isostatic compensation. This alone may explain the lack of large volcanic edifices although the presence of several rugged mountains is puzzling. Another factor may be the lack of density contrast between the lava and crustal rocks. Magma is pumped to the top of large terrestrial shield volcanoes partly by the density contrast between the mantle and basaltic lava. On Io, however, the high rates of volcanism imply that most lava is derived by the remelting of formerly erupted materials. The composition of the lava is therefore probably similar to the surrounding rocks and the density contrast small. This, coupled with the relatively shallow depth of origin of the lava, may prevent generation of the pressure head needed to build large edifices.

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Volcanic eruption plumes on Io

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An umbrella-shaped plume detected above Io confirms that Io is volcanically active. Preliminary analyses of eight such eruptive plumes are presented.

THE detection of an umbrella-shaped plume extending about 280 km above the bright limb of Io[1] was one of the most important discoveries made during the Voyager 1 encounter with the jovian system. This discovery proves that Io is volcanically active at present, and the number and magnitude of these eruptions indicate that Io is the most volcanically active body so far discovered in the Solar System. The source of the heat which drives this volcanic activity is probably the tidal heating mechanism proposed by Peale *et al.*[2].

The Voyager 1 Io data reveal eight eruptive plumes detected on images from 4 days before to 3 days after the Io encounter. Up to 4 days before encounter the resolution was too poor to detect the plumes. In general, the eruptive plumes can only be seen near the bright limb or at small phase angles near the dark limb where they are projected against the dark sky and shine by reflected sunlight. However, most of the plumes are so faint that special processing is required to detect them against the black sky.

The Voyager 1 longitude coverage at the limb is limited. Depending on resolution, at 10° in front of or beyond the bright limb, about 10 km of a 40-km high plume would be visible against the black sky. The surface area between 10° either side of the limb covered by the Voyager 1 pictures with resolution better than 90 km per television line pair represents about 80% of the surface of Io. About 54% of the surface was seen in the limb regions at 10 km per television line pair resolution or better. Considering the faintness of the plumes we feel that only about half of the planet has been surveyed satisfactorily, and other eruptive plumes, particularly smaller ones, may have passed undetected.

Characteristics of plumes and their sources

Figure 1 shows the distribution and approximate diameters of the eight detected plumes, while Table 1 lists their dimensions, locations, and the times at which they were seen. The exact location of Plume 8 is uncertain because of limited photographic coverage but it is located along the error bar in Fig. 1. The dimensions of Plumes 5, 7 and 8 are not well established because of poor resolution and/or the faintness of the plume. Except for the faint Plume 8 at latitude −45°, all observed plumes occur within ±30° of the equator.

Plume 1, the largest, is about 1,000 km wide and 280 km high measured in both clear and UV filters. It is a highly symmetrical umbrella-shaped cloud with a central fountain about 35 km wide near its base (*Plate 5b*, p. 786). When viewed nearly vertically (*Plate 6a*, p. 788) the plume source is surrounded by an elliptical, heart-shaped diffuse area. The centre of this area contains one or two black spots from which the eruption apparently originates. Dark diffuse streaks radiate from these black spots for a distance of about 150 km. A light zone in which topographic features can be seen more clearly than in other areas surrounds the dark streaks. This light zone is surrounded by a dark diffuse region whose outer limit approximately coincides with the plume width. A light diffuse band about 90 km wide occurs on the western edge of the dark region, while on the north and east the dark zone grades outwards into a very diffuse region about 300 km wide. The dark and light diffuse zones which comprise this heart-shaped region probably represent airborne and/or surface pyroclastics. *Plate 6b*, p. 788 is a high resolution (1.5 km per television line pair) oblique image of the source region of Plume 1. In this view the sky was processed separately to show the filamentary structure in the plume off the limb of Io. The dark diffuse radial streaks which form the centre of the plume are associated with three black spots located at the northern end of a highly fractured uplift about 200 km wide. The central portion of this uplift is bisected by a graben 25–50 km wide. The largest black spot is adjacent to a basal scarp encircling the uplift and appears to be associated with, or form part of, an adjacent caldera. Two smaller black spots occur along the basal scarp and a parallel fracture, and appear to be the sources of the dark diffuse material to the west. It is not clear which of these three vents is the source of Plume 1; possibly all three are erupting simultaneously. Several smaller dark diffuse

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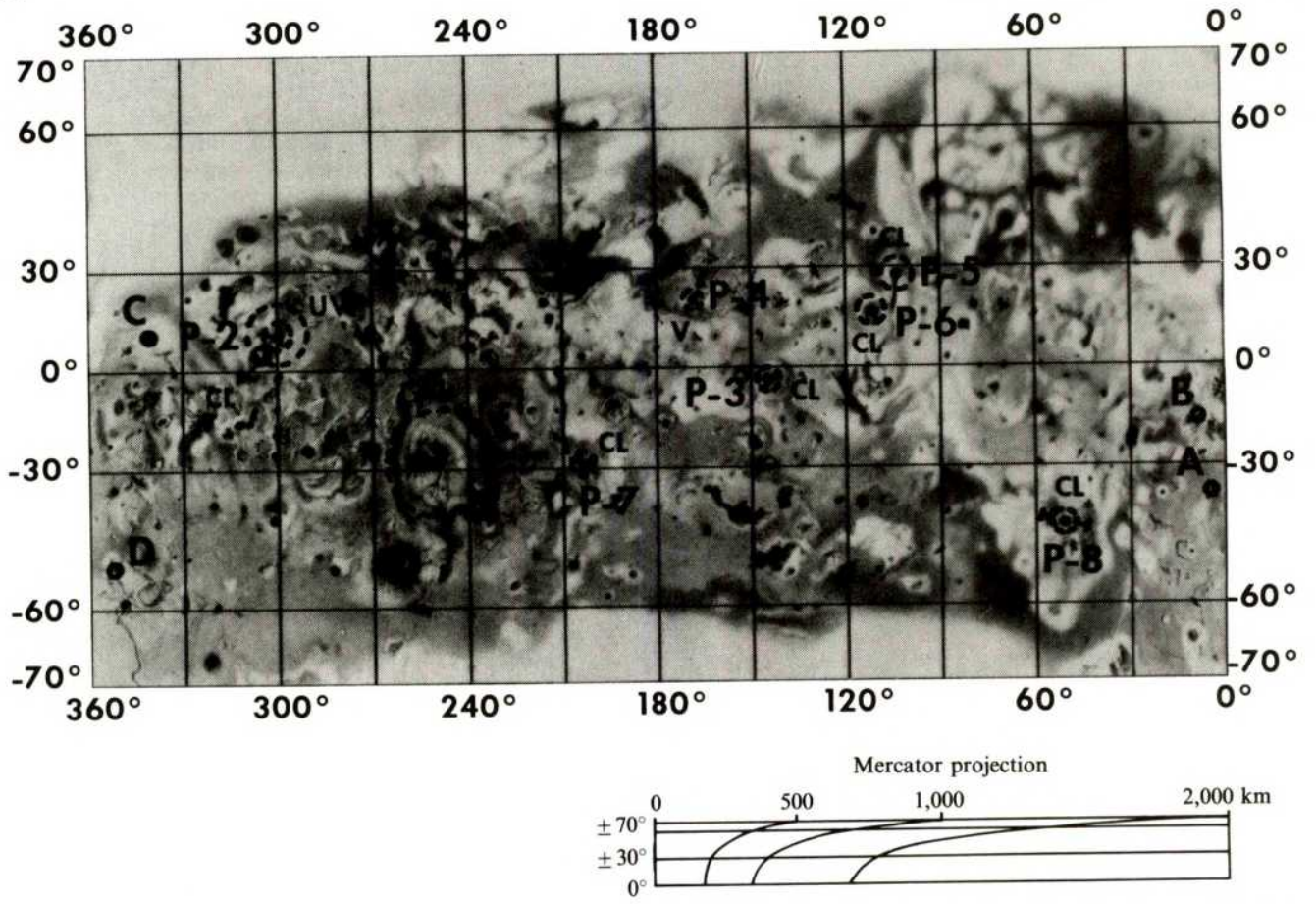


Fig. 1 The location of the eight eruptive plumes and their diameters. Four possibly active eruption sites are indicated at A–D. The latitude/longitude grid is very approximate and contains errors of 10° or more. ●, Eruption plume source; ○, plume diameter; CL, clear filter, UV, UV filter; V, violet filter; ○, possible eruption.

areas, some with bright spots as their source, are probably eruptive centres which may have been active when the picture was taken. The large scale symmetry of the plume suggests that the geometry of the vents is very simple, suggestive of pipe vents or short fissures. All are located along fractures or the basal scarp which itself is probably a fault scarp.

The character of Plume 2 (*Plate 5b*) is different from that of the other plumes. It is a very diffuse cloud lacking the umbrella shape or columnar fountain characteristic of most other plumes. Viewed through the clear filter it is about 100 km high and 210 km wide, but in UV light it is more than twice as large (Fig. 1). Therefore, this plume shows two components: a central core seen in visible light surrounded by a much fainter envelope of material which scatters preferentially in the UV. The central core is probably dark pyroclastic ejecta while the faint envelope may be a reflection from extremely fine particles or possibly resonance scattering by gas[3]. Other plumes such as Plumes 1 and 4 were also observed in the UV but did not show evidence of larger envelopes in this filter. *Plate 5a*, p. 786 is a moderately high resolution picture (~3 km per television line pair) of the source region of Plume 2. The eruption is associated with a black linear strip (A in *Plate 5a*) about 175 km long and 25 km wide. Dark jets of material, probably pyroclastics, appear to be erupting from the west end of the black strip. These streaks form a dark patch with a maximum dimension of about 200 km. This coincides approximately with the width of the core of the plume as viewed in visible light. Although the west end of the black strip seems to be the main eruption site, smaller dark and light streaks issuing from the northern edge of the strip strongly suggest that eruptions occur along its entire length. A bright zone about 420 km in diameter surrounds the main eruption site. Another brightish region is associated with the northeastern part of the black strip. Surrounding these bright zones is a very diffuse region about 800 km in diameter. The outer edge of the plume viewed in UV light lies about halfway between the bright zone and the outer limits of the darker diffuse region. Plume 2 appears to be the result of a fissure eruption occurring along a fracture 175–200 km long. This very long fissure eruption may account, at least in part, for the lack of a columnar fountain and the very diffuse appearance of the plume viewed in profile. The dark material forming the black strip has an irregular outline suggesting it is made up of lava flows which have issued from the fissure.

Plume 3 is a very symmetrical umbrella-shaped plume about 250 km wide and 70 km high (*Plates 5b, 6a and 7c*, pp. 786–789). It is associated with a dark circular area surrounded by a bright white ring shown in *Plate 7b*, p. 789. The brightest part of the central fountain is about 15 km in diameter and coincides with a dark spot at the centre of the inner ring (arrow in *Plate 7a*). The diameter of the plume is about the same as that of the inner dark ring, suggesting that most of the deposition from the plume is concentrated within this area. *Plate 7a* is an oblique view of the eruption showing jets of material being ejected on ballistic trajectories. Some of this ejecta clearly lands on the white ring. Whether the bright ring surrounding the source region is associated with this eruption or is an earlier deposit is not clear. Johnson *et al.*[4] speculate that bright rings may be SO_2 frost deposited from the volcanic gas. The narrowness of the central fountain (~15 km) and the almost perfect symmetry of the plume suggest that this eruption is either a short fissure or more probably a pipe vent eruption.

Plumes 4–8 are not as well documented as Plumes 1–3. Plume 4 (*Plate 5b*) is a high (~95 km), relatively thin column capped by a diffuse asymmetrical cloud elongated to the north. This plume has the largest height-to-width ratio (1.2) and is associated with the southern end of a dark band where dark radial streaks are surrounded by a lighter diffuse region (*Plates 6a and 7b*). Plume 5 was photographed only at very low resolution or when it was

Table 1 Voyager 1 plume observations

Plume no.	Location* lat/long	No. of observations†	Time of first observation	Time of last observation	Duration	Plume* width (km)	Plume* height (km)	Central fountain* width (km)	Plume height/ plume width
1	−29°/249°	4	−16.9h	+3d 1.4h	3d 18.3h	10000(CL)	280(CL)	35(UV)	0.3
2	+10°/300°	8	−3d 6.0h	+3d 1.4h	6d 7.4h	210(CL)	100(CL)	—	0.5(CL)
						585(UV)	210(UV)		0.3(UV)
3	−4°/144°	4	−1d 3.8h	−9.3h	18.5h	250(CL)	70(CL)	15(CL)	0.3
4	+21°/169°	2	−1d 1.6h	−5.6h	20.0h	75(V)	95(V)	20(V)	1.2
5	+27°/105°	4	−1d 11.3h	−11.1h	1d 0.2h	200(CL)	80(CL)	?	0.4
6	+17°/111°	3	−1d 9.9h	−11.1h	22.8h	250(CL)	80(CL)	60(CL)	0.3
7	−29°/203°	2	0.0h	+0.5h	0.5h	180(CL)	120(CL)	?	0.6
8	−44°/ 52°	3	0.0h	+2.0h	2.0h	150(CL)	70(CL)	15(CL)	0.4

CL, clear filter measurement; UV, ultraviolet filter measurement; V, violet filter measurement.
* These values are very approximate pending additional data reduction and more accurate measurements.
† Each observation may be one image or a sequence of several images taken through different filters.

barely protruding above the limb. It has about the same dimensions as Plume 6 and is located about 300 km to the north-east. A dark diffuse region surrounded by a lighter halo is the source area of this plume (*Plate 7b*). Plume 6 is unusual in that it has a very broad central fountain about 60 km wide and 80 km high, surrounded by a diffuse cloud about 250 km wide (*Plate 5b and 7a*). This plume is located over a broad linear black band surrounded by a dark diffuse region about 200 km in diameter (*Plate 7b*). This dark region is ringed by a light area which in turn is surrounded by a very diffuse circular region with an albedo similar to that of the inner ring. The width of the central fountain is similar to the projected (line-of-sight) length of the central black band suggesting that Plume 6 is the result of a fissure eruption fountaining along the length of the black band. The width of the diffuse cloud surrounding this fountain is approximately the same as the outer diameter of the bright ring. Plume 7 (*Plate 5b*) is a tall (~120 km) relatively thin column with a diffuse top about 180 km wide. It has the second largest height-to-width ratio and is associated with a three-component, black diffuse region best seen in *Plates 6a and 7a*. The northern and southern components are circled by a bright ring which in turn is surrounded by a darker diffuse region about 400 km in diameter. This plume has not yet been located accurately enough to determine which of the three components is the eruptive vent. However, both the northern and southern components seem to have dark jet-like streaks and surrounding bright rings suggesting they are the most likely sources. Plume 8 (*Plate 5b*) is very faint and was repeatedly viewed on images with nearly the same viewing geometry. The difficulty in triangulating from these images and the poor resolution coverage of the source region make the location of this plume uncertain (Fig. 1).

Other possible eruptions

All plume sources are characterised by: (1) a very dark or black central area consisting of radial to sub-radial jet-like streaks; (2) an irregular to circular bright ring surrounding the central core; and (3) a very broad diffuse region surrounding the bright ring. Numerous regions, other than plume sources, display these same characteristics. Several of these areas are indicated by arrows in *Plate 7b*. Some of these regions, comparable in size to plume sources, were photographed at the limb but show no plumes. Most were not viewed at the limb and may or may not have been active during the Voyager 1 encounter. Four possibly active eruption sites photographed at high resolution are indicated in Fig. 1. All are characterised by dark jet-like streaks very similar to those associated with Plume 2 and photographed at comparable resolution or better. None of these areas was seen at the limb. Furthermore, numerous small dark or bright diffuse spots and streaks seen on high resolution pictures (better than 4 km per television line pair) may have been active during the encounter. Several of these are shown in *Plate 6b*. The presence of eight enormous eruptions occurring simultaneously almost certainly implies that small eruptions below the detection limits of the television system were also taking place.

There is evidence that at least one region on Io changed substantially during the Voyager 1 flyby. The appearance of the region surrounding Plume 4, particularly to the north, has changed dramatically during the interval between the shuttering of the pictures shown in *Plate 7a and b*. In *Plate 7b* numerous black spots and bands to the right of A are no longer visible in *Plate 7a* taken 5.5 h later. These changes may be due to obscuration of surface detail by airborne or surface eruption products, or they may be a phase effect due to a difference in the scattering properties of the material in this region. *Plate 7a* was taken at a phase angle of 2.5° while *Plate 7b* was taken at a phase angle of 14.8°. These changes are unlikely to be solely the result of the eruption of Plume 4 because of its small size and great distance from the altered areas (see Fig. 1). If this obscuration is caused by an eruption then its source is probably at or near the hub of the dark radiating bands. Furthermore, it must have been either initiated or intensified some time during the 5.5-h period between *Plate 7a and b*. Plume 4 was last seen about 5 h after *Plate 7a* was taken. An eruption large enough to cause these changes should have produced a plume large enough to be visible on that picture. The absence of such a plume suggests that either a large eruption began and terminated within a 10-h period or that the changes are indeed the result of a phase effect. Comparative photometric studies of plumes and this region may help decide between these alternatives.

Duration of eruptions

Eruption plumes were observed by Voyager 1 for 6.5 d; the time during which the resolution was sufficient to detect them. However, not all plumes were observed for this period of time because of limited limb coverage and poor resolution. Table 1 shows the relative sequence of plume observations. Only Plume 2 was observed periodically over this entire period. In all instances where plumes were observed on the limb over several days no detectable changes were seen in the size or intensity of the eruption. However, observations of Plume 3 against Io's disk do suggest a change in the eruption rate between the vertical view in *Plate 7b* and the oblique view in *Plate 7a*. Plume 3 in *Plate 7a* appears darker and more optically thick than in *Plate 7b*. If these changes are real and not an effect of the changing viewing geometry then this implies a large change in the eruption rate over a period of 5.5 h. Plume 3 was observed erupting on the limb about 15 h before *Plate 7b* was acquired and about 2 h after *Plate 7a*.

In an attempt to place limits on the extent and duration of this volcanic activity Voyager 2 will observe Io intermittently for 4 d at a range of $4.76–1.38 \times 10^6$ km, with a corresponding resolution of 88–26 km per television line pair. Io will be imaged continuously for 6.5 h at a range of 1.13 to 1.19×10^6 km with 21 km per television line pair average resolution. Plumes 5 and 6 will cross the bright limb during this period.

Age of recent eruptions

Although most of the numerous circular diffuse areas with black centres may not have been active during the Voyager encounter, they almost certainly represent the former sites of relatively

recent eruptions. In some cases their relative age can be determined by superimposing one diffuse ejecta deposit on another. For example, in *Plate 7b* the ejecta deposit of the eruption site just north of Plume 3 overlies that of the eruption site to the north, indicating that it is younger.

An estimate of the upper limits of the absolute age of relatively recent eruptions can be determined by the volcanic resurfacing rate estimated by Johnson *et al.*[4]. From the lack of impact craters and from calculations for the observed volcanic eruptions they estimate a global deposition rate of about 1 mm yr^{-1} or more. Of course, the local deposition rate will be much greater or less than this value, depending on whether the area is close to or remote from an eruption site. Based on the relatively limited limb coverage, the observed eruption plumes seem to be concentrated within ±30° of the equator. Also, the eruptions appear to be more or less randomly distributed with respect to longitude (Fig. 1). This distribution may be linked in some complex way to body tides on Io. If the equatorial concentration of eruptive plumes is real, then either the intensity or frequency of eruptions, or both, are greater in the equatorial regions than in the polar regions. This would imply that the average deposition rate in the equatorial areas is greater than that in the polar regions. The dark polar regions and light equatorial areas may be partly a result of this difference in deposition rate; the lighter equatorial areas resulting from a greater deposition rate of light pyroclastics and sulphur compounds. This suggests that on average the surface in the equatorial regions is younger than that in the polar regions.

A more or less uniformly distributed layer of pyroclastics ≤1 cm thick should be sufficient to make the boundary between the diffuse ejecta deposits associated with eruption sites and the surrounding terrain indistinguishable. Therefore, if the depositional rate estimated by Johnson *et al.*[4] is applicable to at least the equatorial regions, then on average the eruption sites with well-defined boundaries, such as those indicated in *Plate 7b*, must be extremely young (possibly ≤10 yr). The large areal dimensions of these deposits and their variation in colour and albedo suggest that large-scale changes in colour and albedo patterns take place over relatively short periods of time; perhaps a few years or shorter. Integrated full-disk variations of up to 10% are consistent with the historical data[5].

Conclusions

The largest of the eight volcanic eruption plumes (Plume 1) is about 280 km high and 1,000 km in diameter. When viewed nearly vertically, the eruption sites are characterised by a central dark or black irregular to circular spot usually with radiating dark jet-like streaks. This is surrounded by a series of broad light and dark diffuse rings. The morphology of the plumes seems to be related to the dimensions and geometry of the eruption vent. Very diffuse plumes with no discernible central fountain (Plume 2) or a very broad one (Plume 6) are probably associated with long fissure eruptions. Highly symmetrical, umbrella-shaped plumes with relatively narrow central fountains (Plumes 1 and 3) may be related to either short fissure or pipe vent eruptions.

Many areas on Io display the same surface characteristics as those associated with plumes, suggesting they are also active or former eruption sites. Some of these were viewed at the limb but showed no plumes; others were not photographed at the limb and may have been active during the encounter. Most of the larger of these eruption sites seem to occur in the equatorial regions. Even if these sites were not active during the Voyager 1 encounter, they are all probably the locations of relatively recent eruptions. The pyroclastic deposition rate estimated by Johnson *et al.*[4] suggests that they may be, on average, 10 yr old or less.

The appearance of the region north of Plume 4 changed significantly during a 5.5-h period. These changes may be due to a phase effect caused by different scattering properties of the surface in this region, or to obscuration of surface detail by eruption products from an eruption which took place during this period. If these changes are due to an eruption then the time coverage suggests that it was initiated or intensified, and then terminated or abated during a 10-h interval. This, with the long duration of other plumes, suggests that individual eruptions may be both short-lived and long-lived.

All of the observed plumes except Plume 8 are located in the equatorial regions between ±30° latitude. If this distribution is real then the intensities and/or frequency of eruptions is greater in the equatorial than in the polar regions. It follows that the equatorial surfaces are generally younger than those in the polar regions which in part may account for the difference in albedo between these two regions.

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Erosional scarps on Io

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Irregular or fretted scarps on Io are similar to those found on Earth and Mars. A sapping mechanism involving liquid SO_2 is proposed to explain these complexly eroded terrains on Io.

THE surface of Io exhibits a complex planetwide array of scarps that are typically hundreds of kilometres in length and estimated to be hundreds of metres high. At least four types of scarps can be recognised: (1) circular, semicircular to rectilinear scarps that encompass volcanic collapse depressions or calderas; (2) arcuate to digitate or locally bulbous scarps along flow fronts that emanate from calderas and other vents; (3) long, straight to curvilinear tectonic scarps and narrow linear valleys that are interpreted as normal faults and graben; (4) irregular or fretted erosional scarps in layered terrains. The first three types of scarps are described in accompanying papers and will only be discussed here incidentally to the erosional scarps. Io and Mars are the only bodies other than the Earth which show complexly eroded scarps.

The present consensus about the fretted terrain on Mars is that it formed by internal sapping processes related to the melting of ground ice and the release of water to the surface[1,2]. The water-poor, sulphur-rich surface of Io as revealed by Voy-

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ager 1[3] must have evolved very differently than the surface of Mars. However, there are many similarities in the erosional patterns observed. Mechanisms that require external activity such as fluvial and eolian processes are not considered likely for Io because of the exceedingly tenuous atmosphere[4] and low surface temperatures. The extreme youth of Io's surface[5] suggests that sputtering by particles in the jovian magnetosphere is also inadequate to explain the erosional features observed.

Description of scarps and erosional patterns

The most conspicuous stacks of layered terrain and associated erosional scarps lie in the south polar region of Io (see *Plate 3*, p. 784). Caldera scarps, flow-front scarps and tectonic scarps are also present. The flow-front scarps and tectonic scarps both bound flat-topped plateaus, the largest of which extend to $\sim 10^5$ km^2. The surfaces of most of the plateaus appear to be similar to those of the surrounding lowland plains. Interspersed with the layered terrain and locally forming at its expense, are circular-to-irregular calderas, some of which are surrounded by either flat-topped or hummocky scarp-bounded flows. Thus, some combination of surface flow and tectonic processes is apparently required to explain these stacked deposits.

Our initial speculation was that the regional transfer of volatiles, probably from Io's eruptive plumes[6,7], condensation and subsequent sublimation were involved in the formation and subsequent erosion of the polar plains. The Voyager 1 infrared spectrometer (IRIS) discovered a tenuous SO_2 atmosphere near a plume in the equatorial region[4,8], and the observed partial pressure of the SO_2 (~ 0.1 μbar) is thought to be in equilibrium with SO_2 frost on the surface in the equatorial region. Furthermore, SO_2 frost has been suggested as the cause of an absorption feature near 4.1 μm seen in ground-based spectra[9,10]. The equilibrium temperature at the poles is colder by many tens of degrees than at the equator, and with time the poles should act as cold traps. Some of the SO_2 gas produced in the eruptive plumes at lower latitudes should eventually migrate to and condense out in the poles. Thus, the polar plains and the higher scarp-bounded plateaus are probably abnormally rich in SO_2 just as the polar regions on Mars are rich in CO_2. However, sublimation of SO_2 to explain the erosion pattern in the polar regions of Io is not consistent with the geological relationships observed.

Transection and superposition relationships between individual scarps indicate a complex history of deposition, faulting and erosion. Complex cliff retreat patterns can be seen along the present edges of what are inferred to have been both tectonic and flow-front scarps (*Plate 3*). Extensive stripping back of the original surfaces is observed within the stacked layers. The scarp that surrounds this unit shows scalloped patterns consisting partly of intersecting semicircular alcoves. Somewhat rounded prongs stand out from the erosional fronts, and wedge-shaped reentrants contribute locally to scarp irregularity. Long gash-like to sometimes slightly-sinuous valleys also extend into the edges of the layered terrain. Scattered, highly-irregular to near-equidimensional erosional remnants that are now completely separated from the main erosion front are also present. They consist in part of ragged, generally elongate mesas with flat tops which are concordant with the upper surface of the layer undergoing erosion. Even more eroded, elongate to equidimensional rounded hills of lower relief are scattered within the field of mesas near the centre of *Plate 3*. These mesas and hills are analogous to terrestrial inselbergs in their relation to the main scarp and are evidence of some type of pediplaination process on Io. The vague remnants of similar but more subdued scarps from beneath younger layers attest to multiple cycles of deposition and erosion.

Layered and eroded or fretted terrain is not restricted to the south polar region of Io. Smaller patches of flat-surfaced, layered terrain surrounded by lower but similarly eroded scarps are present in the mid-latitude and equatorial regions. The apparent lower relief of these scarps, however, may be a product of poorer illumination and viewing geometry of these pictures. Well-developed tracts of layered and eroded terrain occur near 10° S, 260° W under somewhat more favourable illumination, but outliers or inselbergs are absent, suggesting that the intensity of erosion falls off away from the poles. Where volcanic vents are tightly clustered and digitate flows of the type described by Carr[11] are abundant, layered and eroded terrain is not particularly evident.

The upper centre of *Plate 3* (near 50° S, 340° W) shows a complex stack of layered units associated with a dark-floored caldera. The uppermost layers show bulbous flow-front morphology and their tops are marked by orientated grooves and ridges, indicative of the flow of relatively fresh viscous materials. The lower units have more planar tops and are surrounded by low reticulate scarps marked by small angular reentrants. These scarps seem to be partly controlled by local structure and to be in an early stage of erosional retreat. Of importance with regard to the problem of scarp retreat on Io is the presence of the small white diffuse 'splotches' that emanate from an eroded section of one of the lower scarps. Earth-based spectra, along with IRIS data, suggest that they are frozen SO_2 on the ground[4,9,10]. Similar features are present at the bases of most of the scarp types recognised on Io, and they are apparently related to alcoves and notches within the scarps themselves. Another significant relationship near the upper centre of *Plate 3* is the dark, 100-km long digitate flow that comes from the base of the lowest scarp in the stratigraphic sequence. This flow, unrelated to an observable caldera, is evidence of the presence of liquid material at shallow depth beneath the layered terrains.

Interpretations

Preliminary calculations indicate that ion bombardment from Jupiter's radiation field and consequent sputtering effects can remove only about 100 m of material in about 4×10^8 yr, an insignificant rate in view of the resurfacing rate[5]. The scarp erosion patterns described here can be explained more realistically by internal sapping and the release of either volatiles or liquids to the surface. On Earth, where water is the dominant agent of erosion, spring sapping combined with mass wasting has an important role in the development of alcoves and reentrants in the cliffs of arid regions[12]. Carr[2] has proposed an internal mechanism for Mars whereby water stored in the subsurface can be liquified and on bursting out on the surface will contribute to scarp retreat and even produce the fluvial features observed. No collected runoff or eolian activity is required in this model to account for scarp retreat.

Unlike Mars, we cannot appeal to ground ice and subsurface water to explain the fretted terrain. Io's surface is rich in sulphur and sulphur compounds and the large expanses of whitish terrain seen in the colour reconstructions probably consist either of frozen SO_2[8-10] or a very white form of sulphur[13] which has condensed out of volcanic exhalations.

Smith *et al.*[7] have shown that SO_2 and sulphur should be solid on Io down to a depth of the order of 1 km or less. Below this level a phase change occurs with SO_2 becoming liquid and sulphur remaining solid. From a depth of about 2 km or less on down to a maximum depth of perhaps 4 km, sulphur is molten[7]. These relationships provide a basis for understanding the erosional scarps and the plumes found at their base as well as those seen in caldera walls and along fresh fault scarps. The subsurface isothermal trends marking the sulphur and SO_2 phase boundaries should approximately parallel the surface topography, except in the immediate vicinity of active volcanic centres. The edges of scarps would then represent the zones of easiest release for pressurised subsurface volatiles and liquids, as the layers described are of sufficient thickness to expect phase changes at depth behind the faces of the scarps. Thus, an artesian pressure would drive liquid SO_2 or sulphur to escape through cracks or fissures along the edges of boundary scarps.

A definite flow of molten sulphur from the base of a scarp might be expected to resemble the dark, 100-km long digitate flow referred to previously. The escape of SO_2 would be more dramatic. At artesian pressures of 15–30 bar, the liquid, close to its freezing point (198 K) is forced outwards along the escape

conduit until, near the surface, the pressure drops to the triple point (1.65×10^{-2} bar). We can follow the energetics using an isentropic path in the temperature–entropy diagram for SO_2 given by Smith *et al.*[7]. At the triple point, the liquid begins to crystallise and the system expands isothermally and isobarically with the formation of SO_2 vapour. An expanding effervescent mixture will now form, with each unit volume of liquid producing 5,000 times its volume of vapour. If the vapour and crystallising liquid remain in contact, the solid–fluid mixture will continue to expand until reaching the ambient atmospheric pressure ($\sim 10^{-7}$ bar) at or just outside the vent. On reaching the ambient pressure, the SO_2 solid–vapour mixture will have a total vapour content of ~20% by mass and an exit velocity of a little more than 350 ms^{-1}. The maximum range that can be reached by SO_2 snow issuing from a vent at this velocity is about 70 km for trajectories inclined ~45° to the horizontal. Depending on the orientation of individual vents, however, much of the distributed SO_2 snow would be expected to fall within a few tens of kilometres of the scarps. This is completely consistent with the scale of the diffuse white features seen in *Plate 3* and we propose that artesian SO_2 volatilisation, solidification and ballistic deposition is the source of these features.

The most likely initial escape route would be along a fresh boundary fault. This type of scarp, which is usually relatively straight and smooth surfaced, would rapidly become scalloped, notched and irregular in appearance, due to the energetic venting of SO_2 gas and snow along its face. This sapping process would work together with slumping and other mass wasting processes to create the erosional patterns observed. The release of the aquifer would continue until local equilibrium was attained. Recharge of SO_2 in the aquifer should cause further venting and continued erosion of the scarps. This process of venting and sapping would continue until the SO_2 is locally depleted (for example, the inselbergs) in which case further cliff retreat could occur only by mass wasting working alone. We thus propose that the fretted terrains on Io and those on Mars are formed by analogous sapping mechanisms in which liquid SO_2 provides the analogue to H_2O in terrestrial (and martian) aquifers.

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The role of SO_2 in volcanism on Io

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Io and Earth are the only planetary bodies known to be volcanically active; the energetics of the eruptive plumes on Io have important structural implications and are closely linked with the presence of sulphur and SO_2.

VOLCANIC plumes observed on Io by Voyager 1 reach heights between 70 and 280 km[1,2], implying vent ejection velocities ranging from several hundreds of metres to nearly a thousand metres per second (Table 1). At least eight vents were active at the time of Voyager 1 encounter and both recent and current activity of others seems likely[2]. Although evidence for volcanism is found on the Moon, Mars and Mercury, Io is the only planetary body other than the Earth known to have active volcanism, and the impressive rate at which volcanic material is being deposited on the surface of this highly active planet has profound implications for its bulk chemical evolution[3]. Here we examine the energetics of the eruptive plumes and the implications for the structure, the ground fluid 'hydrology' and hydrological cycle of Io's crust.

The driving energy for Io's intense volcanism seems likely to be the model proposed by Peale *et al.*[4] where tidal energy is dissipated in a thin crust surrounding a completely molten interior. In this model, runaway melting takes place within a small molten core and is stabilised only when the solid mantle or crust becomes sufficiently thin to conduct away all of the tidally generated heat. Because this mechanism is sustained by the forced eccentricity of Io's orbit which arises chiefly from perturbation by Europa, it probably existed throughout much of geologic time and should continue almost indefinitely. If we assume that the current resurfacing rates[3] are applicable over geological time scales, Io's crust must have been recycled many times over while in contact, at its base, with a convective interior. Thus, early loss and eventual depletion of volatiles from the bulk composition of Io would be expected. In explaining current volcanism on Io, we must consider those materials with vapour pressures sufficiently low to have survived aeons of crustal and internal recycling.

Fundamental to our analysis is the probable presence of SO_2 frost or adsorbed SO_2 on the surface of Io[5,6] and the recognition that SO_2 is a prominent constituent of its atmosphere[3,7–9]. Noting also the discovery of ionised S and O in the Io torus[10,11], the spectrophotometric suggestion of elemental sulphur on its surface[12–15], and the absence of both H_2O bands and the broad absorption features associated with iron-bearing silicates, we conclude that SO_2 is the volatile which drives pyroclastic volcanism on Io and that sulphur and SO_2 are the primary constituents of the uppermost crust and subjacent molten zone. Other consituents are certainly present (Na and K are observed to be escaping from Io), but probably occur in minor quantities.

The mechanism that drives violent gaseous eruptions in terrestrial volcanoes is the rapid expansion of volatiles, primarily H_2O and CO_2, which may either be dissolved in the silicate magma or raised to high temperature (~1,500 K) by contact with magma intrusions. Although vent velocities of hundreds of metres per second have been observed in terrestrial volcanic eruptions[16], interaction with the terrestrial atmosphere limits the heights of volcanic plumes to a few tens of kilometres at the most. This is not the case on Io, where venting gases abruptly enter a near-vacuum environment.

Limiting velocities of a fluid or fluidised system expelled from a volcanic vent can be estimated using a simple model of adiabatic decompression of the system as it moves upwards through a passageway connecting a chamber or arbitrary depth with the surface. Assuming reversible adiabatic flow and chemical equilibrium, mass flow up the volcanic conduit can be considered to be isentropic. For such a simplified process, the thermodynamic path of the erupting material during ascent is easily envisioned on a temperature–entropy phase diagram, shown in Fig. 1 for SO_2.

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Table 1 Heights of volcanic plumes on Io

Io volcanic plumes (no.)	Maximum height above the surface* (km)	Vent velocity (ms^{-1})
1	280	930
2	100	580
2 (UV component)	210	810
3	70	490
4	95	570
5	80	520
6	80	520
7	120	630
8	70	490

* After Strom *et al.*[2].

Several qualitatively different eruption styles are possible, depending on the entropy of the system at the time the eruption begins. High velocities of the system are attained after a vapour phase is formed. This can occur when a fluid system rising from depth is decompressed below the critical point, for cases of high entropy, or when the boundary of the liquid + vapour field is reached, for cases of low entropy. It can also occur if the system remains in place until its temperature is raised (by intrusion of a hot mass) to the point where boiling begins.

If a system such as SO_2 has an entropy less than the critical point entropy when vapourisation begins, and if the vapour and liquid phases do not separate, then, on isentropic expansion during ascent of the system to the surface, vapour will continuously evolve as the system varies along an isentrope passing through the liquid + vapour field (see line ABC in Fig. 1) until the triple point (0.0165 bar for SO_2) is intercepted. At this point, remaining liquid in the system crystallises to a lower entropy solid, and vapour is added simultaneously, causing the system to expand isobarically and isothermally until all the liquid phase disappears. Further decompression along the same isentrope results in condensation of SO_2 ice from the vapour phase; complete expansion to 0 K would result in condensation of all the vapour.

Assuming complete thermodynamic equilibrium and steady-state flow, the specific kinetic energy available for fluid motion at any pressure or temperature on ascent of the system to the surface is the difference between the initial specific enthalpy, H_0, and the enthalpy of the products, H minus the specific potential energy gained by rise in a gravity field. For one dimensional flow,

$$\frac{u^2}{2} = H_0 - H - \int g\,dh \qquad (1)$$

where u is the velocity of the system; g the gravitational acceleration; and h the height of the system.
The enthalpy of the products is

$$H = xH_v + (1-x)H_{lors} \qquad (2)$$

where H_v and H_{lors} are the enthalpies of the vapour, liquid or solid phases, and x is the mass fraction of vapour present; x is obtainable from the isobaric chord ratios in Fig. 1 or, numerically, from

$$x = \frac{S - S_{lors}}{S_v - S_{lors}} \qquad (3)$$

where S, S_v, S_{lors} are the total entropy, and vapour, liquid, or solid entropies respectively.

As a specific example of flow at an entropy less than the critical point entropy, consider flow from a liquid SO_2 reservoir at 40 bar and 393 K (representing flow initiating from contact with pure sulphur on its liquidus temperature of 393 K at approximately 1.5 km depth). The entropy of the SO_2 system is 2.90 J g^{-1} K^{-1} (point A in Fig. 1) and its initial specific enthalpy is 530 J g^{-1}. Formation of vapour in the SO_2 system initiates upward movement of the two-phase (liquid + vapour) system and the material flows up the volcanic vent with continuous evolution of vapour and increasing velocity until the triple point is reached. At this point, the SO_2 constitutes a liquid–vapour fluidised system consisting of 45% (mass fraction) vapour and, neglecting small values of $\int g\,dh$, is moving at 430 m s^{-1} (Table 2). If it is assumed that the vent discharges at pressures in

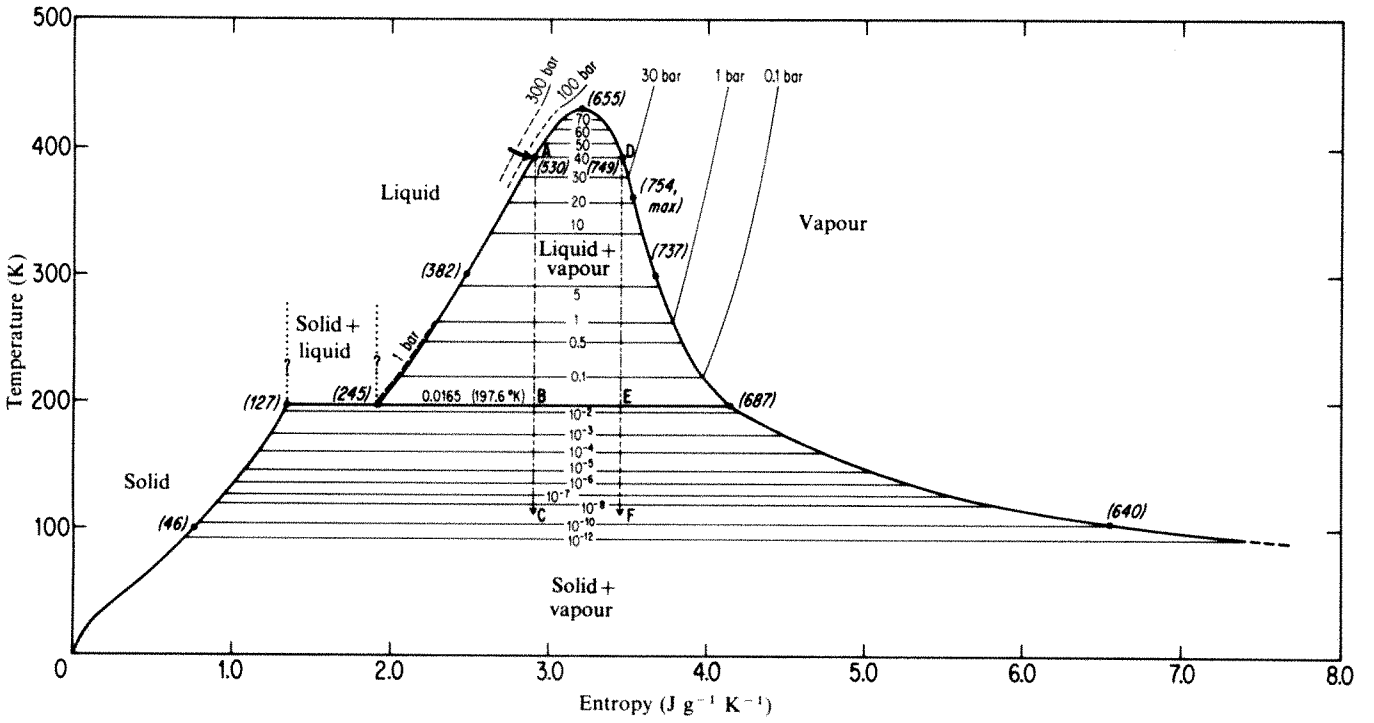


Fig. 1 Entropy–density relations in the SO_2 system. Heavy solid lines indicate the phase boundaries; heavy dashed or dotted lines are extrapolated beyond data sources; light lines represent isobars. Liquid–vapour equilibrium data from Braker and Mossman[26] adjusted to entropy equal 0.0 for the solid phase at 0 K by using the triple-point entropy and the enthalpy of fusion of Giauque and Stephenson[27]. The solid sublimation curve was calculated from the heat capacities of Giauque and Stephenson by numerical integration. The vapour curve on the righthand side was calculated from heats of sublimation provided by D. D. Wagman (personal communication). The 1 bar and 30 bar isobars and all isobars in the liquid + vapour region are from vapour pressures listed by Braker and Mossman; the 0.1, 100, and 300 bar isobars are from data of Canjai and Manning[28]. Isobars in the solid + vapour region are interpolated from vapour–pressure data provided by Wagman. Heavy arrow is pressure–temperature path of the sulphur liquidus, which intersects the SO_2 liquid–liquid + vapour phase boundary at point A. Isentropes ABC and DEF, shown with dot-dash lines, are discussed in text. Specific enthalpies are shown in parentheses.

Table 2 Mass fractions of vapour and velocities of SO_2 fluidised systems expanding isentropically from an initial state at 393 K and 40 bar

Pressure (bars)	Low entropy case (expansion from A in Fig. 1)		High entropy case (expansion from D in Fig. 1)	
	x	u(m s^{-1})	x	u(m s^{-1})
30.1	0.11	—	0.95	124
14.4	0.25	93	0.89	246
8.0	0.32	149	0.86	320
1.17	0.40	260	0.79	461
0.107	0.44	370	0.73	571
0.020	0.44	421	0.70	625
0.0165	0.55*	433*	0.75*	637*
1.9×10^{-3}	0.52	488	0.70	689
9×10^{-5}	0.48	550	0.64	752
7×10^{-7}	0.44	610	0.58	815
8×10^{-9}	0.41	680	0.52	870
4×10^{-12}	0.35	737	0.40	965
0.00	0.00	1030	0.00	1224

*Calculated at the triple point when the liquid phase has just disappeared.

the range 10^{-7}–10^{-12} bar at the ground surface (that is, that the isobars of the atmosphere are not displaced large distances upward over the discharging vent) then, from the temperature–entropy diagram (Fig. 1), it can be concluded that the triple point is reached at depth before the fluidised system emerges from the surface. On the other hand, if the surface isobars are greatly displaced by the flow (the eruption plume 'punches through' the Io atmosphere), the triple point might be reached above the ground surface. At the triple point, the liquid droplets crystallise as the system expands isothermally and isobarically with an increase in vapour content to 55%. Further expansion is accompanied by condensation of SO_2 'snow' from the vapour phase; at the time of exit to surface pressure, the vapour content of the system has decreased to 45–40% (depending on the surface pressure assumed) and, for the case considered, the velocity at the vent is 600 to 700 m s^{-1}. As the plume expands above the surface, SO_2 snow continues to form and the column continues to accelerate; if the plume were to break through the Io atmosphere and expand to approximately zero pressure and 0 K, the enthalpy converted to kinetic and potential energy would be equivalent to a velocity of 1.03 km s^{-1}, if the energy released were represented entirely by the kinetic energy term.

A different process occurs if the SO_2 system has an entropy higher than the critical point entropy on initiation of the eruption. Such a higher entropy could be attained either by (1) heating the SO_2 by incorporation of hot (sulphur) fragments or lapilli; or (2) segregation in the reservoir of the higher entropy vapour from the liquid SO_2, for example, across the 40 bar isobar in the temperature–entropy diagram; or by (3) intrusion of the heat source to shallow depth where the pressures are <40 bar. For any of these cases, the two-phase field is entered at the boundary with the vapour field rather than at the boundary with the liquid field, and isentropic expansion follows a path such as DEF shown in Fig. 1. The initial ascent and expansion of the system are accompanied by condensation of SO_2 liquid from the vapour; at the triple-point the condensed liquid freezes and further ascent is accompanied by condensation of solid from the vapour.

As a specific example of flow at an entropy higher than the critical point entropy, consider the same reservoir as discussed previously at 393 K, 40 bar, but with the vapour phase continuously segregated from the lower-entropy liquid (corresponding to the vapour side of the 40 bar isobar in Fig. 1). The entropy of the vapour is 3.46 J g^{-1} K^{-1} (point D) and its initial specific enthalpy is 749 J g^{-1}. As the vapour accelerates upwards, liquid is continuously condensed until triple point conditions are reached (point E). At this point, the SO_2 system consists of 70% vapour and is moving at 625 m s^{-1} (again neglecting changes in potential energy). At the triple point the liquid freezes, the vapour content increases to 75%, and the system expands isobarically. Further ascent of the system is accompanied by condensation of SO_2 'snow' from the vapour phase. At the time of exit to surface pressure, the vapour content of the system has decreased to 55–50% and the exit velocity is in the range 850–950 m s^{-1} (for surface pressures of 10^{-7}–10^{-12} bar). The theoretical maximum velocity obtainable (by expansion to zero pressure) is 1,224 m s^{-1}.

If acceleration of the fluidised system during expansion above the surface of Io is neglected, the maximum height above the surface reached by a parcel of the system leaving a vent at some exit velocity, u, will depend on the direction of its trajectory relative to the local vertical, which in turn is determined by the geometry of the vent itself. If we assume that the trajectory is purely ballistic, that most of the gas and entrained solids are ejected directly toward the local vertical, and that we can neglect the gravitational perturbations due to Jupiter, a given exit velocity, u, can be related to a maximum height, h, above the surface reached by the ejected material by

$$h = 2GM\,(V^2 - u^2)^{-1} - r_0 \qquad (4)$$

where V is the two-body velocity of escape, r_0 the radius of Io, M the mass of Io, and G the gravitational constant. Taking 8.91×10^{22} g and 1,820 km for the mass and radius of Io and ignoring perturbations due to Jupiter, the velocity of escape from the surface of Io is 2.55 km s^{-1}. Assuming the plume expands to pressures of 10^{-7}–10^{-12} bar, the maximum heights reached by the decompression of pure SO_2 from the starting conditions at 393 K amd 40 bar are 130–170 km for the low entropy case and 230–310 km for the high entropy case.

When computing actual escape trajectories, we cannot ignore the effects of Jupiter. For example, particles ejected vertically from the surface of Io directly towards or away from Jupiter with a velocity of 2.28 km s^{-1} would reach the radius of the co-linear lagrangian points (L_1 and L_2) ~8,700 km above the surface and thus escape into circumjovian space. A more comprehensive discussion of this three-body problem as applied to Io is given by Smyth and McElroy[17]. When treating the near space within 300 km of Io's surface, however, the magnitude of Jupiter's perturbations remains <0.003 and the effects on equation (4) are negligible.

A slightly more stringent requirement on the vent exit velocities than the height of plumes is given by the maximum distance to which material has been deposited on the surface of Io by the plumes. Each deposit records the maximum range over the life of the plume. In the case of Plume 1, the most distant limit of the recognisable deposit is 700 km (22°) from the eruptive centre. The minimum velocity at which material can be ejected to this range on a simple ballistic trajectory is given by

$$u = \left(\frac{2r_0 g_0}{\cot(\phi/2) + 1}\right)^{\frac{1}{2}} \qquad (5)$$

where g_0 is the gravitational acceleration at the surface (179 cm s^{-2}); and ϕ the range in planetocentric angular distance from the vent. For a range ϕ of 22° for the limit of the deposit of Plume 1, u is 1.03 km s^{-1}.

Significant departures from purely ballistic trajectories of material in the plumes are caused by expansion of the gas in the plumes and probably also by shocks. Expansion in the plumes provides a moderate increment of velocity after exit from the vent, whereas shocks influence the flow in the gas and details of the shapes of the plumes and trajectories of initially entrained particles[18]. We can conclude, however, that SO_2 gas and condensed particles can be accelerated to velocities required to explain the highest of the observed plumes, starting from temperatures close to the sulphur liquidus, so long as the plumes are nearly free of entrained initially cold material. A temperature slightly higher than the melting point of monoclinic sulphur at 40 bar (393 K) may be required to explain the most distant deposits of Plume 1.

Addition of cold solid material to the fluidised system expanding up the vent, by spalling of the walls of the vent, for instance, will decrease the velocity of the system. If all this material is entrained, the kinetic energy of the fluidised system

must be distributed through a larger mass. If the mass of the system is doubled, for example, the velocity will decrease by a factor of $\sqrt{2}$. On the other hand, if liquid sulphur is entrained, the decrease in velocity is very much less than for cold wall rock. This is because the enthalpy of the sulphur droplets becomes converted to kinetic energy as the fluidised system expands and cools. Entrained sulphur is probably reduced to particles comparable in size to volcanic ash produced in violent gaseous eruptions in terrestrial volcanoes of the order of 1 mm or less. Drops of this size will very quickly come to approximate thermal equilibrium with the gas, releasing both the heat of fusion, $\sim 38\ J\ g^{-1}$, as the drops freeze, and about $0.75\ J\ g^{-1}\ K^{-1}$, as the frozen sulphur ash cools to temperatures of the order of 100 K. The total specific enthalpy released by cooling liquid sulphur to 100 K is about 260 J, which is about half of the specific enthalpy released by adiabatic expansion of the entraining SO_2 gas. Hence, fairly large mass fractions of initially liquid sulphur can be entrained in the eruptive column without seriously decreasing the vent exit velocity of the plume.

Observations made by Voyager I are consistent with the interpretation that elemental sulphur is a common constituent of the surface of Io. Abundant flows of low relief, ranging in colour from red to yellow, radiate from many caldera-like features[19,20]. These flows have lengths of up to several hundred kilometres, and many apparently have very low gradients. They were formed, therefore, by fluids of viscosity comparable to or less than the viscosity of lunar basaltic lavas, $\sim 10^2$–10^4 P[21]. Molten sulphur at all temperatures is a fluid of appropriately low viscosity[20]. Moreover, because of the low melting point of sulphur, the rate of heat loss from sulphur flows would be two orders of magnitude lower than for silicate lava flows, which further facilitates the development of comparatively great flow length.

Many calderas are occupied by material of very low albedo resembling lava lakes[1,19,22]. In one case, a black horseshoe-shaped 'lava lake' ~200 km across (*Plate 1*, p. 782) has a model-derived temperature about 150 K above that of the surrounding surface[23]. Thermal observations suggest that this feature is an active lake of molten sulphur with a partial, very thin, chilled crust. Of special interest is that although this 'lake' lies within the fallout area of Plume 2, no fallout deposit is recognisable on its surface. The albedo of the 'lake' is appropriate for molten sulphur at temperatures in the range of 500 K and above [20]. The black colour of the liquid may be partly preserved in chilled crusts by quenching [20]. We interpret all the black features resembling lava lakes distributed across the surface of Io as active or recently active lakes of molten sulphur.

Except at the 'lava lakes' and possibly other active lava surfaces, the surface temperature of Io is well below the freezing point of SO_2 (198 K at 1 bar). The widely distributed white patches, illustrated elsewhere[1], could well be optically thick deposits of SO_2 frost, although a very white form of elemental sulphur has also been suggested[20]. SO_2 may also be adsorbed on sulphur (or other constituents) on the yellow parts of Io's surface. Any plume-supplied SO_2, leading to a surface pressure somewhat in excess of the mean daytime (130 K (ref. 23)) vapour pressure of solid $SO_2 \sim 1.4 \times 10^{-7}$ bar (Fig. 3), would result in precipitation of SO_2 frost and the build up of frost deposits that are stable through the day. Frost would tend to accumulate preferentially in areas that remain coldest during the diurnal temperature cycle. At a given latitude the areas that remain coldest are those with the highest albedo—the white patches themselves. This positive feedback mechanism may explain the patchy distribution of the frost and the fact that lava flows recently extruded onto the surface apparently remain frost-free (at least during the day).

Models of the crustal structure of Io, which are necessary for a theoretical understanding of the dynamics of the plumes, can be derived from an estimate of mean density of the satellite, estimates of the rate of heat production by tidal dissipation, and the interferences about the surface composition. The mean density of Io, $3.53\ g\ cm^{-3}$ (ref. 1), suggests that the satellite is composed predominantly of ferromagnesian silicates and that it has a substantial iron or iron sulphide core. The model for Io proposed by Peale *et al.*[4] before the Voyager discovery of volcanism contained a uniformly melted interior surrounded by a 20 km thick silicate crust with an implied surface heat flux of $\sim 500\ erg\ cm^{-2}\ s^{-1}$. More detailed models of the crust depend critically on the assumed ratio of free sulphur to silicates in the crust. As discussed by Carr[19], sulphur dissolved in silicate magmas exsolves as the magmas rise to the surface and, in terrestrial volcanoes, condenses on the surface or in near surface voids. This condensed sulphur is then remobilised as a melt if it is buried and reheated, a process which has occurred on Earth where sulphur lava flows have been noted. Buildup of the Io crust by repeated extrusion of sulphur-saturated silicate lavas would result in continued enrichment of sulphur in the upper part of the crust by remobilisation and upward migration of the free sulphur both as a gas and as a melt.

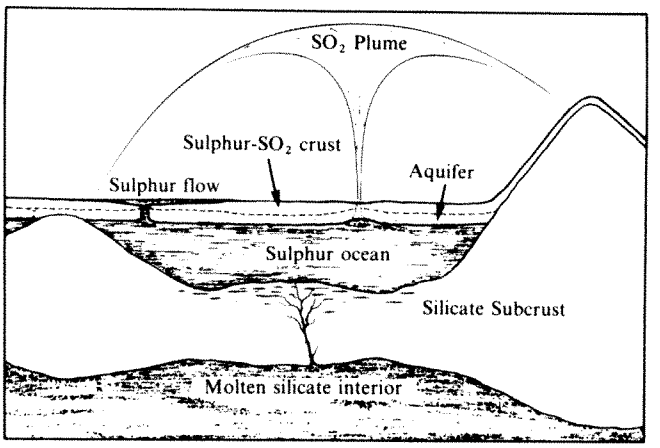


Fig. 2 Proposed crustal structure for Io. Heights and thicknesses are not drawn to scale. The uppermost layer, which also covers all solid silicate topography, is composed of a mixture of solid SO_2 and sulphur. The subjacent layer is a porous solid sulphur aquifer through which liquid SO_2 flows freely. Below this is a molten sulphur 'ocean' which rests on a solid silicate subcrust overlying a uniformly melted silicate interior. Where the upper crust is less buoyant, molten sulphur will flow over the surface. A plume is created when liquid SO_2 pours into an open vent left by a receding molten sulphur intrusion. Intruded silicate sills build up at the bottom of the sulphur ocean.

Carr [19] suggests that both sulphur and silicate lava flows are present around many caldera-like eruptive centres on Io. If this is so, the solid crust of Io consists predominately of silicates, with a subordinate amount of sulphur concentrated in the upper part. However, injection of a column of silicate magma into a solid sulphur upper crust would quickly lead to melting of a volume of sulphur larger than the volume of the silicate column; the higher density silicate magma could not then rise buoyantly through the fluid sulphur unless it were expanded by vesiculation to a density less than that of molten sulphur ($\sim 1.8\ g\ cm^{-3}$).

We prefer a model in which the upper crust consists largely of elemental sulphur and SO_2 and overlies a subjacent layer of molten sulphur possibly several kilometres thick (Fig. 2). In the upper crust, sulphur is present both as lava flows and as interstratified pyroclastic deposits. The rate at which temperature increases with depth in the upper crust depends both on the local coefficient of thermal conductivity of the sulphur and the frozen SO_2 and the value of the net outward heat flux. Of the two, the latter is by far the more difficult to estimate. In our model, we use a conservative value for the heat flux generated by tidally dissipated energy in the silicate lower crust, $100\ erg\ cm^{-2}\ s^{-1}$. Of this total, using resurfacing rates estimated by Johnson *et al.*[3], $25\ erg\ cm^{-2}\ s^{-1}$ escapes through volcanism, leaving 75 $erg\ cm^{-2}\ s^{-1}$ to be conducted upward through the crust. The lower crust (a few km to ~20 km depth) is composed mainly of a complex of silicate sills that have been intruded successively at the base of the molten sulphur layer. These intrusives are more

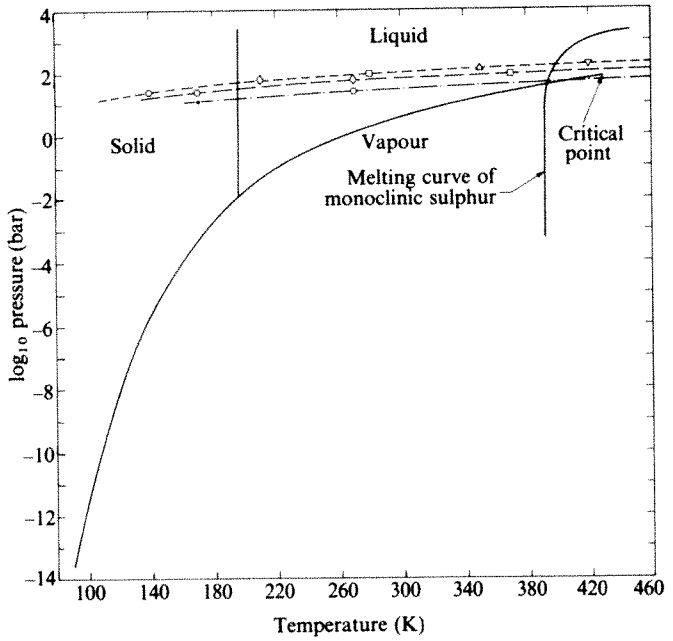


Fig. 3 Pressure–temperature phase diagram for SO_2 showing the relationship of three assumed geotherms on Io to the boundaries of the phase fields. Boundaries of phase fields are derived from data given by Braker and Mossman[26], Giauque and Stephenson[27] and Wagman. The geotherm 200 K km^{-1} is believed to be close to the actual pressure–temperature profile in the upper (sulphur) crust of Io. Depths plotted on the geotherms are calculated for densities of 1.45 g cm^{-3} up to 1 km and 1.89 g cm^{-3} below 1 km (corresponding to a porous sulphur–SO_2 permafrost zone to 1 km and a zone of porous sulphur saturated with liquid SO_2 below 1 km). ⊙, 0.5 km; ○, 1 km; ◇, 2 km; □, 3 km; △, 4 km; ▽, 5 km; ---- 70 K km^{-1}; —— 100 K km^{-1}; —·—· 200 K km^{-1}.

and more thermally metamorphosed towards the base of the lower crust where, again, complete remelting defines the base.

Recycling of the sulphur upper layer is independent of the cycling of the silicate lower layer. Temperatures of sulphur melts erupting at the surface will tend to be close to the melting point of sulphur.

The limiting thickness to which the sulphur upper layer can evolve is that at which the pore pressure at the base of the sulphur layer is just equal to the mean sulphur vapour pressure of the silicate magmas intruded at its base. Studies by Haughton *et al.*[24] suggest that silicate magmas with sulphur vapour pressure high enough for the sulphur upper layer to evolve to several kilometres thickness (S_2 vapour pressure ~100 bar) must have very low contents of FeO.

SO_2 frost precipitated on the surface of Io tends to be buried under pyroclastic fallout from the eruptive plume systems, and, if sufficiently insulated by pyroclastic deposits, it can also be buried beneath sulphur flows. Thus, as volcanic material accumulates on the surface, some of the frost deposits are trapped and carried down in the volcanic cycling of the crust. As temperatures rise with increasing depth of burial, the trapped SO_2 probably migrates to some extent and recrystallises in irregularly distributed bodies of ground ice.

Assuming the nominal heat flux given above and a temperature-dependent, thermal conductivity of $3–5 \times 10^{-3}$ W cm^{-1} K^{-1} for the permafrost zone, the 198 K isotherm (freezing level of SO_2) will lie at depths between 500 and 1,000 m; depths to this isotherm will be greatest at the poles and least at the equator. Below this isotherm the trapped SO_2 is liquid and forms an interstitial ground fluid that, if sufficiently abundant, tends to saturate all permeable strata. The SO_2 ground fluid freezes out wherever it reaches the 198 K isotherm and tends to seal the base of the overlying SO_2 permafrost zone to produce an effective 'aquaclude'.

The base of the SO_2 liquid zone will be defined either by contact with molten sulphur or by intercept of the local geotherm of Io with the SO_2 vapour field (Fig. 3). For thermal gradients of the order of 200 K km^{-1} or somewhat steeper consistent with the heat flow and thermal conductivity we have adopted, the SO_2 liquid zone will be ~500–1,000 m thick, and it will be underlain by a thin SO_2 vapour zone. If the total abundance of SO_2 in the crust is high enough, the SO_2 liquid zone may be saturated or nearly saturated throughout.

If the SO_2 liquid zone is saturated, it will be an artesian system, driven by the pressure of liquid sulphur or SO_2 vapour at the base of the zone. In this case, liquid SO_2 will flow towards any locus where fluid escapes and where there is a corresponding pressure drop[9]. If many such loci of fluid escape are distributed over the surface of Io, the regional flow of SO_2 ground fluid will tend to be up the pressure gradient, towards the shallowest parts of the 198 K isotherm. Hence, the regional flow will tend to be from the polar regions to the equator, possibly from the regions of white patches to frost-free regions and, most importantly, from regions of average thermal gradient towards active plume vents.

All but one of the known eruptive plumes on Io occur at latitudes $\lesssim 30°$ (ref. 2). Assuming that the plumes are driven by SO_2, this distribution suggests that the SO_2 liquid zone is, in fact, nearly saturated and that regional artesian flow occurs from the polar regions to the equatorial regions. If only a small fraction of the SO_2 liquid zone were saturated, most or all of this zone at low latitudes might be above the regional SO_2 liquid table and insufficient SO_2 would be available to form the eruptive plumes.

The melting point of sulphur is probably reached at depths of the order of 1,500 m below the surface. In places where convective flow of the SO_2 ground fluid can occur, the thermal gradients will be less steep and the top of the molten sulphur layer may be deeper. Where the sulphur is thick enough, the molten sulphur constitutes a sulphur 'ocean' or 'thiosphere'[20] extending downwards to the lower silicate crust, possibly several kilometres below. The solubility of SO_2 in liquid sulphur is ~0.02 (mass fraction): thus SO_2 may also be transported over global distances by currents within the sulphur ocean.

The upper crust of solid sulphur and solid and fluid SO_2 rests buoyantly on the sulphur ocean. The upper crust is buoyant because of pore space in the SO_2 permafrost zone, probably on the order of several tens of per cent, and the admixture of low density solid SO_2 (~1.6 g cm^{-3}), in the solid sulphur (~2.0 g cm^{-3}), and because of the low density of the liquid SO_2 (~1.5 g cm^{-3}) that fills the pore space in the SO_2 liquid zone. As the SO_2 liquid zone is probably sealed at the base of the permafrost, pore pressure in the liquid SO_2 can approach the lithostatic pressure. Hence, if the SO_2 liquid zone is saturated, collapse of pores is retarded until the SO_2 vapour zone is reached. Wherever the base of the sulphur upper crust exceeds the density of liquid sulphur, (1.8 g cm^{-3}) as result of loss of pore space, it will tend to founder into the sulphur ocean.

The overall buoyant freeboard of the sulphur crust is maintained by delamination or foundering of the base but probably remains small. Wherever the freeboard is negative, liquid sulphur can rise to the surface to form a sulphur volcano. Rapid rise of sulphur along a new fissure will result in chilling of the sulphur against the fissure walls. A layer of frozen sulphur along the chilled margins probably seals the fissure against inflow of liquid SO_2. In most cases, cessation of sulphur magma flow at an immature vent probably results in complete freezing of sulphur in the vent and no loss of SO_2 fluid at the surface.

At more mature conduits, where prolonged flow of sulphur has heated the vent walls (perhaps those vents that feed lava lakes) there may be a different denouement of a sulphur volcano. When local foundering at the base of the sulphur crust occurs, the crust will bob up buoyantly, and the level at the top of the liquid sulphur column will abruptly subside relative to the surface. Because the top of the liquid SO_2 zone surrounding a mature vent may be near the surface, withdrawal of the sulphur column allows the SO_2 to flow into the vent, initiating a 'phreatic' eruption. By this means a plume is born. The suggested mechanism for initiating the plumes is analogous to the mechanism of rare phreatic eruptions at Kilauea, Hawaii, which

occur after major withdrawals of lava in the caldera that allow groundwater to flow into the vent [25]. Where the flow of SO_2 ground fluid is high enough, the top of the liquid sulphur column will be eroded by the expanding fluidised SO_2 system, and the base of the expanding system may descend deep into the sulphur crust. The plume may be extinguished when the liquid SO_2 available to drain into the vent is exhausted or, more likely, when the rate of upwelling of liquid sulphur in the vent exceeds the loss to the fluidised column at the top. This could occur after the SO_2 liquid table around the vent has been deeply drawn down by prolonged discharge. The rising liquid sulphur column will then shut off the SO_2 flow.

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Dynamics of volcanic plumes on Io

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Ballistic and aerodynamic models are proposed to explain the volcanic plumes on Io, with particular reference to Plumes 1 and 3 which seem to have the same origin.

VARIOUS clues suggest that aerodynamics play a role in the volcanic plumes on Io which were revealed by the cameras on Voyager 1[1]. These are: (1) the formation of a more or less circular ring or double ring about the vent, the region of the ring being a bland one obviously covered over by frost or crystals or entrained particles from the plume; (2) the filamentary structure of the upper regions of Plume 1 (*Plate 6b,* p. 788) as shown in a picture taken near the limb; and (3) abrupt bends in each of the individual dark filaments along trajectories in Fig. 1 which shows Plume 3 taken as it was becoming visibly active on the disk.

The ballistic model

We assume that the rate of flow of gas is sufficiently small from our volcanic vent so that gas and solid particles become completely decoupled over a distance small compared with the maximum height reached by the particles and remain decoupled. In that case a convenient model starts all particles through a circular orifice with a common velocity diverging from a common point below the centre so that the velocity vectors uniformly fill a cone with a vertical axis and opening out toward the zenith. We shall show that this model does not fit the observed structure of the plumes.

Let r denote radial distance (Fig. 2), z the height above the surface, t the time, z_0 the depth of the vertex of the launch cone, v_0 the effective launch velocity at that depth, and t_0 the effective epoch of passage through the vertex. We neglect the curvature of Io's surface and the variation of its acceleration of gravity g, with height. The equations of motion and initial conditions are:

$$\left.\begin{aligned} &d^2z/dt^2 = -g, \quad d^2r/dt^2 = 0, \quad dz/dt|_{t=t_0} = v_0\cos i, \\ &dr/dt|_{t=t_0} = v_0\sin i, \quad z|_{t=t_0} = -z_0 \quad \text{and} \quad r|_{t=t_0} = 0 \end{aligned}\right\} \quad (1)$$

where i denotes the launch angle measured from the zenith.

Elimination of the time and solution for the two values of i which will dispatch particles through a given position (r, z) yields:

$$\cot i = \frac{v_0^2}{gr}\left\{1 \pm \left[1 - \left(\frac{gr}{v_0}\right)^2 - 2\frac{g(z_0+z)}{v_0^2}\right]^{1/2}\right\} \quad (2)$$

The two solutions coalesce at an upper envelope at which the square root vanishes

$$z_M = -z_0 + \frac{v_0^2}{2g} - \frac{gr^2}{2v_0^2}, \qquad \cot i_M = \frac{v_0^2}{gr} \quad (3)$$

where the subscript M denotes the upper envelope.

The outer edge of the launch cone is at i_0 and this sets a limit on the envelope at

$$r_1 \equiv \frac{v_0^2}{g}\tan i_0, \qquad z_1 = \frac{v_0^2}{2g}(1-\tan^2 i_0) - z_0 \quad (4)$$

where the subscript 1 denotes this ring.

The curve for $i = i_0$ will act as a lower bound for one of the two solutions for cot i (lower sign) out to (r_1, z_1):

$$z_m = -z_0 + r\cot i_0 - \frac{gr^2}{2v_0^2}\operatorname{cosec}^2 i_0 \quad (5)$$

where the subscript m denotes this lower bound for populated double solutions for i. This lower bound should provide an internal surface within the plume. Beyond and below (r_1, z_1) the surface equation (5) becomes an outer bound on the plume terminating at $(r_m, 0)$:

$$r_m = \frac{v_0^2}{g}\sin i_0\left[\cos i_0 + \left(\cos^2 i_0 - \frac{2gz_0}{v_0^2}\right)^{1/2}\right] \quad (6)$$

Should z_1 lie below Io's surface, the surface, equation (3), would terminate at $(r_M, 0)$:

$$r_M = \frac{v_0^2}{g}\left(1 - \frac{2gz_0}{v_0^2}\right)^{1/2} \quad (7)$$

The surface, equation (5), also has an inner intersection with Io's surface at the radius r_p, of the orifice:

$$r_p = \frac{v_0^2}{g}\sin i_0\left[\cos i_0 - \left(\cos^2 i_0 - \frac{2gz_0}{v_0^2}\right)^{1/2}\right] \quad (8)$$

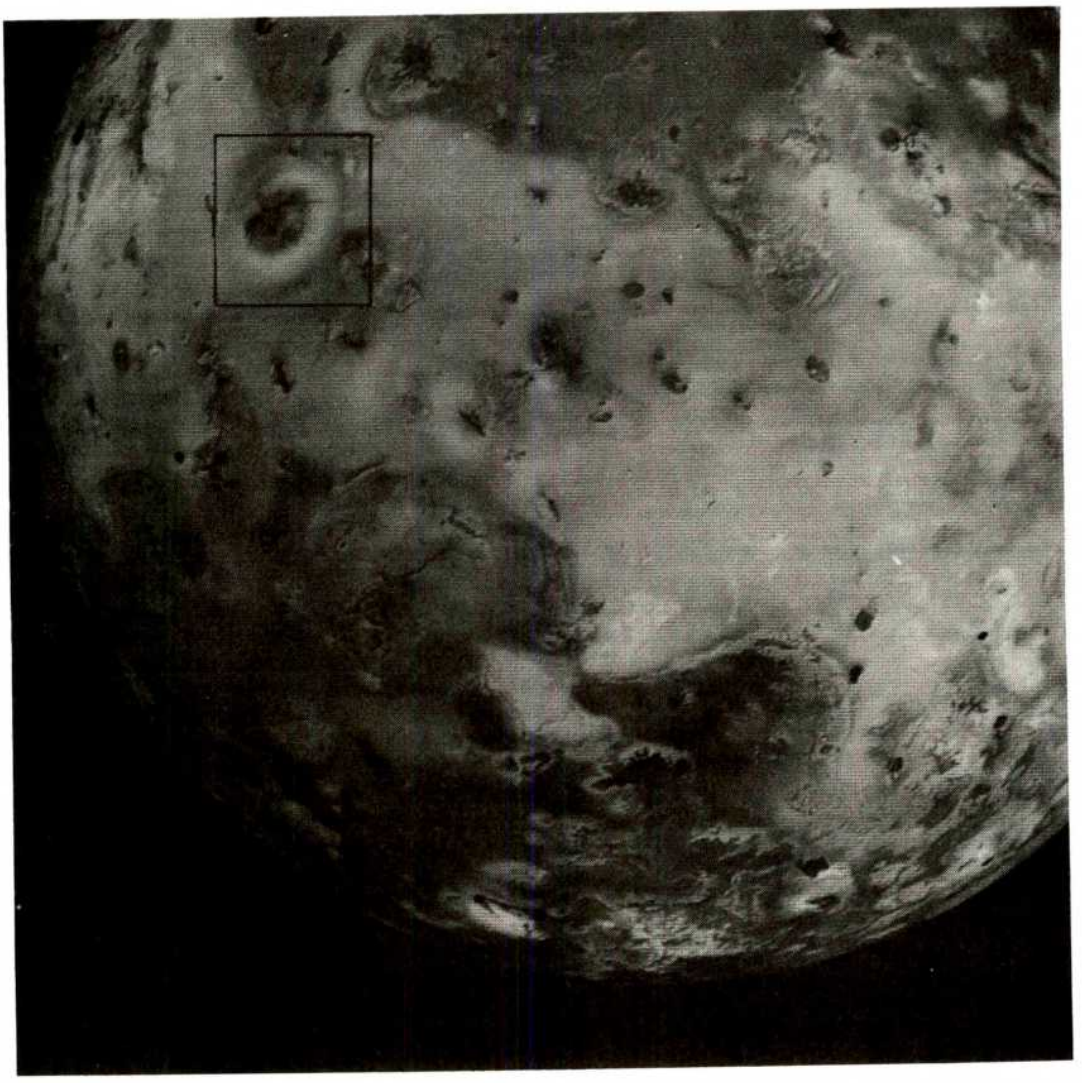


Fig. 1 Box shows Plume 3 at onset of activity as seen from Voyager 1 at 1979 March 4 d 22 h 38 min 12 s UT (FDS 16372.360).

This is the radius of the base of the stalk of the plume.

Measurements of images obtained by the imaging system of Voyager 1 yield the outer radius, r_M or r_m, the radius of the stalk of the plume, r_p, and the height of the plume, h_p (z_M at $r=0$):

$$h_p = \frac{v_0^2}{2g} - z_0 = \frac{v_p^2}{2g} \tag{9}$$

where v_p is the velocity at departure from the vent. Equations (7), (8) and (9) have the solution

$$\tan i_0 = \frac{2h_p}{r_m + r_p} - \left[\frac{4h_p^2}{(r_m+r_p)^2} - \left(\frac{r_m - r_p}{r_m + r_p}\right)^2\right]^{1/2},$$

$$\frac{v_0^2}{g} = (r_m + r_p)\,\mathrm{cosec}\,2\,i_0 \quad \text{and}$$

$$z_0 = \frac{v_0^2}{2g} - h_p \tag{10}$$

There are two plumes which appear to be overwhelmingly from one small vent in an almost horizontal orientation. They are Plumes 1 and 3 discussed by Strom *et al.*[2]. Table 1 presents the results of fitting these measurements on these plumes and includes a comparison of the observed transition point (r_1, z_1) with the predicted point for Plume 3. Note the blatant failure of our model to fit Plume 3 with r_1, z_1 (see *Plate 5*, p. 786) at the outer edges at ground level.

A fundamental difficulty with the ballistic model is that we need an additional force to turn the trajectories of the particles and we are thus led to consider an aerodynamic model.

Jet and shock disk or aerodynamic model

We now consider as an alternative that the combination of particle sizes and rate of gas flow is such that the particles are entrained and transported by the gas into the central portion of the top of the plume with the particles being released into ballistic trajectories after passage through the first part of their descent.

Expansion from a vent at sonic speed cannot produce a Prandtl–Meyer expansion (ref. 3, p. 281–283 and Fig. 4.22, p. 282) into a vacuum unless every molecule freezes or condenses on contact with the surface. Any flow reflected from the nearby surface will produce a shock front near the edge of the stalk of the plume which will initially constrain the horizontal expansion (ref. 4, p. 420–422 and Fig. 203, p. 421). In the absence of a sufficient ambient atmosphere this weak recompression shock will disappear with increasing height and allow a free expansion into a cone at an asymptotic Mach angle:

$$i_0 = \arctan\,(1/M_0) \tag{11}$$

where M_0 is the corresponding Mach number (see Fig. 3).

The very rapid decompression indicated by the above discussion should leave a very cold gas with negligible thermal energy travelling at an initial velocity, q_p. This leads us to treat an elementary parcel of gas as following a ballistic trajectory until recompression sets in. At that point we expect the presence of a shock front and shall discuss the effects of the presence of such a front.

Consider an element of a vertical cylinder of radius, r, azimuthal angular extent $d\phi$ and vertical extent dz. For this we use

Table 1 Fits to plumes 1 and 3 for the ballistic model

Plume no.	r_m (km)	r_p (km)	h_p (km)	i_0 (deg)	v_0^2/g (km)	z_o (km)	v_p (km s^{-1})	r_1 (km)	z_1 (km)	r_1 (km)	r_2 (km)
1	500	17.5	280	28.05	623	32	1.00	332	191	—	—
3	125	7.5	70	25.74	169	15	0.50	82	50	125	0

equation (5) in the general form without the subscript m on z, and the subscript 0 on i and with q as replacement for v_0:

$$z = -z_0 + r\cot i - \frac{gr^2}{2q_0^2}\operatorname{cosec}^2 i \tag{12}$$

We take the partial derivative with respect to i;

$$|dz| = \left|1 - \frac{gr}{q_0^2}\cot i\right| r\operatorname{cosec}^2 i\, di \tag{13}$$

The velocity of flow across the element $r d\phi |dz|$ is $q_0 \sin i$. Density variations will then cancel those of $r\,dz$ to maintain continuity so that the minimum density will occur at the maximum of $r|dz|$. We find the minimum versus r to lie at:

$$r_M = \frac{2q_0^2}{3g}\tan i, \qquad z_M = \frac{4q_0^2}{9g}\left(1 - \frac{1}{2}\tan i\right) - z_0 \tag{14}$$

These relationships define a ring on a surface of minimum temperature and pressure along the trajectories. The recompression that must follow along these streamlines in highly supersonic flow will take the form of a stationary shock front (ref. 3, p. 263–264). We assume that the shock front will lie at the density minimum in its steady state. Elimination of i yields the equation for the shock front:

$$z_M = \frac{4q_0^2}{9g} - z_0 - \frac{1}{3}\frac{q}{q_0^2}r^2 \tag{15}$$

We compare the slope of the frontal surface with respect to r with that for the trajectory

$$\frac{dz}{dr} = \cot i - \frac{2}{3}\sec i \operatorname{cosec} i, \qquad \frac{dz_M}{dr} = -\frac{4}{9}\tan i \tag{16}$$

and we then require that the flow be into the shock front:

$$\frac{dz}{dr} > \frac{dz_M}{dr}$$

which is equivalent to:

$$\tan^2 i < 3/2 \tag{17}$$

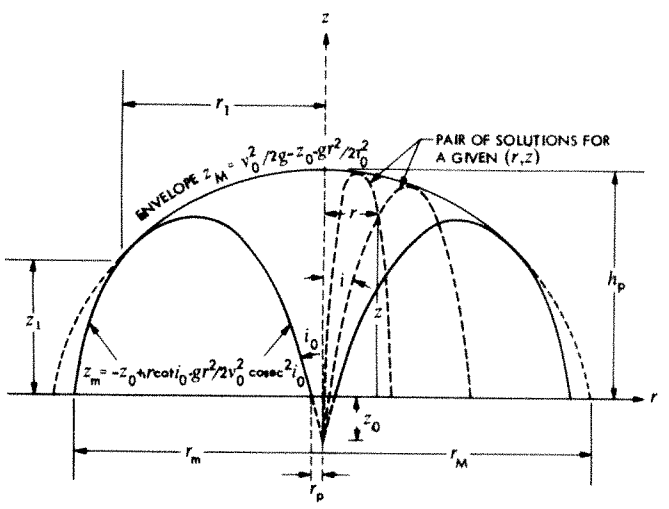


Fig. 2 The geometric model.

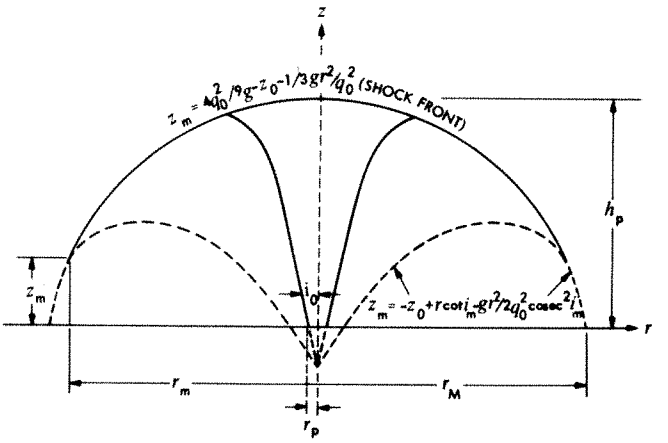


Fig. 3 The aerodynamic model.

By equation (14) this limit corresponds to:

$$r_m < \left(\frac{2}{3}\right)^{1/2}\frac{q_0^2}{g}, \qquad z_m > \frac{4q_0^2}{9g}\left(1 - \frac{\sqrt{6}}{4}\right) - z_0 \tag{18}$$

and the shock surface or curved shock disk extends out to this limit or to:

$$r_m = \frac{2}{3}\frac{q_0^2}{gM_0}, \qquad z_m = \frac{4q_0^2}{9g}\left(1 - \frac{1}{2M_0}\right) - z_0 \tag{19}$$

if $M_0 > (3/2)^{1/2}$ so that this edge pre-empts the limit.

Conditions immediately above the shock

It is possible to resolve the velocity just below the shock disk into components normal and parallel to the shock front. Imposing conditions of conservation of mass, momentum and energy across the shock is the next step (ref. 4, p. 25–33) which yields the conditions immediately above the shock. Even for a perfect gas the temperature above the shock will be much cooler than at the vent and the situation becomes more extreme for sulphur dioxide with both crystals and vapour present. In that case a rather large density jump with evaporation of some of the crystals occurs across the front.

We assume that the shocked gas forms a rather cool, thin and relatively dense layer draining off the dome provided by the curved shock disk. We assume that departure from the dome is essentially along the ballistic trajectory tangent to its limit—a crude approximation.

We further assume that the gas, at least, spreads out to reach this limiting trajectory which passes through the point given as a limit in equation (18). The trajectory is characterised by:

$$\cot i_m = (2/3)^{1/2} \tag{20}$$

Substitution of this parameter into equation (12) for the trajectory and solution for $z = 0$ yields the outer radius of the plume at its intersection with the surface:

$$r_M = \frac{\sqrt{6}}{5}\frac{q_0^2}{g}\left[1 + \left(1 - 5\frac{g_0^2}{q_0^2}\right)^{1/2}\right] \tag{21}$$

The corresponding value of the radius of the stalk at its base r_p, is

$$r_p = \frac{q_0^2}{g}\sin i_0 \cos i_0\left[1 - \left(1 - \frac{2gz_0}{q_0^2}\sec^2 i_0\right)^{1/2}\right] \tag{22}$$

derived in the same manner. The height of the shock h_p, is given by z_m for $r = 0$:

$$h_p = \frac{4}{9}\frac{q_0^2}{g} - z_0 \tag{23}$$

which we take as the height of the plume.

Table 2 Fits to Plumes 1 and 3 for the aerodynamic model

	Observed	From (21), (22)*		From (18)	
Plume no.	i_0 (deg)	q_0^2/g (km)	z_0 (km)	r_m (km)	z_m (km)
1	30	550	29	449	65
3	45	137	7	112	17

	From (19)		From (23)		
Plume no.	r_m (km)	z_m (km)	h_p (km)	q_m (km s^{-1})	q_p (km s^{-1})
1	212	145	215	0.33	1.00
3	91	39	54	0.16	0.51

* Equation nos given in parentheses.

Comparison and observations

Solution of equations (21) and (22) yields the results in Table 2. The quantity q_m is the velocity just below the shock on the axis of the plume computed by application of the conservation of energy which yields:

$$q = [q_0^2 - 2g(z_0 + z)]^{1/2} \quad (24)$$

The disagreement between the computed height of the plume from the measured r_m, r_p and the measured height probably occurs because the actual trajectories beyond the shock disk are lower than the ballistic trajectories used. This is also indicated by the outer bend in the filaments appearing in Fig. 1. The inner bend is, of course, imposed by the shock front.

The thermodynamic diagram for sulphur dioxide prepared by Smith *et al.*[5] and their discussion make it clear that we are in a regime in which both the solid and the vapour are present in roughly equal amounts. In that case passage through the shock front will take place at a ratio of specific heats, close to unity with a large density increase, significant sublimation of entrained crystals of sulphur dioxide and that the temperature immediately above the front will be much lower than that which would occur for a perfect gas. The downward flow would involve expansion into a volume only a few times larger and recompression on reaching the surface could produce another shock front in the shape of a ring with lateral escape, unless most of the gas immediately crystallises onto the surface. The observations do not show such a front so it is either very close to the surface or does not exist owing to rapid crystallisation onto the surface.

Comparison of *Plate 7b*, p. 789 with Fig. 1 strongly suggests that we are witnessing variable rates of injection of another substance (sulphur droplets?) into a steady gaseous flow (sulphur dioxide).

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Volcanic resurfacing rates and implications for volatiles on Io

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Resurfacing rates and surface ages on Io are estimated, together with the material ejection and deposition rates of the active volcanic plumes.

THE discovery of current volcanism on Io[1,2] raises important new questions about the history of volatile material on Io, the rates of material deposition, the age of the currently observed surface, the loss rates of volatiles, and the ultimate fate of volatiles. This article presents an initial comparison of resurfacing rates based on two separate observations and implications for supply of material to the jovian torus. In estimating the resurfacing rates and surface ages we first examine the lack of recognisable impact craters down to the limiting resolution of about 600 m. Second, we estimate from imaging data the material ejection and deposition rates of the active volcanic plumes.

Removal of impact craters

To estimate the age of Io's landforms and hence their removal rate based on the absence of impact craters we must assume some flux of impacting bodies. Although such an estimate has large error bounds we are asking order-of-magnitude questions: is the resurfacing rate mm per yr, mm per millenium, or mm per aeon? Are the surface landforms hundreds of thousands or hundreds of millions of years old? Two methods have been used to estimate current relative impact rates for the terrestrial planets—Mercury, the Moon and Mars. The first models the distribution and impact rates from telescopically observed asteroids, comets and meteor showers[3,4]. The second compares the variations in the number of small impact craters (a few kilometres diameter) formed on smooth plains on these bodies following the extremely rapid decline in impact flux ~4,000 Myr ago[5]. Both these techniques suggest that the impact fluxes in the Solar System over a heliocentric range of 0.4 to ~1.5 AU have been roughly uniform, within a factor of two, during the past 10^9 years or so. The extrapolation to the jovian system suggests that fluxes there cannot be different by more than an order of magnitude from those in the inner Solar System without violating the apparently uniform character of the inner fluxes. Hence for our order of magnitude estimates we will use fluxes quoted for the Moon and Mars[5] realising that they might be in error by as much as a factor of 10 due to undiscovered populations, Jupiter gravity effects, and so on.

Figure 1 shows the three steps used to estimate that resurfacing rate which will just erase the expected impact craters.

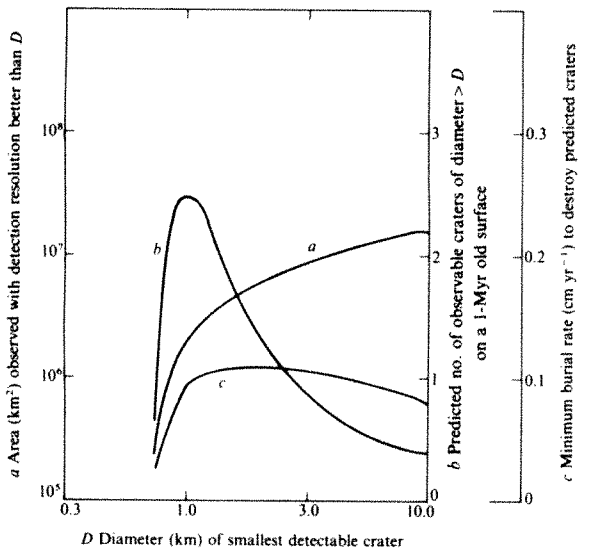


Fig. 1 Estimate of resurfacing rate to destroy impact craters. Shown as a function of crater diameter are: *a*, the areal coverage (in km²) of Io obtained by Voyager 1 on which a crater of diameter *D* or larger could be detected; *b*, the number of craters larger than *D* that would have been seen in the Voyager 1 picture collection if Io's surface were 1 Myr old, assuming a lunar cratering size frequency spectrum and rate; *c*, the minimum rate of burial (or erosion) required to remove the craters so that none are now visible.

Different estimates can be made for different crater sizes. For instance, although larger craters are less frequent, and on the average older, they may take longer to destroy. Furthermore although smaller craters are more frequent they require higher resolution pictures to be recognised. Since in general we have less coverage at the higher resolutions, there is a higher probability of seeing a larger crater than a small one in many cases. Figure 1 shows that for the Voyager 1 pictures the probability of observing a 1 km crater is higher than that for a 600 m crater, although the latter would be far more abundant on the surface. The minimum resurfacing values in Fig. 1 are calculated assuming that the average crater diameter larger than D (the diameter of Io) has a diameter $\sim D$, that the craters have a depths of $1/5D$ and that burial by a blanket twice their depth is required to obscure them. The best estimates come from observations of 1–5 km craters, which should be statistically common and easily observed. Our value of the required resurfacing rate 10^{-1} cm yr^{-1} and should be reliable within a factor of 10. Hence our lower limit is 10^{-2} cm yr^{-1}. Also, resurfacing can be performed by a variety of processes including deposition by volcanic surface flows[6], volcanic plumes[7] and surface erosion[8]. Do the observed active plumes, which may be the most active of these processes, provide material at a rate consistent with these burial rates?

Depositional rates of volcanic plume material

To estimate the resurfacing rate from observations of current volcanic activity we start with the dimensions of plumes of volcanic ejecta[7] (r_p is the radius of plume; h_p the height of plume: see Table 1 in ref. 7). The visibility of the plumes in Voyager 1 images implies an optical depth of the order of at least unity in the denser parts of the plume along an optical path of scale $\sqrt{2}r_p$. The plumes are dark when seen in projection against the disk and scatter more strongly at shorter wavelengths when seen off the disk[2]. Accordingly we assume the particles to be small, so their cross section for extinction, σ_p, can be written as

$$\sigma_p = \frac{2\pi^2 K}{\lambda} a^3 \tag{1}$$

where a is the root mean cube of the particle radii, λ the wavelength and K a constant of order unity[9]. Thus, we are considering extinction due to particles with $a < \lambda$, but are ignoring contributions from large particles and Rayleigh scattering $a \ll \lambda$. For an idealised cylindrical volume circumscribing the plume, we then take the optical depth, τ, to be

$$\tau = \sqrt{2} r_p \sigma_p n_p = \frac{2\sqrt{2}\pi^2 K}{\lambda} a^3 r_p n_p \tag{2}$$

where n_p is the number density of particles.

If we take f_p to be the volume fraction of the cylindrical volume actually filled by plume structure, the total number of particles in the plume, N_p, is

$$N_p = f_p \pi r_p^2 n_p h_p \tag{3}$$

and the total mass of particles, M_p,

$$M_p = m_p N_p = \frac{4}{3}\pi a^3 \rho_p N_p \tag{4}$$

where ρ_p is the individual density of the particles.

Solving equation (2) for n_p and substituting in equations (3) and (4) we find that:

$$M_p = \frac{\sqrt{2} f_p \tau}{3K} \rho_p \lambda r_p h_p \tag{5}$$

which is independent of particle size a.

The resurfacing rate due to n plumes, R (cm s^{-1}), can then be written in terms of M_p and the time of flight of the particles, t_p. If $R \equiv \sum_{p=1}^{n} R_p$, then:

$$R = \frac{\sum_{p=1}^{n} (M_p/t_p)}{4\pi R^2 \rho_p} = \frac{\tau}{6\pi K} \frac{g^{1/2}\lambda}{D^2} \sum_{p=1}^{n} f_p h_p^{1/2} r_p \tag{6}$$

where D is the diameter of Io and we have used the ballistic flight time given by $t_p \equiv 2\sqrt{2} h_p^{1/2} g^{-1/2}$, g being the acceleration of gravity on Io ($\sim$180 cm s^{-2}). Gas density in the densest parts of the plumes might be enough for drag forces to be important but in the thin parts near the top from which the particles fall back to the surface the ballistic, free fall velocities are supersonic with respect to Io's tenuous atmosphere and the ballistic flight time should be close to the actual flight times of the particles. For example, the ballistic time is about 10^3 s from 100 km altitude.

Taking $\tau \geq 1$, $\rho_p \geq 2$ g cm^{-3}, $K \sim 1$, and $\lambda = 4.8 \times 10^{-5}$ cm, Table 1 gives lower limits for the quantity M_p/f_p for each plume, the total for all plumes and R_p/f_p. Using the same quantities in equation (6) yields a value for R/f, if we assume $f_p \equiv f$ for all plumes.

$$\frac{R}{f} \geq 1.1 \times 10^{-10} \quad \text{cm s}^{-1}$$

The value for R depends on the estimate of f, which must await closer geometric analysis of the images. Assuming that 10% or more of the volume is filled by plume structure we obtain

$$R \geq \sim 3.5 \times 10^{-4} \text{ cm yr}^{-1}$$

This lower limit on the rate is a factor of 100 lower than the lower limit derived from the cratering argument earlier. Our assumptions have been extremely conservative, however. In particular, the derived plume resurfacing rates refer only to a limited size range of particles in plumes actually observed by Voyager 1; larger particles (up to millimetre sizes) could be a significant portion of the mass delivered to the surface. We have also neglected resurfacing due to condensation of the gaseous component of the eruptions, which could at least be equivalent to the particulate mass[10]. Also, considerable resurfacing must be done by volcanic flow activity, for which there is abundant evidence in the images[2,6]. Finally, there are many suggestions in the imaging data of numerous smaller gas/particulate venting below the level detectable as plumes. This low level activity, if constant, could be one of the most important contributors to resurfacing. Taking these factors into account, we conclude that the resurfacing rates required by the crater arguments are in general agreement with the observed level of volcanic activity, but that resurfacing mechanisms in addition to particulate

($a < \lambda$) deposition by the eight known volcanoes are probably necessary unless recent cratering rates at Io have been 100–1,000 times lower than current inner Solar System rates (which we consider unlikely). We will use 10^{-1} cm yr^{-1} as our nominal rate and 3×10^{-4} cm yr^{-1} as an extreme lower limit.

Implications for volatile history

The degree of resurfacing described above has profound implications for the geochemistry and geophysics of Io. It implies that large portions of the crust or interior of Io have been turned over or recycled many times in geologic time. We will now investigate the implications of this rapid resurfacing for volatile production rates and volatile loss from Io.

Knowledge of the volatile composition around Io comes primarily from ground-based studies of neutral and ionic species around Io, in the magnetosphere inside Io's orbit, and from Voyager 1 remote sensing and *in situ* analyses of the plasma torus. Materials identified in the torus include (refs in parentheses), Na I (11), Na nuclei (12), K I (13), S II (14), S III (15), S IV (15), S nuclei (12), O II (15–17), O III (10), O nuclei (12), H (18) and possibly S_2^+ or SO_2^+ (17) (see ref. 19 for a summary table). There are obviously still large gaps in our knowledge of the material in Io's vicinity and the inner magnetosphere. However, it seems clear that Io is the most probable source of most, if not all, of this material. The bulk of the data indicates that S and O are the most abundant species, Na less abundant but still significant and other species present only in trace amounts (leaving aside the issue of hydrogen). Thus, despite possible fractionation effects and observational selection factors, we should look for these elements in the material currently escaping from Io. We can estimate escape rates for some of these species assuming Io is the source. For Na, a rate of $\sim 10^8$ cm^{-2} s^{-1} is required to produce the observed cloud[20,21]. Hydrogen was estimated at 10^{10} cm^{-2} s^{-1} (ref. 18) from Pioneer 10 data and the lack of Lyα emission in the Io torus during the Voyager 1 encounter[15] suggests this is an upper limit at present. Injection of S and O ions into the magnetosphere supplies energy which, if radiated away by the observed UV emission features requires a supply rate of $\sim 10^{10}$ cm^{-2} s^{-1} for these ions[15].

Sulphur has long been regarded as a likely candidate surface material for Io, primarily to explain the very steep decrease in Io's reflectance at short visible wavelengths[22–24]. The volcanism provides an obvious source for sulphur and sulphur compounds, since these are some of the more abundant materials produced in terrestrial volcanism[25–27]. Sulphur flows are proposed as one source of coloured features on Io's surface[28]. Sulphur compounds, particularly S and SO_2, have been suggested as the driving gas for the explosive volcanisms[2,10] and SO_2 has been identified in absorption in infrared spectra from the IRIS experiment on Voyager 1[29]. What is seen in the torus is thus in general agreement with the characteristics of Io and its volcanism (see also ref 19).

We have no direct evidence concerning the water content in Io's volcanism. Water is, of course, a common constituent in terrestrial volcanism (mostly recycled groundwater), and Io may well have had a significant water content at formation[30,31]. However, based on the absence of any indication of the strong IR H_2O absorptions near 3 μm, Pollack *et al.*[32] place a limit of 10% fractional coverage of water ice on Io and 10^{-3} relative abundance of bound water. Since the areas covered by individual plume ejecta range up to ~10% of the observed disk of Io, water condensation over the area affected by the volcanoes should have been observed; it seems that water must be a minor constituent of Io's volcanism or absent altogether.

We can now take the estimated resurfacing rates and the observed supply rates to the torus to draw some conclusions about the history of volatile supply on Io. Assuming an average of 20 AMU for the species involved, resurfacing rates of 3×10^{-4} cm yr^{-1} and 10^{-1} cm yr^{-1} supply $\sim 10^{12}$ atoms cm^{-2} s^{-1} and $\sim 3 \times 10^{14}$ atoms cm^{-2} s^{-1} respectively. It is clear that the current escape rate of $\sim 10^{10}$ cm^{-2} s^{-1} can remove only a fraction of the material being brought to the surface by volcanism.

Table 1 Plume dimensions and resurfacing parameters

Plume no.	r_p* (km)	h_p* (km)	M_p/f_p ($\times 10^9$ g)	R_p/f_p ($\times 10^{-12}$ cm s^{-1})
1	500	280	≥63	≥66.1
2	105	100	≥4.7	≥8.3
3	125	70	≥4.0	≥8.27
4	37	95	≥1.6	≥2.85
5	100	80	≥3.6	≥7.07
6	125	80	≥4.5	≥8.84
7	90	120	≥4.9	≥7.79
8	70	70	≥2.2	>4.63
Total			≥89	113

* From ref. 6.

Io has only a very tenuous atmosphere. Limits of surface pressure range from 10^{-6} bar (stellar occulation) to 10^{-8} to 10^{-11} bar from ionospheric models[33,35]. If only 1% of the material in Io's volcanoes were volatile, then gas would be being supplied at rates of 1 to >200 times greater than the escape rate. Thus, to avoid building up an atmosphere beyond the observed limits, we must conclude that either there are few or no volatiles associated with Io's volcanoes or that virtually all of the volatiles produced are being recycled back into the surface. An extremely low volatile content is unlikely since some gas is needed to drive the observed eruptions[2]. Thus we conclude that whatever gases are being produced are probably being condensed or otherwise reincorporated onto the surface and only minor amounts are escaping from the volcanoes directly or from a tenuous atmosphere.

The observation of SO_2 gas over a volcanic plume[29] and the possibility of an atmosphere containing SO_2 in equilibrium with solid SO_2 at some temperature[19,29] are consistent with the above arguments. In addition condensed volcanic gases explain some of the puzzling features of Io's spectrum[32,36]. Hapke[37] has suggested, based on transmission spectra of various volcanic materials, that H_2S, SO_2 and possibly S_2O might explain some of the features in Io's reflection spectrum. Fanale *et al.*[38] and Smythe *et al.*[39] have demonstrated that diffuse reflection spectra of SO_2 frost explain the absorption near 4.0 μm very well.

Some of the albedo features on Io's surface may also be understood in terms of condensation phenomena. Inspection of Fig. 12, 14*a*, 16, 17*a* and 18 in ref. 2 shows that the deposits of small particles or ash is dark and highly localised. We may match this process with the more localised bright rings outside the inner cores of volcanically active areas (Fig. 18*a* and *b* in ref 2). We propose that the bright rings are formed on the outer edges of hot volcanic areas where the surface temperature is low enough to allow sulphur and/or SO_2 frost to deposit from the volcanic gas with the outer limit being set by the outer limit of higher vapour density associated with the plume. An interesting example is the dark area south of Plume 2 shown in *Plate 1*, p. 782 see Strom *et al.*[7]. The bright deposit from this plume does not cross this dark 'lava lake.' We suggest that this is due to high temperature of this feature[40] preventing condensation of SO_2.

An important aspect of Io's volcanism is that is has probably been going on for $(4–4.5) \times 10^9$ yr (ref. 41). The above range of inferred resurfacing rates implies the supply of anywhere from ~0.1 to ~10 times the total mass of Io over geological time. This is another way of viewing the conclusion reached earlier that significant amounts of Io's crust and interior have been recycled several times. The current escape rates, on the other hand, only amount to a loss of 10^{-4} of the mass of Io over geologic time. Thus the currently observed rates do not imply significant loss of material from Io since formation. The presence of abundant heavier volatiles such as sulphur is not therefore particularly surprising since only 1% of Io's total sulphur content (if near solar abundance) would have been lost at these rates. However, the ease with which escape occurs may be a strong function of the volatiles involved, and escape rates could have been very

different earlier in Io's history. In fact it seems likely that the large volumes of material brought up by the volcanic activity must have originally contained larger amounts of volatiles such as H_2S and H_2O which were lost through dissociation, ionisation and H escape. This would explain the apparent absence of H_2O combined with active current degassing of sulphur compounds (see also ref. 19).

Implications for supply to plasma and neutral torus

The observation of volcanic eruption plumes on Io also raises the question of what processes are responsible for injecting material into the neutral clouds and plasma torus associated with Io. An initial question is whether material is directly escaping in the eruption process. The height of the observed plumes suggests ejection velocities of ~ 1 km s^{-1}, below the 2.5 km s^{-1} escape velocity. Since particulate material in an eruption is entrained with the gas, particle velocities greater than the gas velocity are unlikely. Velocities of 1 km s^{-1} are already high for this type of eruptive activity, and higher velocities are unlikely[10]. Thus it seems that direct ejection of particulate material with velocities greater than the escape velocity is not responsible for supplying material to the magnetosphere.

Atmosphere loss by either thermal or non-thermal mechanisms of gas supplied by the volcanoes is potentially a major source for the observed escape flux. Jeans escape of sulphur from an atmosphere with a surface pressure of 10^{-10} bar, for instance, would supply $\sim 10^{10}$ atoms cm^{-2} s^{-1} from the critical level of ~ 500 km if the exospheric temperature were 1,200 K and sulphur were a major constituent at that level. Note that the volcanic eruptions may supply material to altitudes near to or even above the exobase. Thus the volcanic activity may bring gases to a level where they will be exposed to escape by thermal or non-thermal mechanisms.

Non-thermal escape mechanisms such as magnetospheric sweeping of ions or dissociative recombination reactions have been suggested as strong possibilities in Io's environment[42–44]. These may dominate thermal escape, and here again the volcanic eruptions may play a major role. If Io is 'protected' by the interaction of the co-rotating plasma with a possible Io magnetic field[45], then the volcanoes may on some sides of Io supply material above the stand-off altitude, allowing interaction with and sweeping by the magnetosphere. Also, although direct escape of particulates from the volcanoes is unlikely, it is possible that some of the very fine micrometre or submicrometre particles in the volcanic plumes may become charged in the plasma environment and be removed by magnetic sweeping. This is at least potentially an important source of dust in the inner magnetosphere. Direct sputtering (see ref. 42) of dust in the plumes by magnetospheric particles is also possible, although the surface area exposed in plumes is less than Io's surface area.

The processes described above may be sufficient to account for current losses of sulphur and oxygen to the torus and for the escape of hydrogen earlier in Io's history. The problem of sodium escape is more difficult, at least partially because we have considerably more information about the neutral species, whereas we only observe the magnetospheric ions for most other species. Three points particularly deserve comment: the apparent constancy of sodium supply since 1973, the observed velocity distribution of the sodium atoms, and the asymmetry of escape required by the cloud geometry.

A constant supply of sodium by volcanoes is not ruled out by the observed volcanic activity, which certainly suggests a rather high average level of activity. However, considering the short lifetime against ionisation implied by observations (20–50 h, ref. 46), major fluctuations in volcanic activity (by a factor of ≥ 2) should have been observed in the sodium data if the sodium escape is directly linked to the volcanoes.

Hapke[37] has suggested that Na (and K) are supplied to the torus by evaporation from hot silicate lavas ($T \sim 1{,}450$ K), possibly carried to great altitudes in volcanic plumes. This seems unlikely for several reasons. First, no temperature of this magnitude has been observed on Io[40], and models for explosive gas volcanism start with much more modest temperatures[10]. Second, any hot silicate which could be ejected by such a gas eruption would cool considerably during its 10 min trip to the top of a plume. Finally, the large Doppler width of the sodium emission lines (~ 5 km s^{-1} FWHM) and the high velocity asymmetry discovered by Trafton are evidence against thermal escape[47,48], even if the volcanic activity supplies sodium or sodium compounds to the atmosphere. Since only neutral sodium is involved, non-thermal mechanisms involving ionisation are not possibilities.

Sputtering of atoms from the surface by impacting magnetospheric ions has been suggested as the sodium supply mechanism by Matson and coworkers[42]. Before Voyager, the primary difficulties with this mechanism were the very low efficiencies for sputtering by protons (although ice may be an exception[49]) which were then thought to be the major positive ions in the magnetosphere, and the possible thermalisation of sputtered atoms in Io's atmosphere, requiring yet another energetic event to supply escape energy. The discovery of large densities of sulphur and oxygen ions in the inner magnetosphere has removed the difficulty with yields. For a density of 10^3 cm^{-3} the flux of ions at Io's position in the co-rotational direction is $\sim 6 \times 10^9$ cm^{-2} s^{-1}. Whether these ions impact Io directly or are precipitated into it by an Io magnetosphere, the energy of the impact will be comparable to the co-rotational energy, ~ 500 eV for sulphur at Io's orbit. Heavy ions in this energy range have substantial sputtering yields of the order of unity or greater[50]. Ions of this energy can also withstand several collisions with atmospheric molecules without losing a significant fraction of their energy. Thus the known magnetospheric ion population is sufficient to supply the required sodium flux even for modest sodium abundances in the surface (1–10%).

The major issue remaining is whether sputtered atoms escape Io or are merely supplied to a thermalising atmosphere for later escape. An SO_2 atmosphere (or any other atmosphere that meets the criteria discussed above for volcanic gases that can be reincorporated in the surface) may be considerable at night[19,29]. Thus this class of atmospheric model may allow atmospheric escape of gases in the daytime and sputtering of surface material at night. Note that sulphur should also be sputtered in some quantity (along with other surface materials), so that volcanic supply of daytime gases might represent a modulation on a lower level, a more steady supply rate.

The shape of the cloud and the velocity profile asymmetry seem to require asymmetric escape of neutrals from Io, most likely from the inner hemisphere, either the trailing or the leading portion thereof[51,52]. This asymmetry could in principle arise either from asymmetric source or sink processes. The volcanoes are more or less uniformly distributed in longitude[2,6], and the night/day effect suggested above averages out to uniform sputtering over an orbit if no other processes operate. Interaction with the magnetosphere remains the best candidate for producing this asymmetry either by creating preferential regions for ion precipitation or possibly by shielding neutrals from electron ionisation in regions inside an Io 'magnetosphere'[45]. It is possible that a day/night modulation combined with a magnetospheric asymmetry could produce the east–west sodium anomalies reported by Bergstralh *et al.*[53] and Goldberg *et al.*[54], but more detailed models will be necessary to study these possibilities.

Conclusion

The resurfacing rates implied by observations of active volcanism on Io are sufficient to explain the lack of recognisable impact craters on Io's surface. The implied rates of material supply to Io's surface require major recycling of Io's upper crust and mantle and that volcanic volatiles be condensable under Io's surface conditions, consistent with the observation of SO_2[29] and models of Io's volcanism[10]. Atmospheric escape of a small

fraction of the volcanic gas can supply the S and O in Io's torus and these same species, as energetic heavy ions, may be responsible for the supply by sputtering of high velocity neutral sodium.

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Sulphur flows on Io

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The colour patterns observed on Io indicate extensive surface flows of quenched molten sulphur

Io has the reddest surface in the Solar System, with the possible exception of Amalthea, which may in fact, be coloured by material lost from Io. Io also displays the largest variation of colour with planetocentric longitude of any other object in the Solar System[1]. Voyager 1 close-encounter photomosaics have clarified both the coloration of Io and its surface variation (see, for example the colour images in Smith *et al.*[2]). Although the Voyager 1 photometric fidelity is not better than 10%, general colour patterns are clear. The surface is mottled with dozens of large, and, probably, thousands of small randomly distributed dark patches, which in most cases are adjacent to red or orange coloured terrain. Equatorial and temperate latitudes are rich in these bright colours. Polar terrain tends to be darker, confirming earlier groundbased impressions. There are also extensive yellow, and white or blue-white, provinces on the satellite's surface. Many of the dark spots are active or recently active shallow volcanic constructs[2–6]; seven of the volcanoes have been observed by Voyager 1 in violent eruption. The pattern of albedo features points strongly to a succession of complex effusive and fluvial events. Some dark localised diffuse features exhibit radiating patterns implying downslope flow, such as in *Plate* 2, p 783. In this image there is a hint of sinuous anastamosing channels, ~300 km long, which are blood-red with some traces of black material down the principal channel. One channel is red proximate to the caldera, fading to yellow distally. In general, dark flows are short and diffuse, red flows longer, and orange and yellow flows tend to be distributed in sheets of considerable area.

A crude similarity between the integrated-disk colour of Io and the spectrum of elemental sulphur led Wamstecker *et al.*[7] to suggest the presence of abundant sulphur on the ionian surface. Sulphur has also been identified in the Io-related circumjovian torus both by Earth-based observations[8] and by Voyager 1 experiments[9,10]; SO_2 has been identified in the gas phase by Voyager 1 IR spectroscopy[11]; and it has been suggested that SO_2 frost may explain some of the broadband IR spectral features in the integrated disk of Io[12,13]. After water and CO_2, sulphur and its compounds are generally the first or second most abundant effluents in terrestrial volcanoes, and fumaroles, in some cases comprising tens of per cent by mass[14]. Where fumarole temperatures are $\lesssim 650$ °C, sulphur gases tend to be the major constituents after steam, and the corresponding volcanic constructs are called solfataras. Sulphur and its compounds have been thought[2,15] to drive the volcanic plumes because of the absence on Io, unlike the other galilean satellites, of surface water frost in groundbased IR spectroscopic observations. Io has been substantially devolatilised. H_2S is rapidly photodissociated and, because of its very low freezing point, hydrogen has an opportunity to escape efficiently from Io by processes such as Jeans evaporation, or magnetospheric sweeping. As Io becomes more and more oxidising, S_n and SO_2 become prime candidates for the principal volcanic effluents. Ionian effusive flows and sulphur volcanoes may be driven by the lithostatic pressure gradient and explosive decompression of volatiles[16], with the energy derived from tidal dissipation dependent on the resonance lock with Europa[17]. However, the following discussion is independent of the mechanics of Io volcanism.

Sulphur coloration

There are perhaps dozens of sulphur allotropes with differing physical, chemical and optical properties derived from differences in both the packing and the bonding of polyatomic sulphur; their melting points vary from 110 to 119 °C. Because the melting point of sulphur is ~1,000 °C less than that of

silicate, a stochastic distribution of thermal events in the interior of Io should melt sulphur—already known to be abundant near the surface—more often than it should melt silicates. At its melting point liquid sulphur is yellow, intensifying to orange at about 150 °C, red at about 180 °C, and black at about 250 °C (see ref. 18). At the normal boiling point, 444 °C, the composition is mostly cyclic S_8 with some S_6 and S_2; at 1,000 °C, sulphur is largely S_2. Therefore, the species of sulphur in the ionian atmosphere depends strongly on the temperature in the volcanic vent and the rate of cooling. The chemistry of liquid S_n, with $2 \leq n \leq 22$ at least, is complex and incompletely understood[19,20].

At 25 °C, solid sulphur shows a steep absorption edge[18] at about 2,850 Å which moves at lower temperatures to shorter wavelengths at about 2.3 Å K^{-1} and at higher temperatures (until the melting point) to longer wavelengths at about 2.0 Å K^{-1}. Thus, at the mean ionian surface temperature, the absorption edge is near 2,500 Å and at the melting point, near 3,000 Å. This absorption edge is partly responsible for the yellow coloration of solid sulphur at room temperature; at liquid nitrogen temperatures orthorhombic sulphur is snow-white because the wings of the absorption edge are sufficiently far into the UV.

Remarkably, if liquid sulphur is rapidly quenched or freeze-dried to low temperatures, its colour is preserved. The faster the freezing, the better the preservation; the higher the initial temperature of the melt, the deeper the hue. These colours have three sources[18]: (1) the S_n absorption edge in the violet or near UV; (2) an absorption peak near 4,000 Å due to S_3; and (3) an absorption peak at 5,500 Å due to S_4. S_3 and S_4 are typically present in abundances of ~1% in the frozen melt; they are trapped metastable species and their influence declines markedly at post-quenching temperatures $T \gtrsim 180$ K. Therefore, away from calderas and other localised heating, these metastable species are preserved on Io and should affect the coloration of the surface. The colour of quenched liquid sulphur depends on its thermal history and associated metastable allotropes, as well as on impurities.

Terrestrial flows of molten sulphur—such as at the Japanese volcano Siretoko-Iosan[21]—are chocolate brown. However, this is unlikely to be due to a sulphur allotrope; a minimal abundance of organic matter of 1 part in 10^5 can colour sulphur brown[18]. The absence of brown coloration on Io would suggest that virtually no organics are present, a conclusion consistent with substantial devolatilisation and escape of hydrogen during Io's history.

The dominant absorption edge of sulphur continues to move to longer wavelengths, through the entire visible spectrum, between the melting point and the normal boiling point, at a rate of approximately 6 Å K^{-1}, or more than twice the rate for the solid[18]. Nelson and Hapke[22], in a prescient paper, suggested that the integrated-disk coloration of Io is due to quenched liquid sulphur and even proposed, before Voyager, "volcanic fumaroles" as one possible means of melting the sulphur. They suggest that the observed 3300 Å absorption feature on Io is due to quenched liquid sulphur. The above temperature-dependence of the sulphur absorption edge means that a 3,300 Å absorption feature requires liquid sulphur which had been raised to about 155 °C before quenching, that is, temperatures high enough to produce vivid orange-red coloration.

Viscosity, colour and flow time

Figure 1 shows the temperature-dependence of the dynamic viscosity, ν, of liquid sulphur and a rough indication of its colour-dependence. In this model the black patches in and around the volcanic calderas are quenched liquid sulphur originally at $T \gtrsim 250$ °C. The connection of such temperatures with volcanic mechanics is discussed in ref. 16. As in terrestrial volcanoes, the incidence of melt in the caldera should be intermittent; there is no Voyager IR evidence for extensive regions with temperatures above the melting point in any one of the few calderas examined to date[23]. Because of its comparatively high viscosity (comparable to olivine basalts), black and red-black

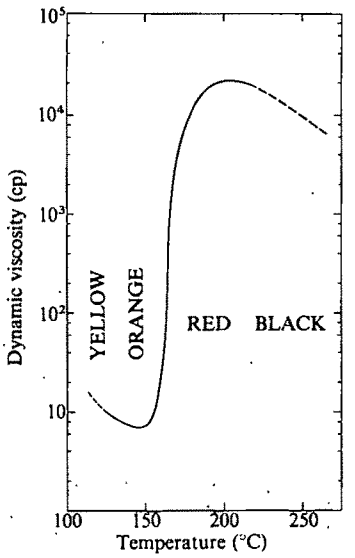


Fig. 1 Viscosity (after the *International Critical Tables*) and colour of molten sulphur as a function of temperature.

liquid sulphur will flow sluggishly and therefore be confined to the vicinity of the caldera. The liquid will cool by radiation and evaporation, the colour will change to red and orange, and the viscosity will drop dramatically, permitting substantial downslope flow and suggesting that the red sinuous deposits are frozen sulphur rivers. When the sulphur melt cools to the orange-yellow and yellow range, just above the melting point, the viscosity is less than 10 times that of liquid water (1 cp at 20 °C), and the melt which is not contained by topographic relief has the opportunity to spread laterally and to form extensive yellow and orange-yellow sheets by flooding the lowland plains. Because of the Stefan–Boltzmann temperature dependence, cooling is about an order of magnitude more rapid in the black and red than in the orange and yellow, and the deposits of the latter coloration should cover more ionian surface area than the former, as is observed. The increase in area covered by the low-viscosity low-temperature melt leads to still faster radiative cooling. Fan-shaped flows, running from red-black interiors to orange-yellow exteriors, are also consistent with the model.

The increase of viscosity with temperature above 157 °C, atypical of most liquids, is produced by the rupturing of the cyclic octatomic form of sulphur to produce long and complex sulphur chains and polymers, with fragmented octagonal forms prominent. At $T > 187$ °C this long-chain structure begins to dissociate thermally. Thus the most interesting and complex sulphur chemistry on Io should occur in the red provinces.

Flow of a sulphur melt in a cylindrical channel on Io will be laminar when the Reynolds number $(V\rho d/\nu) < 2{,}000$, where V is the velocity of flow, ρ the density of liquid sulphur (1.8 g cm^{-3}) and d the channel width. For $d \sim 100$ m, and red liquid sulphur, the condition reduces to $V < 10$ cm s^{-1}, which may be plausible for high-viscosity flow. Narrower channels are more likely to be laminar. For laminar flow, Poiseuille's equation indicates that the discharge $Q \propto \nu^{-1}$, for a fixed channel diameter and a fixed pressure drop per unit length. The shallow slopes on Io and the lower acceleration due to gravity make both V and Q correspondingly lower than on Earth. A red flow at $V \sim 10$ cm s^{-1} will traverse 100 km in a period of weeks. But for orange and yellow liquid sulphur, ν is so small that the laminar flow condition is unlikely to be met. Thus, black and red liquid sulphur on Io should mobilise slowly and may exhibit laminar flow; while orange and yellow sulphur melts should be correspondingly much faster and fully turbulent. The surface of Io attests to a very complex sequence of overlapping flows. Both the ubiquity of sulphur flows and the likely volcanic mechanisms[16] point to the tapping of an underlying ocean of liquid sulphur, for which the name 'thiosphere' is here proposed.

Consider the cooling of a single eruption of liquid sulphur, sufficient to fill a channel 100 km long, 100 m wide and 10 m

Table 1 Minimum thicknesses d, and rough turnover times t_t, of films of quenched liquid sulphur for $\tau \sim 1$ at $\lambda \simeq 5{,}000$ Å

Colour	Original T	d	t_t
Yellow	≃120 C	tens of centimetres	centuries
Orange	≃140 C	centimetres	decades
Red	≃170 C	millimetres	years
Black	≳250 C	tens of micrometres	days

deep. The sulphur mass is $M \sim 2 \times 10^{14}$g and the radiating surface area is $A \sim 10^{11}$ cm^2. The cooling time will then be $t_c \gg [(c_p \Delta T + L)M]/[\sigma \bar{T}^4 A]$, where c_p is the specific heat at constant pressure of liquid sulphur $\sim 9.5 \times 10^6$ erg g^{-1}, $L \simeq 3.8 \times 10^8$ erg g^{-1} is the latent heat of fusion of sulphur, σ is the Stefan–Boltzmann constant, ΔT is taken as ≃500 °C, and the average radiative temperature during the flow $\bar{T}$ is taken as 400 K. The result of this crude calculation is $t_c \gg 2$ months.

Considerably thinner films of liquid sulphur from much more modest eruptions can significantly colour the surface. Table 1 gives rough estimates (data from refs 18 and 24) of the thickness of quenched liquid sulphur necessary to provide significant optical depth in visible light. Because of the high viscosity of hot liquid sulphur, very large deposits should accumulate near the calderas; however, extremely thin quenched films initially at such high temperature would be enough to provide the observed coloration. In contrast, the yellow intercrater plains must be covered by at least tens of centimetres of quenched liquid sulphur.

Table 1 also shows the turnover time t_t, for variously coloured deposits to be laid down, assuming a mean planetary deposition rate of volcanic effluvia (other than surface flows) of 10^{-1} cm yr^{-1} as estimated by Johnson *et al.*[15]. Because the deposition rates are not well known[15], and in any case should be much larger than the planetary average near the calderas and somewhat less than the planetary average in the intercrater plains, these figures are very rough. The gross silicate productivity ratio[25] of fragmentary ejecta to lava for all volcanic systems on Earth is ~5. If a similar ratio is applied to sulphur on Io, then the mean liquid sulphur flooding rate would be $\geqslant 10^{-2}$ cm yr^{-1}, implying (Table 1) that albedo changes in dark circumcaldera regions can take place in periods less than 2 weeks, and changes in red flows in periods less than years. Volcanoes are concentrated at equatorial latitudes[5], as are the deposits here hypothesised to be recently frozen sulphur melts. Synoptic observations of the surface of Io may uncover large-scale surface albedo and colour changes—particularly in the deeply coloured regions. There are several other possible sources of variable features not involving liquid flows, including the direct deposition of solid volcanic effluvia in the immediate vicinity of calderas and the surface condensation of gas phase effluvia, including SO_2 and S_n. The white deposits on Io might be S_n, rapidly cooled from just above the melting point, but frozen SO_2 is a very promising candidate. The white deposits, like the volcanoes, are also concentrated towards equatorial latitudes.

Decay of sulphur chromophores

A related question concerns the timescale for decay of the sulphur chromophores, particularly the metastable allotropes, at ionian ambient conditions. The colour of quenched liquid sulphur at room temperature persists 'indefinitely' (M. Gouterman, personal communication). Conservatively, we interpret 'indefinitely' as $\gg 1$ month $\sim 3 \times 10^6$ s. We adopt exponential colour decay statistics of the form $t = t_0 (\exp (E/kT) - 1)$, where E is the bond energy of the chromophore, and where the equation satisfies the boundary conditions that $t = \infty$ at $T = 0$ and $t = 0$ at $T = \infty$. We then readily find that the characteristic time for chromophore decay at $T = 135$ K greatly exceeds the lifetime of Io for $E > 1$ eV. Thus, provided the ionian chromophores are caused by chemical bonds stronger than van der Waals forces or hydrogen bonds (roughly ~0.1 eV), the coloured deposits on the surface of Io should persist for geologically significant periods of time, from a reaction kinetics standpoint. Charged particle impact and UV radiation damage might, however, change the coloration in shorter times.

Sulphur coverage

Typical ballistic flight times for solids in the ionian volcanic plumes are ~1,000 s. With the minimum mass ejection rates of Johnson *et al.*[15] we calculate productivities for Io volcanoes of 4×10^6 to 3×10^5 g s^{-1}, which is comparable to the Mt Pelee or (1924) Kilauea eruptions, but less than the Surtsey and much less than the Krakatoa eruptions[14]. What is striking about Io volcanism is not the individual production rates but the number, frequency, altitude (because of the low atmospheric pressure) and sulphur chemistry of the events. It is of interest to calculate what the mean surface area covered by molten sulphur is at a given moment. Microwave occultation measurements made by Pioneer 10 derive[8,26], from the ionisation profile of the nightside ionian atmosphere, a surface pressure of neutral constituents, but for an incorrect composition model. An earlier upper limit, derived from a stellar occultation, of 10^{-6} bar[27] is consistent with the Voyager discovery[11] of 10^{-7} bar of SO_2. We conservatively assume that the atmosphere is composed mainly of S_2, in vapour pressure equilibrium with a sulphur melt and that the gas phase volcanic effluvia are so hot that they produce significant lateral spreading before they chemically react or freeze out. Rough values for the vapour pressure over black liquid sulphur near 250 °C are then ~10 mbar; over red liquid sulphur ~1 mbar, and over yellow ~0.1 mbar. Therefore, in the three cases, for the mean atmospheric pressure to be 10^{-6} bar the fractional coverage of black melts must be $<10^{-4}$; for red, $<10^{-3}$; and for yellow $<10^{-2}$. To reach 10^{-7} bar ($[S_2] \sim [SO_2]$) these values must be lowered by one order of magnitude. Thus only a tiny fraction of the surface of Io can be molten at present, although that fraction may be much larger than the corresponding fraction for Earth. Accordingly, the existence of a major flow may be accompanied by a significant increase, at least locally, of surface pressure. Even if 10^{-3} of the surface is at $T \simeq 300$ °C its relative contribution to the net thermal emission from Io will be small.

Vertical relief of several kilometres, tending to localise around calderas on Io, has been recognised[6]. These may be silicate extrusions through the underlying thiosphere. But it is of interest to examine the possibility that they are constructions largely of elemental sulphur which is otherwise in such abundance on the ionian surface. In the Peale *et al.* model[17] of tidal heating in the interior of Io the melting point of sulphur is reached only several kilometres below the surface. Thus, sulphur relief features on Io could have roots extending subsurface only by an amount equal roughly to their height above surface. The tensile and yield strength of such sulphur constructs could be as low as 10^8 dyn cm^{-2} for the density of sulphur and the low acceleration due to gravity on Io and the constructs would still not be crushed by their own weight. (Typical tensile and yield strengths for silicates at room temperature are in excess of 10^9 dyn cm^{-2}). However, the density of solid sulphur exceeds the density of the liquid; if the solid were porous to account for flotation, the strength of the solid would decline markedly, and melting of such a sulphur iceberg at its base would require a rapid production rate for such constructs to maintain the observed steady state population. On the whole, silicate relief on Io may be more likely than sulphur relief (see ref. 4), although surface flows are very probably liquid sulphur and not silicates.

Conclusions

The variegated black, red, orange, yellow and (perhaps) white coloration on Io seems explicable in terms of liquid sulphur, produced in ionian volcanoes and solfataras, and quenched at Io ambient temperatures ≃135 K. A number of liquid sulphur flow fronts and frozen rivers appear in the Voyager images. The darker hues may be regions of past sluggish laminar flow, the lighter hues of fast turbulent flow. Opaque layers are at least tens

of micrometres to tens of centimetres thick, depending on colour, and, at present volcanic eruption rates, should have lifetimes against covering by airborne effluvia between days and centuries. The time scale for thermal degradation of chromophores at ambient Io temperatures is significantly longer. Thus coloured liquid flow fronts would have been covered over by solid volcanic effluvia in geologically very short periods of time unless new fluid flows occurred in the same interval. Since the fluvial patterns observed are almost certainly surface rather than atmospheric flows, this suggests that the average renewal time scale of the colour and albedo (but not topographic) features on Io is $\lesssim 10^3$ yr. In this sense it is the youngest solid surface in the Solar System. The white deposits on Io might be orthorhombic sulphur or SO_2 or other atmospheric effluvia deposited from the atmosphere. However, they must be laid down fast enough not to be covered by flows of molten sulphur. The coloured regions of Io may be where liquid sulphur flows dominate the condensation and deposition of volcanic effluvia; while the white regions represent the opposite case. The dark poles of Io may represent a non-uniform distribution of previous volcanic events, concentrated towards high latitudes. Alternatively, the lower polar temperatures may produce more rapid quenching of hot sulphur melts, and the preferential preservation of their deep colour. From the deduced neutral atmospheric surface density of Io and the vapour pressure of gas phase sulphur above a melt we provisionally calculate an upper limit to the present fractional surface area of Io which is molten between 10^{-2} and 10^{-5}. Even with the high value for this fraction, the time interval between the appearance of successive major flows of molten sulphur on Io must be less than a decade for the renewal of the entire surface in colour and albedo in $<10^3$ years to be accounted for. But this is consistent with the turnover times calculated independently in Table 1. Thus arguments from volcanic deposition rates and from the vapour pressure over sulphur melts are both consistent with the notion that the surface of Io changes significantly in geologically very short periods of time. Systematic and synoptic observations of Io over several years, as by the Galileo mission, promises the discovery of major surface changes.

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Photometric evidence on long-term stability of albedo and colour markings on Io

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Historical measurements of the albedo and colour of Io are examined for evidence of major resurfacing events.

THE Voyager discovery[1,2] of intense volcanic activity on Io raises the possibility that endogenic processes could alter the large-scale distribution of colour and albedo on the surface of this satellite in extremely short times on a geologic scale. Estimates of the resurfacing rates on Io from the observed volcanic eruptions and from the lack of observed impact craters have led Johnson *et al.*[3] to estimate average resurfacing rates of at least 0.1 mm yr^{-1}, probably ~1 mm yr^{-1} or more. In a region where an eruption is taking place, the rate could be two or more orders of magnitude larger. Given the size of the observed eruptions[4], it is not difficult to imagine deposition of an optically thick layer of fresh pyroclastics or recondensed volatiles over tens of thousands of square kilometres on Io in times as short as a few weeks.

Precision measurements of the brightness of Io and the other Galilean satellites have been carried out several times since the pioneering photoelectric work of Stebbins and Jacobsen[5,6] 50 yr ago; similar measurements of colour extend back to the work of Harris[7] in about 1950. This article reexamines the historical measurements of the albedo and colour of Io to determine whether there is evidence of variations, or if not, to set a limit on major resurfacing events resulting in hemispheric brightness changes during the past half century.

Historical data

Extensive broad-band, unfiltered photoelectric measurements of the galilean satellites were first carried out in 1926 and 1927 (refs 5, 6); in the 1950s, less extensive observations on the *UBV* system were undertaken[7]; and by the mid 1970s extensive sets of observations in *UBV* and *uvby* had been obtained by several observers[8–11]. Because the brightness of the satellites varies with both orbital longitude (rotation) and solar phase angle, many observations are required to separate the functional dependence of brightness on these two parameters. (Alternatively, all of the data necessary to define the mean brightness and rotation curve can be obtained over a very small range in phase, say 5–7°, neglecting the phase dependence.) The available observations have been reviewed elsewhere[10–12], and the present results are derived from those discussions.

Table 1 summarises the measurements of brightness. In order to minimise variations due to differences among photometric

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Table 1 Historical brightness of Io (relative to Ganymede)

Date	Ref.	Mean mag*	Amplitude	Longitude of maximum	Longitude of minimum
1926–27	6	0.24†	0.10‡	100±20	270±20
1951–54	7	0.33	0.18	?	300±30
1973	8	0.43	0.19	95±10	320±10
1973	9	0.41	0.11	100±10	310±15
1974	10	0.42	0.18	100±10	310±10
1974	9	0.40	0.13	100±10	300±15

* $[\bar{V}(\text{Io}, \alpha = 6°) - \bar{V}(\text{Ganymede}, \alpha = 6°)]$.

† As transformed by Harris[7]. Since these observations were unfiltered, the transformation introduces an unknown uncertainty of at least 0.1 mag.

‡ The measured amplitude was 0.20; the value here is based on the assumption that Stebbins and Jacobsen[5,6] measured primarily blue light, and Millis and Thompson[9] found in 1973–74 that the amplitude in B was about 0.1 mag less than the amplitude in V.

systems and choice of standards, we have normalised all Io photometry to average values for Ganymede obtained by the same observers during the same time period. We thus assume that Ganymede is unchanging, as indicated by the antiquity of its heavily cratered surface[2]. Because the Stebbins and Jacobsen[5,6] observations were unfiltered, their data do not transform properly to the standard V system. We have listed the mean V magnitude as transformed by Harris[7], but we emphasise the difficulty of achieving a proper photometric transformation of these disparate data to a common system for an object as red as Io. Thus, the absolute brightnesses, even when normalised to Ganymede, remain uncertain to perhaps ±0.05 mag. In contrast, the amplitude of the light curve should be measured to ±0.03 mag, and the longitudes of maximum and minimum to ±10° (standard deviations).

There is substantial difference in the mean magnitudes listed in Table 1 for 1926–27 and 1951–54, in comparison to more recent data, suggestive of intrinsic changes in albedo. Unfortunately, the changes are not large enough to exclude the possibility that they are due to systematic effects, resulting from problems in the observations or in differing colour response of the instruments. Some observers[9,10,13,14] have suggested that Io has undergone a monotonic brightening with time; however, we do not see conclusive evidence of a long-term trend of this sort.

The data in Table 1 on light curve amplitude and shape are probably more secure than those on mean magnitude. The amplitude agreement is excellent, except for the 1926–27 data which yield a smaller amplitude in blue than has subsequently been seen, again suggestive of change but insufficient to demonstrate it conclusively. The mean longitude of the minimum, from Table 1, is ~300°; with a spread from 270° to 320°. The presence of a dark trailing hemisphere on Io is clear, and all data since Stebbins and Jacobsen agree on a deep minimum close to 300°. There is no evidence in these data for any clear temporal trends in the location of dark deposits, and the 50 yr stability of the albedo variations with longitude is large relative to calculated resurfacing times for Io.

A less extensive but significant data set exists for longitudinal variations in colour. Starting with Harris' observations[7] and continuing through more recent spectrophotometric observations[8,9,15,16], all observers have consistently noted that the dark longitude zone near 300° is much redder than the rest of the surface. This general agreement is reinforced by detailed spectral studies over a more limited time span. In particular, medium resolution (4–25 nm) spectrophotometric differences between the bright and dark hemispheres measured by Johnson in 1969[15] and similar measurements reported by Nelson and Hapke[16] during 1975–77 agree within 5% over a range of wavelengths from 300 to 700 nm.

Perhaps the most sensitive way of looking for changes on a surface is by means of polarisation. The degree of polarisation is sensitive not only to the albedo of the surface material, but also to its texture, and can be measured to accuracies of at least 1‰ for objects such as Io[17]. The most extensive set of recent photoelectric polarisation measurements of Io are those of Zellner and Gradie (personal communication), who find high scatter in some of the Io polarimetry. Although the scatter is difficult to interpret in terms of specific changes on Io, it is probably real, and is evidence of detectable changes on the satellite.

All of the photometric data reviewed here show unusually high scatter for Io. In the past, these apparent variations have been rationalised as being due to difficulties in observing Io in the presence of scattered light from Jupiter. Now, we tend to accept at least part of the variation at face value as indicating major changes affecting areas on the satellite of $\sim 10^4$ km^2. However, the overall stability remains striking, with no indication of changes in total albedo over 50 yr $\geq$10%, and with the longitudinal asymmetry of albedo and colour preserved to within the accuracy of the measurements.

Interpretation

Is the degree of observed photometric stability of Io consistent with the resurfacing rates deduced from Voyager 1 observations? In order to be detected, changes in colour and albedo must be of an extent and magnitude sufficient to alter the hemispherically averaged optical properties measured by Earth-based photometry. Therefore what are the albedo and colour contrasts typical of volcanic deposits on Io?

The level of contrast in blue light (478 nm) can be judged from Voyager 1 frame FDS 16368.38, centred on longitude 150° (see *Plate 7*, p. 789). The highest normal reflectivities in this image are 0.76 for extensive white areas that may be SO_2 frost[18]. The darkest major regions, which are concentrated near the poles, have a reflectance of 0.20; near the centre of the disk, reflectances range down to about 0.30. Thus, contrast ratios (bright/dark) of factors of two to three are present, with both light and dark material apparently associated with eruptive activity. As shown in *Plate 7*, these contrasts are present at relatively small spatial scales. Io has a spotty appearance, with spots typically a few hundred kilometres across.

For a contrast ratio of two, a change affecting 2% of the disk, corresponding to a feature with a linear dimension of about 500 km, would produce an integrated brightness change of 0.01 mag. This dimension is characteristic of the largest eruption-associated surface markings seen by Voyager 1; in principle, then, photometry of 0.01 mag precision might detect changes associated with a single very large eruption if it produces material contrasting strongly with the pre-existing surface. However, this level of precision is rarely, if ever, attained in photometry of Io because of scattered light from Jupiter, and the observations shown in Table 1 indicate only that no changes >0.1 mag have taken place for over 50 yr, and none >0.03 between 1973 and 1974. The area corresponding to 0.1 mag is 20% of the disk, or ~1,500 km. A history of volcanic eruptions such as those seen by Voyager 1 randomly spaced in longitude would not be expected to result in albedo changes of this magnitude even if the material deposited had the very high contrast level assumed for this estimate.

Colours can be measured photometrically with more precision than albedos, but the historical record on colour measurements is meagre. Examination of blue (478 nm) and orange (590 nm) Voyager images shows that the red areas are 50% more reflective in orange than in blue, while the white areas are essentially neutral. Thus, the covering of a white area by red volcanic deposits, or of red material by white deposits, should produce a measurable (0.02 mag) change in disk-averaged B–V colour for areas as small as 700 km on a slide. Careful photometry might thus detect volcanically produced colour changes, but only at the limit of sensitivity of the technique.

There remains the problem, not of detecting changes in the average albedo and colour of Io, but of understanding the presence of major longitudinal differences in the first place. If the surface is constantly renewed by volcanic activity, and if the volcanoes are well distributed in longitude, why should the dark, reddish material be found concentrated in equatorial latitudes

preferentially on the trailing hemisphere over a 50-yr time span? At the resurfacing rates deduced from Voyager 1 of ~1 mm yr^{-1}, an essentially new surface, from the optical point of view, should be generated within a few decades at most. The preservation of the observed longitudinal photometric asymmetry for 50 yr therefore suggests one of the following three explanations: (1) a single eruptive centre (such as P-1), tapping a particular magma source, has continued to dominate the volcanic deposition on the trailing hemisphere for at least 50 yr; (2) there exists a persistent hemispheric compositional dichotomy in the magma source regions, such that darker material is deposited preferentially on the trailing side in spite of changing individual eruptive centres; (3) the photomeric asymmetry is caused by exogenic modification of the surface deposits, for instance, by magnetospheric interactions. We cannot at present distinguish between these three possibilities, which have quite different implications for the nature of volcanism on Io.

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Identification of gaseous SO_2 and new upper limits for other gases on Io

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Gaseous SO_2 has been identified on Io. The estimated abundance of 0.2 cm atm is consistent with an atmosphere in equilibrium with solid SO_2 at the local surface equilibrium temperature. Preliminary upper limits for several gases, including sulphur compounds and other terrestrial volcanic emissions have been derived. Io is apparently depleted in hydrogen, carbon and nitrogen.

ONE objective of the Voyager IR experiment (IRIS) is to search for and analyse atmospheres on the jovian satellites[1]. The possibility of an atmosphere on Io has been studied for some time[2–6], and as a result, upper limits have been established for many gases and for the total surface pressure. The possible existence of various sulphur compounds on the surface[7,8], together with the discovery of active volcanism[9,10] suggest that further investigations should include searches for additional gases which are products of terrestrial volcanism, and sulphur compounds in particular.

Previous spectroscopic investigations of the atmosphere of Io have been restricted to the visible[2,3] and near IR[6]. Although it contains the rotational and vibrational signatures of many gases, the thermal IR region has not been investigated because of difficulties in obtaining high-quality spectra. The Voyager 1 IR spectroscopy experiment, however, has now made this region available.

During the flyby of Io, over 200 IR spectra of the satellite have been obtained at a spectral resolution of 4.3 cm^{-1} and spatial resolutions down to 50 km (ref. 11). The useful spectral range of these data extends from 180 cm^{-1} to an upper limit which depends on surface temperature: about 650 cm^{-1} at 90 K and 1,100 cm^{-1} at 130 K. One of the most interesting subsets of the data covers an unexpectedly warm region containing a volcanic feature near which a plume (Plume 2 of Smith *et al.*[10]) originates. Spectra of adjacent regions show surface brightness temperatures near 130 K at 250 cm^{-1}, with a slight rising trend with increasing wavenumbers. However, spectra over the warm region show brightness temperatures in excess of 210 K at large wavenumbers. This effectively extends the useful spectral range of the data by increasing the wavenumber at which the signal-to-noise ratio equals unity to beyond 1,900 cm^{-1}.

Identification of SO_2

Seven spectra over the 'hot spot' were averaged to provide the data for this analysis. The portion of the resulting spectrum between 1,000 and 1,500 cm^{-1} is shown in Fig. 1 and an expanded version in Fig. 2. We identify the feature between 1,320 and 1,380 cm^{-1} as the ν_3 band of SO_2. In addition to ν_3, SO_2 has two other IR-active fundamental bands in the IRIS spectral range: ν_1 at 1,152 cm^{-1} and ν_2 at 518 cm^{-1}. The strengths of the ν_1, ν_2, and ν_3 bands are in the ratio 1:1:8, respectively[12]. As a result of this, of lower thermal contrast at lower wavenumbers, and of possible additional radiative transfer effects, only the strongest band is clearly evident in the IRIS spectrum. A faint suggestion of ν_1 seems present in Fig. 1. The ν_2 band lies in a region strongly contaminated by presently unidentified spectral features; however, no clearly identifiable structure assignable to ν_2 is apparent.

To confirm the identification of ν_3 and to obtain an estimate of the sulphur dioxide abundance, a simple model consisting of a single homogeneous layer of SO_2 at 130 K has been used to generate synthetic comparison spectra. Line positions, strengths, and collision halfwidths are from A. Goldman (personal communication). Doppler and lorentzian broadening are both taken into account. Several abundances, with their corresponding surface pressures, have been considered; in keeping with the homogeneous model, the pressure used in the computations is taken as half of the corresponding surface pressure. A continuum level is chosen which matches that of the data on both sides of the band. Resulting synthetic absorption spectra for SO_2 abundances of 0.1 and 1.0 cm atm (corresponding to surface pressures of 5.2×10^{-8} and 5.2×10^{-7} bar, respectively) are also shown in Fig. 2. The band contour in the data is

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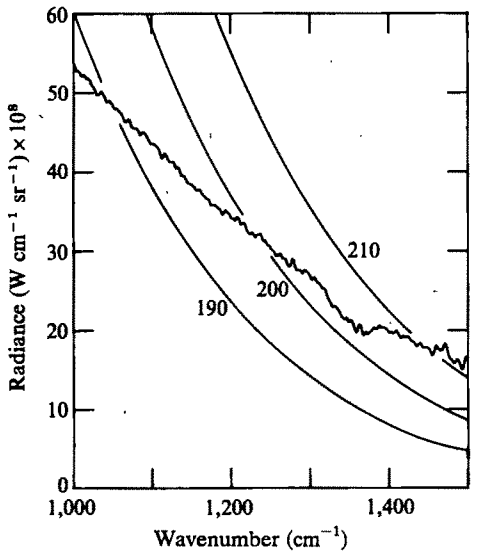


Fig. 1 Portion of Io spectrum over hot spot. The weak ν_1 (1,150 cm^{-1}) and the stronger ν_3 (1,361 cm^{-1}) bands of SO_2 fall in this spectral range. The smooth curves represent black body spectra at the indicated temperatures.

rather well reproduced; a best fit seems to lie between the two calculated curves shown, but closer to the one representing 0.1 cm atm. We adopt 0.2 cm atm as the most likely abundance, with an estimated probable error of a factor of 2. Refinement of this abundance estimate requires a more realistic radiative transfer model.

Upper limits for undetected constituents

Several other gases were searched for in the Io spectrum, but were not detected. Upper limits were calculated using an expression derived from the radiative transfer equation[1]:

$$u = \frac{nN}{[B(T_s) - B(\bar{T})]} \times \frac{\Delta\nu}{S(\bar{T})}$$

Here u is the upper limit abundance; n is the margin above the noise level (N) which is considered adequate for detection ($n = 2$ is adopted); $\Delta\nu$ is the instrumental resolution (4.3 cm^{-1}); $[B(T_s) - B(\bar{T})]$ is the thermal contrast between the lower boundary and the atmosphere, where B is the Planck function, T_s is the background brightness temperature, and $\bar{T}$ is the mean atmospheric temperature; $S(\bar{T})$ is the line strength of the atmospheric constituent. Where possible, intervals with strong Q-branches were chosen. The total line strength used was obtained by summing all lines within the appropriate 4.3 cm^{-1} resolution element. For calculation purposes, two mean gas temperatures, 130 and 250 K were chosen; the first is for a gas in equilibrium with the surface surrounding the 'hot spot', and the second is for a warm atmosphere model[13]. The background temperature, T_s, is taken from the measured Io spectrum. Because of the nonisothermal character of the spectrum, evident in Fig. 1, T_s is a function of wavenumber. The temperature dependence of the line strength and the variation of the noise level with wavenumber have also been taken into account.

Preliminary upper limits for 11 gases, including four sulphur compounds (COS, CS_2, SO_3, and H_2S), are listed in Table 1. The line strength for COS is taken from Penner[16]; that for CS_2 is derived from Robinson[17] and Smith[18]. The strength for SO_3 is obtained from Bent and Ladner[19] and from Majkowski *et al.*[20], as follows. The latter group measured the absorption coefficient of ν_3 at 1,391 cm^{-1}. From this the absorptions of the fundamentals ν_2 at 500 cm^{-1} and ν_4 at 532 cm^{-1} were obtained by scaling the transmission spectra of Bent and Ladner.

The strongest band of H_2S in the spectral range of the data (ν_2) is relatively weak; hence, the upper limit has not been derived from the IRIS spectrum, but from an extension of the analysis of the 1971 occultation of Beta Scorpii by Io. From the geometry of the eclipse and the rapid disappearance of the stellar signal, Bartholdi and Owen[2] showed that an upper limit to Io's surface pressure in atmospheres is given by $2\times10^{-13}\times(T/M)^{3/2}/\rho$, where T, M, and ρ are the temperature, molecular weight, and refractivity of the gas. Assuming that the gas is in equilibrium with the surface, and noting that the occultation points are near the terminator, we take $T = 110$ K. The refractivity of H_2S is approximately 6.3×10^{-4} (ref. 21). Thus, an upper limit to the surface pressure is 2×10^{-8} bar, which corresponds to an upper limit abundance of 0.07 cm atm. A better limit for H_2S may be obtained from the IRIS data when line strengths for the pure rotation spectrum become available.

We have generated the line positions and strengths for CO_2, CH_4, and NH_3 from standard quantum mechanical formulations using molecular constants based on laboratory data. For O_3 and H_2O, the line positions and strengths of McClatchey *et al.*[22] are used.

Discussion

The region of Io observed during these measurements contains three features: Plume 2 (ref. 10), a warm region at ~290 K which covers roughly 10% of the instrument field of view, and a remaining background area at ~130 K (ref. 11). The observed SO_2 could originate from any or all of these sources.

Since sulphur dioxide is generally the major sulphur bearing component of terrestrial volcanic gases[23], an obvious source for the gas is the volcanic plume. The resurfacing rate due to this plume can be used to estimate the amount of gas within it. By assuming that the optically thick (particulate) portion of the plume occupies 10% of the cylinder defined by the plume boundaries, and that the gas-to-solid mass ratio is 2, the data of Table 1 of Johnson *et al.*[24] indicate that the plume contains at least 10^9 g of gas. As uncertainties in the resurfacing rate and in the gas-to-solid ratio are unlikely to provide an error of two orders of magnitude, 10^{11} g seems to be a safe upper limit to the gas content of the plume. Over the warm area, which provides the best thermal contrast, the derived SO_2 abundance implies 2×10^{11} g of SO_2. Thus, only in the most extreme case would the plume contribution be a significant fraction of the total.

The temperature of the warm area is far above the melting point of SO_2 (198 K); explosive boiling and extensive condensation as clouds would be evident if a substantial amount of liquid SO_2 were present[25]. Theoretical arguments[26,27] suggest that a cooling sulphur extrusion is more likely. Outgassing of SO_2 originally dissolved in such a melt will certainly occur; however, once the material has cooled this extensively (95 K below the melting point), such activity should be much reduced due to the solidification of at least the surface layer; further data (see below) suggest that this is the case.

From spectra recorded near to, but not including the hot spot, the surface temperature of the surroundings is determined to be ~130 K. At this temperature, accumulation of solid SO_2 by

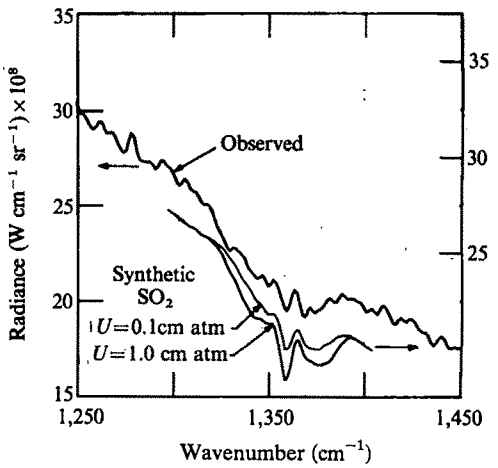


Fig. 2 Comparison of Io and synthesised spectra in the vicinity of the ν_3 band of SO_2. The synthesised spectra represent absorption by homogeneous layers of 0.1 and 1 cm atm of pure SO_2 gas at 130 K and pressures of 2.6×10^{-8} and 2.6×10^{-7} bar, respectively. The measured and calculated spectra are vertically displaced for clarity.

Table 1 Upper limits of various gases on Io

Gas	Band used (cm^{-1})	Vapour pressure abundance (cm atm) above solid at 130 K	Upper limit (cm atm) for T_{gas} = 130 K	Upper limit (cm atm) for T_{gas} = 250 K
COS	ν_1 859	510*	3.8×10^{-4}	1.5×10^{-4}
CS_2	ν_3 1,535	1.4×10^{-1}*	3.5×10^{-5}	2.8×10^{-5}
SO_3	ν_2 497	1.6×10^{-6}*	6.5×10^{-5}	2.4×10^{-5}
H_2S	—	56	— 7×10^{-2}†	—
CO_2	ν_2 667	1,050	1.5×10^{-4}	5.1×10^{-5}
O_3	ν_1 1,042	‡	1.9×10^{-3}	1.1×10^{-3}
N_2O	ν_1 589	4,780	7.4×10^{-3}	2.2×10^{-3}
H_2O	Rot. 254	6.4×10^{-7}*	9.2×10^{-3}	1.9×10^{-4}
CH_4	ν_4 1,306	‡	1.0×10^{-2}	1.7×10^{-3}
NH_3	ν_2 931	11	1.4×10^{-3}	3.4×10^{-4}
HCl	Rot. 206	1.8×10^{-4}	3.8×10^{-3}	3.7×10^{-4}

With the exception of H_2S, the analysis is based on the absence of spectral features in the Io spectrum containing a hot spot. The H_2S limit is based on a stellar occultation (see text). For comparison, abundances of gases in equilibrium with their solid phases are included. These are evaluated from ref. 15 unless otherwise indicated.

* Based on data extrapolated from ref. 14.

† From stellar occultation using T_{gas} = 110 K (see text).

‡ Above melting point.

precipitation of solid particles from the plume, by direct condensation of the plume upon contact with the surface, and possibly by extrusion of liquid SO_2 through surface fractures[25] may occur. Indeed, the 4.1 μm feature in spectra of Io obtained from the Earth[28,29] has been identified as the $\nu_1+\nu_3$ band of solid SO_2[30,31]. Thus, it seems likely that an SO_2 atmosphere in approximate equilibrium with the vapour pressure of solid SO_2 could become established.

The surface pressure corresponding to our derived abundance of SO_2 is 1.0×10^{-7} bar, with an uncertainty of a factor of 2. Excellent agreement therefore exists with the vapour pressure of SO_2 at 130 K (1.4×10^{-7} bar), as determined by Wagman[32]. However, our measurement is significantly higher than the 10^{-8}–10^{-9} bar limit set for the neutral atmospheric pressure from the Pioneer 10 occultation measurements[4]. On the other hand, our observation was made near 13.00 h LT, whereas the Pioneer occultations occurred near 05.30 and 17.30 h LT. For these local times and comparable low latitudes, IRIS measurements indicate surface temperatures below 110 K. Extensive diurnal surface temperature variations, coupled with the strong dependence of SO_2 vapour pressure on temperature, will produce very steep gradients in atmospheric density with local time, and on collapse of the SO_2 atmosphere to an exosphere on the night side of Io; extreme cases of such behaviour, which also show the effects of trapping, are evident in exospheric models for the lunar atmosphere[33,34]. At 110 K, the vapour pressure of SO_2 is 2.5×10^{-10} bar, which is more compatible with the Pioneer results. It is more difficult to reconcile the present determination with the much lower neutral atmosphere estimates by Johnson, Matson and Carlson[5].

Recent theoretical work[24,35] also supports the presence of SO_2 in the volcanic plumes and as a neutral atmosphere; sulphur dioxide seems capable of providing the sulphur and oxygen to the Io torus, and of supporting the measured ionosphere.

A strongly blue component, assigned to Rayleigh scattering by particulates, has been observed in images of the volcanic plumes[10,24]. Minute particles of condensed SO_2 could account for this. Close inspection of the IR data and the synthetic spectra in Fig. 2 reveals a possible weak absorption between 1,320 and 1,340 cm^{-1}. In the solid state, the ν_3 band of SO_2 is shifted into this region, as is shown in Fig. 3. Comparison of the feature of the solid with the data does not show complete agreement; hence, no identification can yet be made. We are now conducting laboratory studies to provide quantitative information on this band.

Unfortunately, with two exceptions, it has not been possible to map the suggested SO_2 atmosphere over the planet because the surface is generally too cold to provide an adequate signal in the strong ν_3 band. The exceptions are two additional smaller hot spots observed on the dark side of Io. Based on preliminary pointing information, these occur at (32°, 103°) and (28°, 194°). Taking account of the 425 km diameter IRIS field of view at the time of the observations, the first of these can be identified with Plume 5 (ref. 10); to date, no plume has been identified with the second. In both cases, the surface temperature surrounding the hot spot is below 100 K. Although the signal-to-noise ratio in these spectra is poor, no signature of ν_3 is apparently present; this is consistent with the low vapour pressure of 3.9×10^{-12} bar for SO_2 at this temperature. It also suggests that outgassing from the material at 290 K is not at present a major source of SO_2.

The atmosphere will be affected by factors such as local gradients associated with global temperature variations, and loss mechanisms due to ionisation, surface chemical reactions, and burial by the dominant gas constituent. However, if it is assumed that production mechanisms, such as plumes and extrusive activity, have been active long enough for a steady state condition to be established, then if loss processes are not significant, the vapour pressures of volatiles become important in the consideration of upper limits. If the vapour pressure exceeds the pressure corresponding to the derived limits, then the limits represent true upper limits on possible gaseous constituents, and thereby on their solid phases.

The limits for CH_4, NH_3, H_2S and N_2O of our preliminary results in Table 1 are improvements over previous determinations by Fink *et al.*[6]; limits for CO_2 and H_2O are improvements over values obtained by Bartholdi and Owen[2]. Note that the presence of an SO_2 atmosphere does not affect the derivation of the H_2S upper limit, since at the appropriate gas temperature (assumed equal to the surface temperature of ~110 K), the vapour pressure of SO_2 is so low that it will not be detectable; the vapour pressure of H_2S, however, will support a substantial atmosphere. As indicated in Table 1, for all of the gases except H_2O and SO_3, the limits correspond to pressures far below the respective vapour pressures at 130 K. Thus, we conclude that Io is deficient in these materials. The low abundances of carbon and nitrogen containing gases are significant, the implied depletion being consistent with the lack of C and N in the Io torus[37].

The vapour pressures of H_2O and SO_3 at 130 K place these gases substantially below our detection threshold, so the present limits only imply that there are no strong non-equilibrium sources of these gases within the instrument field of view. The sensitivity of the IRIS instrument to condensed phases on the satellite surface is much reduced by very low thermal contrast. Thus, the absence of near-IR absorption features of H_2O still provides the best upper limit for this constituent[29]. Combined with the low abundance of H_2S, however, it seems that Io is also deficient in hydrogen. The low H_2S abundance also conflicts with Hapke's suggestion that this gas should be present on Io[38].

Conclusions

The thermal IR spectrum of Io indicates the presence of a sulphur dioxide atmosphere which is in equilibrium with solid SO_2 at the local surface temperature. A likely source of this

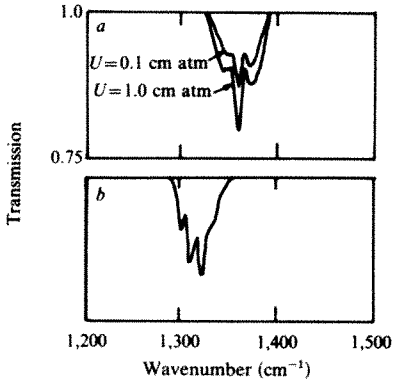


Fig. 3 *a*, Transmission spectrum of SO_2 gas computed from line-by-line integration. *b*, Measured SO_2 ice spectrum. Adapted from ref. 36.

material is volcanic activity. Losses to space may account for the sulphur and oxygen observed in the Io torus; condensation and precipitation may account in part for the burial of impact craters. Upper limits for several other gases have been set, which indicate a depletion of hydrogen, carbon, and nitrogen in the satellite; this is consistent with the absence of these species in the torus. All of these results provide strong support for major sulphur volcanism[26,27] driven by tidal interactions[39].

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Note added in proof: Interestingly, many of our conclusions have also been reached by W.-H. Ip and W. I. Axford (*Nature*, submitted).

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The stability of an SO_2 atmosphere on Io

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Measurements of Io's volcanism and the plasma torus suggest that SO_2 is a major constituent of its atmosphere.

Recent Voyager 1 measurements on Io's volcanism and the plasma torus suggest that Io could have a thermosphere with SO_2 as the major gas at a surface density of 10^{11}–10^{13} cm^{-3} on the dayside. The bulk of the atmosphere is most likely protected from Jupiter's co-rotating plasma at Io's orbit by either a magnetosphere of Io or by slowing down of the plasma in a comet-like interaction. Yet the scavenging rates above the magnetopause or ionopause are high enough that this atmosphere must be continually replenished by the volcanic activity, otherwise the atmosphere would disappear rapidly. Other minor constituents expected are argon, SO_3, S_2, O, S, and CO_2.

The maintenance of a well developed ionosphere observed on Io[1,2] has been puzzling due to the relatively strong ram pressure of Jupiter's co-rotating plasma at Io's orbit which would sweep away any gravitationally bound ionosphere[3]. If the ionosphere were protected, models proposed using NH_3 and Na (refs 3–6) would explain the observed ionospheric densities. The recent Voyager encounter of Io has yielded a large quantity of diversified data (see Table 1) from which an entirely different picture of Io is unfolding and which compels us to revise our thinking about Io's atmosphere and ionosphere.

Active volcanism observed on Io[7,8] could provide a continuous source of gases from Io's interior. The velocity of the ejected material is ≤ 1 km s^{-1} (ref. 7), comfortably less than the escape velocity of 2.56 km s^{-1} on Io. Hence, any direct escape is negligible. The resurfacing rate due to the dust ejected from the volcanoes is at least 10^{-3} m yr^{-1} (ref. 9). A comparable amount of gas would be needed to drive the dust out of the volcanoes. This leads to a gas outflux of $\sim 10^{14}$ molecules cm^{-2} s^{-1} averaged over the globe. In comparison, a supply rate of only $\sim 10^{10}$ cm^{-2} s^{-1} for ions of S and O is needed into the plasma torus[10] if ion co-rotation energy is the primary source of heating the torus electrons. An estimate of the total gas supply rate can be made by requiring that the observed maximum plasma density of 4,000 cm^{-3} (ref. 11) be maintained over the diffusion time of ions out of a 2 R_J thick plasma torus (R_J = radius of Jupiter). Eviatar *et al.*[12] have estimated a diffusion time of 20 days to drift 1 R_J in radial sense at Io's orbit. This leads to a supply rate of $\sim 10^{11}$ cm^{-2} s^{-1}. Mass loading would, however, reduce the velocity of ions and hence increase the diffusion time. Based on Voyager 1 plasma data which suggest massive mass loading of co-rotating plasma by Io, the estimated supply rate is $\sim 10^{12}$ cm^{-2} s^{-1} (ref. 23), still two orders of magnitude less than the gas outflux from the volcanoes.

This inventory leads to an obvious but important conclusion. Only a small fraction of what is coming out of Io's volcanoes is escaping; the bulk of it is being recycled. There must be an atmospheric reservoir which maintains the gases until they have had a chance to recycle, perhaps by condensation to the surface on the nightside and subsequent chemical reactions.

Such an atmosphere must be protected from the co-rotating plasma in order to be stable. The comet-like interaction proposed by Cloutier *et al.*[3] is still a promising approach, in which case the ionopause could be regarded as the atmospheric boundary beyond which all the neutrals are ionised and lost. Another attractive mechanism has been proposed recently by Kivelson *et al.*[13] which invokes an intrinsic magnetic field of Io and a well developed magnetosphere which would provide a magnetic barrier to the co-rotating plasma and hence, shield the atmosphere.

Atmospheric constituents diffusing across the magnetopause or the ionopause boundary (~200 km in the upstream direction) will be subject to ionisation by hot electrons in the plasma torus and subsequent scavenging by the co-rotating plasma. According to solar-wind induced mass loss formalism of Michel[14], mass loading of the co-rotating plasma will limit the maximum swept flux to 6×10^8 cm^{-2} s^{-1}, about 1/40th of the magnetospheric wind itself. The Michel limit apparently does not apply since the

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Table 1 Constituents detected in the vicinity of Io and in the associated plasma torus

Experiments	Measurement region	Constituent	Density (cm^{-3})	Temperature (K)	Ref.
Ground-based telescopes	Cloud around Io	Na	10		20
	Cloud around Io	S^+		$T_i \sim 10^4$	21
Pioneer 10 UV experiment	Incomplete torus at Io's orbit	H			22
Pioneer 10 radio occultation experiment	Pre-sunset ionosphere	e	6×10^4		1
	Pre-sunrise ionosphere	e	9×10^3		2
Voyager UV experiment	Plasma torus remote sensing	S^{2+}	95		
		S^{3+}	55	$T_i \sim 10^5$	10
		O^{2+}	850		
Voyager plasma science experiment	Plasma torus 5.3 $R_J < r < 6.3$ R_J *in situ*	O^{2+}			
		S^{2+} or O^+	Total ~ 2,000	$T_i \sim 10^5$	15
		S^+			
		S_2^+ or SO_2^+			
Voyager planetary radio astronomy experiment	Plasma torus *in situ*	e	2,000–4,000	$T_i \sim 10^5$	11
Voyager imaging experiment	Volcanoes	Dust umbrella			7, 8
Voyager IR experiment	A volcanic plume	SO_2			19

needed flux input from Io into the plasma torus is comparable to the co-rotating plasma flux. Either Jupiter's magnetic field can impart sufficient momentum to sustain such a large flux input, or the co-rotation would cease at Io's orbit due to this mass loading. In any case the Io torus measurements from Voyager 1 imply that a gas flux of 10^{11}–10^{12} cm^{-2} s^{-1} averaged over the globe could be lost from Io into the plasma torus.

Only the dayside atmosphere is subject to escape while the cold nightside and poles would serve as a sink of atmospheric gases. However, as shown in Fig. 1, even the dayside atmosphere will be completely protected over about half the Io's orbit when the dayside is towards the downstream direction. The scavenging of atmospheric gases is maximum at the eastern elongation and minimum at the western elongation.

The scavenging lifetimes of H, C, N, and O for the volatile escape can be estimated as follows. The atmospheric densities implied by the ionosphere are low enough that we can assume that each constituent is in diffusive equilibrium. In this case the depletion time, defined as the time it takes for the scavenging process to deplete the atmosphere by $1/e$ is given by $T_D = T_i \exp(z_b/H_i)$, where T_i is the lifetime against ionisation by plasma torus electrons, z_b is the magnetopause or ionopause altitude, and H_i is the scale height for the ith constituent. In the hot plasma torus region ($T = 10^5$ K) near Io, the measured electron density is ~2,000 cm^{-3} (refs 11, 15) which corresponds to an electron flux of 4×10^{11} cm^{-2} s^{-1}. The energy of these electrons ~10 eV, is, however, marginal for ionisation of most constituents and this flux therefore represents an upper limit to the flux available for ionisation of atmospheric constituents. The cross-section for ionisation by low-energy electrons near the threshold is $\sim10^{-16}$ cm^2 for most constituents. The resulting ionisation rate is 1–4×10^{-5} s^{-1}, probably closer to the lower value. Assuming an exospheric temperature of 1,000 K and $z_b = 200$ km, we obtain the atmospheric depletion times listed in Table 2. We caution that these lifetimes are based on the assumption that all neutrals diffusing across the boundary are ionised and lost. This is true if sufficient ionising flux is available and if the atmospheric column density above z_b does not exceed $\phi_{max}T_i$, where ϕ_{max} is the maximum flux that can be carried by the co-rotating plasma. If $\phi_{max} = 10^{11}$ cm^{-2} s^{-1}, the likely flux in view of the Voyager 1 data, the above assumption is valid for an SO_2 atmosphere with a surface density of $\leq10^{11}$ cm^{-3} averaged over the globe. At higher densities the depletion times will be longer but the differential escape of atmospheric constituents will be maintained.

It is immediately apparent that the lifetimes are so short that the atmosphere must be replenished continuously. Note that the lighter gases escape more rapidly than the heavier ones, as a consequence of the diffusive separation assumed in our model. It is interesting, however, to compare these lifetimes to Io's orbital period which is 1.77 days. Hydrogen, which has a depletion time shorter than the orbit period, will escape so rapidly that it has little chance of recycling at the surface. A large fraction of C, N, and O will also escape, but some will be recycled. Heavier elements, such as S, will be mostly recycled.

The implications of this differential loss on the evolution of Io's volatile content are significant. As a guide, it is instructive to consider the composition of gases ejected by the Earth's volcanoes. Rubey's[16] inventory of volcanic gases from Kilauea and Mauna Loa includes following median values of volume percentages: H_2O(73.5), CO_2(11.8), N_2(4.7), SO_2(6.4), SO_3(2.3), S_2(0.2), H_2(0.4), CO(0.5), Cl_2(0.05), and A(0.2). Io may have had similar composition of gases coming out of its volcanoes in early geological history. Since Io most likely has a molten interior[17], fresh material will be brought out to the surface by convective currents and hence, Io must have lost practically all of its hydrogen content over geological time through volcanic activity and subsequent escape. This could explain why no H_2O is observed on Io. Heavier gases CO_2 and SO_2 have much longer depletion times, but their escape would proceed through the rapid photodissociation of these constituents into atomic elements and their subsequent escape. The conspicuous absence of N(<10% of O) in the plasma torus[15] and the absence of energetic nuclei of both C(<3% of O) and N(<7% of O) in the jovian magnetosphere near Io[18] suggest that most of CO_2 and N_2 have also escaped from Io in addition to H_2O. Thus, NH_3 and N_2 previously suggested from the theoretical models[3–6] must be ruled out. Remarkably, SO_2, the next most abundant gas in the Earth's volcanoes, has been observed above one of the hot spots on Io by the Voyager IR experiment[19]. This provides a very important milestone in the current state of evolution of Io and is understandable in terms of the above model. Most likely, SO_3, S_2, and argon have also been retained. Atomic oxygen and atomic sulphur should be present as photodissociation products. Some CO_2 may also be present as a minor constituent.

Table 2 Atmospheric depletion times on Io

Atmospheric constituent	Depletion time (d)
H	1.2
C	2.0
N	2.2
O	2.4
S	4.9
A	6.9
H_2O	2.6
NH_3	2.5
CO_2	8.3
SO_2	20.0

Table 3 SO_2 atmosphere on Io

Surface Temperature T(K)	Vapour pressure[25] P(bar)	Surface density n(cm^{-3})
135	1.9×10^{-6}	1.4×10^{14}
130	5.8×10^{-7}	4.3×10^{13}
125	1.5×10^{-7}	1.2×10^{13}
120	3.5×10^{-8}	2.8×10^{12}
115	6.7×10^{-9}	5.6×10^{11}
110	1.0×10^{-9}	9.0×10^{10}
100	1.0×10^{-11}	1.0×10^{9}
90	2.1×10^{-14}	2.2×10^{6}

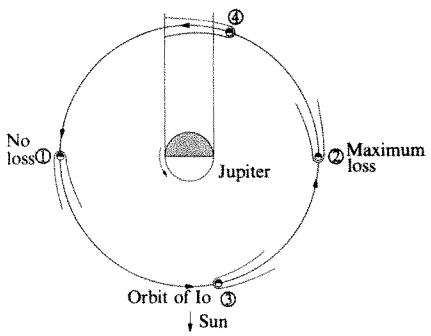


Fig. 1 The geometry of a magnetosphere protecting Io's atmosphere from Jupiter's co-rotating plasma at Io's orbit. Only the dayside would have a substantial atmosphere; hence the loss rate varies over Io's orbit. There is essentially no loss at the western elongation ①, while the scavenging rate above the magnetopause or the ionopause is maximum at the eastern elongation ②. Intermediate loss rates are expected at locations ③ and ④, which correspond to the location of Io in its orbit at the time of the Pioneer 10 radio occultation experiment and the Voyager 1 encounter, respectively.

In fact, the identification of gaseous SO_2 on Io, the evidence for solid SO_2 on the surface of Io[24], the measurement of sulphur and oxygen ions in the plasma torus[10,15], and the possible presence of SO_2^+ in the plasma torus[15] tempt us to consider SO_2 as the major gas in Io's atmosphere. Indeed, the upper limits to various candidate gases by the Voyager IR experiment[19] are consistent with this scenario. We can further examine this possibility by testing whether an SO_2 atmosphere is consistent with the observed density and structure of Io's ionosphere and the relative S to O abundance in the plasma torus.

For this purpose, we consider the dusk or pre-sunset ionosphere measured by the Pioneer 10 radio occultation experiment[1], since in our model the dusk side ionosphere is protected while the dawn side ionosphere is facing the co-rotating plasma and is subject to compression or a complex intermixing of plasmas. The sunlit SO_2 atmosphere will be ionised by the solar UV radiation shortward of 1,000 Å producing SO_2^+. In the photochemical equilibrium, the SO_2^+ ions will be lost by recombination with electrons and the SO_2^+ ion scale height is twice the SO_2 scale height. A fit to the electron density profile above the peak will require an exospheric temperature of 1,100 K. The ionosphere peak will occur at a level where the absorption optical depth $\tau = 1$. Requiring a $\tau = 1$ at 75 km and at solar zenith angle of 81° (the values corresponding to the Pioneer 10 radio occultation experiment), the SO_2 density is 2×10^9 cm^{-3} at this level. Assuming an SO_2 ionisation rate of 5×10^{-8} s^{-1} (analogous to CO_2) and an SO_2^+ recombination rate of $\sim10^{-7}$ cm^{-3} s^{-1}, the corresponding SO_2^+ ion density at the peak is 2×10^4 cm^{-3}, a factor of three less than the observed value. However, the SO_2 gas is a strong IR radiator, hence a cool SO_2 thermosphere ($T = 200$ K) analogous to the cool CO_2 thermospheres of Venus and Mars may not be unlikely. In this case, $\tau = 1$ will occur at a level where SO_2 density is 8×10^9 cm^{-3} and the corresponding peak SO_2^+ density is 4×10^4 cm^{-3}. Note that the exobase in this case is at ~105 km, not much above the ionosphere peak; thus the ion temperature could be substantially higher than the neutral temperature to match the observed ionosphere scaleheight. The peak electron density is still 30% less than the observed value which may be due to an uncertainty in the assumed ionisation cross-sections, recombination rates, and solar UV fluxes. Alternatively, the balance of ionisation may be provided by the precipitation of energetic electrons (~10 keV) or protons (~10 MeV) as suggested previously by McElroy and Yung[4] for a molecular atmosphere. These uncertainties, however, should not introduce more than a factor of 10 error in the required SO_2 density at the ionosphere peak.

An extrapolation to the surface in a single slab thermosphere approximation yields an SO_2 surface density of 10^{11}–10^{12} cm^{-3}. The vapour pressure of SO_2 at 110–117 K appropriate near the evening terminator (J. Pearl, personal communication) will maintain this surface density (see Table 3). Although this is an order of magnitude estimate for the surface density, it is remarkable that an SO_2 atmosphere in thermal equilibrium with the surface is consistent with the observed ionosphere. In this case, the SO_2 surface density near the subsolar point ($T\sim$ 130 K, J. Pearl, personal communication) could be as high as $\sim10^{13}$ cm^{-3}, which is consistent with a column of 0.2 cm atm (surface density $\sim5\times10^{12}$ cm^{-3}) at 1 p.m. local time on Io observed by the Voyager IR experiment[19].

The relationship between the torus ion densities and the atmospheric S and O abundance will require a photochemical model, but a rough estimate can be made as follows. Total sulphur ion densities add up to ~150 cm^{-3}, whereas total oxygen ion density is 850 cm^{-3} (ref. 10). Assuming that all O and S atoms above the magnetopause boundary are converted to ions, this implies a density ratio [O]/[S] = 3 at 200 km. For a 1,000 K exosphere, this scales to comparable O and S densities below the ionosphere peak at 75 km which is what one would expect since most of the SO_2 dissociation would take place within one scale height below the ionosphere peak.

In conclusion, a stable SO_2 atmosphere on Io shows promise if the volcanic activity is continuous. If, however, the volcanoes stop erupting, the atmosphere will be depleted at a rapid rate. Note that the atmosphere must be protected in order to be stable, either by a magnetosphere or by slowing down of the co-rotating plasma in a comet-like interaction. Both of these cases lead to a differential escape of gases which is required by the present state of Io's atmosphere.

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Significance of absorption features in Io's IR reflectance spectrum

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Absorption features in Io's reflectance spectrum suggest that frozen SO_2 molecules are present on the surface of Io as free frost; this may have important implications for Io's atmosphere.

Io'S deep 4.1-μm absorption may be due to frozen SO_2 molecules incorporated in or adsorbed on sulphur allotropes and other surface material, and present as free frost. Shallower absorption features near 3.8 μm and 2.6 μm may be due to H_2S trapped in surface material. We show here that the presence of SO_2 may have important implications for Io's atmosphere and transient phenomena; and suggest that any abundant metal-containing compounds are spectrally bland in the 2.0–5.0 μm range, such as halides or sulphides.

Among Io's many remarkable properties is a reflectance spectrum showing a steep dropoff in the UV, a high visible and near-IR albedo, and the absence of major absorptions in the 1.0–3.0 μm region[1,2]. It has been suggested[3–5] that those properties could be explained if the surface were largely covered with an anhydrous mix of sulphur and a variety of salts, mainly sulphates. Fanale *et al.*[3,4] further argued that such a unique surface could have formed by endogenic activity, specifically the percolation of aqueous solutions to Io's surface from a warm or hot interior, followed by H_2O loss to space[3,4]. Wamsteker *et al.*[6] also argued that elemental S was a major constituent and pointed out that the non-sulphur component had a visible spectrum resembling that of Saturn's rings.

The so-called 'evaporite' hypothesis[3,4] in which aqueous solutions are the carrier requires an efficient process of subsequent surface dehydration because IR spectra of Io[7–9] place increasingly severe limits[2,9] on the abundance of H_2O in any form in the optical surface. Charged particle sputtering has been suggested[10] as a surface dehydration mechanism. The presence of a major absorption near 4.1 μm has been reported and its interpretation is considered here.

Voyager observed intensive current volcanic activity at many sites on Io[11,12], and just before Voyager's encounter, Peale *et al.*[13] had predicted extensive tidal heating of Io by Jupiter. Fanale *et al.*[3,4] had considered only radioactive decay as a current heat source on Io which led to the prediction that Io had undergone extensive devolitisation but would currently possess a 400 km solid shell overlying molten silicate[4]. Although they speculated that ordinary magmatism might occur at the surface, the migration of aqueous solutions seemed more compatible with their thermal model—hence the postulated carrier role for H_2O. Now, however, with theory[13] and observation[11,12] suggesting a very shallow solidus and rapid surface renewal[14] it seems unnecessary to postulate any current efficient dehydration mechanism. Io-forming material may have originally contained substantial amounts of bound H_2O[3,4] and a greater role for H_2O earlier in Io's history is still an exciting speculation, though beyond the scope of this note.

With the dehydration problem mitigated, it remains to explain the deep 4.1-m band without violating other constraints imposed by Io's spectrum or other observations.

Figure 1 shows Io's 0.3–5.2 μm reflectance spectrum and those of some possible Io surface constituents. Our laboratory spectra from 2.4 to 5.3 μm were obtained using a spectrometer constructed from two main components: (1) a chopped quartz halogen light source run in a low voltage d.c. mode, and (2) an indium antimonide photometer having a two-segment circular variable filter—the same instrument used to record the satellite spectrum[7,8]. The spectra were taken using sublimed sulphur as a reflectance standard. The circular variable filter consisted of two segments, the first of which had a wavelength range of 2.37–4.52 μm with $\Delta\lambda/\lambda \sim 0.01$. The second had a wavelength range of 3.9–7.7 μm with $\Delta\lambda/\lambda \simeq 0.025$. The laboratory spectra were recorded on a chart recorder, then digitised and processed by a computer. Processing amounted to matching the two halves of the spectrum and then ratioing each spectrum to the composite sulphur reflectance standard. Wavelength calibration was obtained from distinct absorptions in the known bands in the KNO_3 reflectance spectrum. In cases where samples were dehydrated, evacuated containers with sapphire windows were used, otherwise the samples were run in Petri dishes open to the air. The laboratory spectra in the 0.33–2.5 μm region were taken with a spectrogoniometer and environment chamber using a Halon standard and similar computer processing (Halon is a white reflectance standard for the 0.3–2.5 μm spectral region that does not absorb water). Again, the same instrument used in obtaining the Io spectrum[2,15] was used in the laboratory.

Spectroscopic arguments against major coverage of Io's surface by many candidate surface materials including rock-forming silicates, many frosts, certain salts, and other phases have been reported previously[3–5]. When the deep 4.1-μm band was first observed it was speculated that it might be an overtone band of an anion of a salt such as a carbonate or a nitrate[7,9] since a major absorption at 4.0–4.1 μm is present in the spectra of both groups of minerals. However, carbonates and nitrates also exhibit other bands—some almost as deep as the band around 4 μm—in the region 3.5–3.6 μm (ref. 16) which are not present in Io's spectrum (see Fig. 1). Also, Kieffer *et al.*[17] have recently shown that altering the grain size and temperature of the carbonates and nitrates does not significantly change the ratio of the band depth at 3.6 μm to that at 4.1 μm. With these possibilities largely voided, we reconsidered the possibility that elemental sulphur alone might explain Io's deep 4.1-μm absorption. Ordinary 'flowers of sulphur' is S_8 and there are no major absorptions for S_8 in the 2.0–5.0 μm region[7]. In fact, we use S_8 as a near IR reflectance standard. However, Nelson and Hapke[18] have shown that S_2, S_3 and S_4 have very different visible and UV spectra, and that a mixture of these polymers can explain some of Io's reflectance characteristics in the visible and UV better than pure S_8, a mix including polymeric chains such as S_4 and S_2. Furthermore, they stated that heating S_8 to >160 °C and quenching produces a mix containing S_2, S_3, S_4 and possibly S_6. Thus to check for possible 4 μm absorption, we prepared an altered sulphur sample by heating sulphur in air until it reached a temperature of ~200 °C. It was then quenched in liquid nitrogen, ground in liquid nitrogen and kept under liquid nitrogen until the spectrum was run. At run time the sample was transferred to a sapphire window container which was subsequently evacuated. Several spectra were taken at ~5 min intervals over a period of 75 min as the sample warmed to room temperature. The processed spectra show a large water band at

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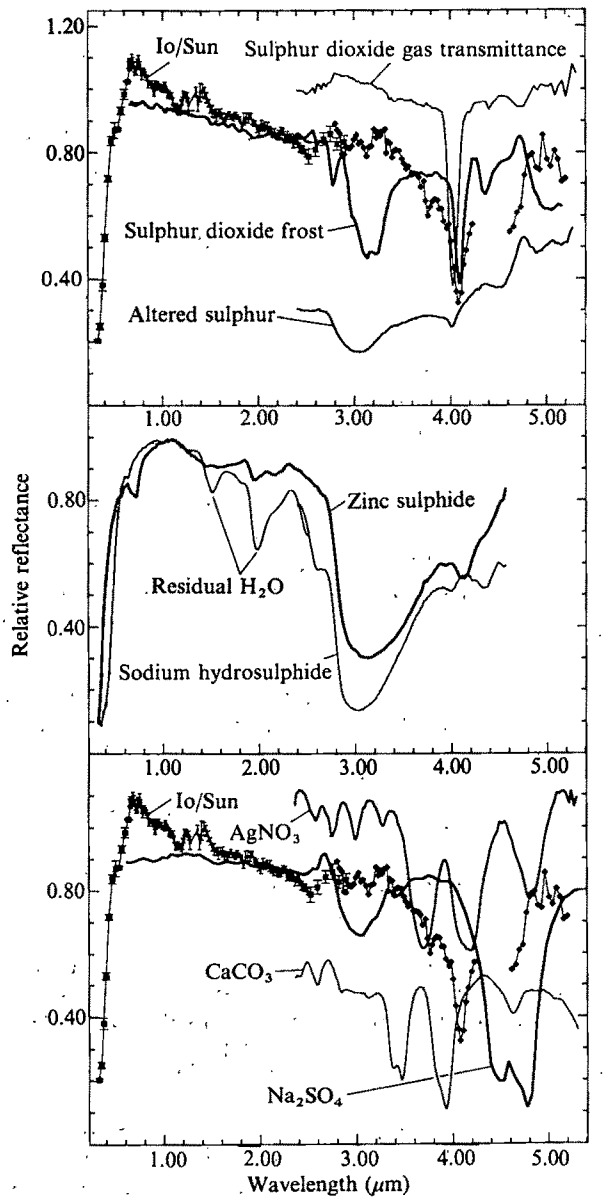


Fig. 1 The reflectance spectrum of Io (leading side, except the Cruikshank 1979 data[8] from 2.9 to 5.2 μm which are mixed orbital phases) is compared with various laboratory spectra. The Io composite spectrum is from Clark and McCord[2] and contains data from McFadden *et al.*[15] (0.325–0.65 μm), Clark and McCord[2] (0.65–2.5 μm), Pollack *et al.*[9] (2.5–3.0 μm), and Cruikshank[8]. The Na_2SO_4 spectrum (H. H. Kieffer, personal communication) is plotted as reflectance. All other laboratory spectra are from this study. The SO_2 frost, ZnS, NaSH and Na_2SO_4 are plotted as reflectance, whereas the other laboratory data are scaled for better clarity. The Io data are scaled to 1.0 at 1.02 μm (the geometric albedo of Io at 1.02 μm is approximately 0.90). The absorption bands at ~3 μm in the laboratory data are due to a small amount of water in the samples.

3.0 μm due to contamination during handling of the cold sample, and three bands at 4.03, 4.4 and possibly at 5.0 μm, with the 4.03-μm band being the sharpest and most prominent. The shape of the spectrum, as well as the apparent band depths, changed radically while the sample was warming. The final (room-temperature) spectrum is shown in Fig. 1. This suggested contamination by a spectrally active species such as H_2S or SO_2 as an alternative to the intended generation of polymeric sulphur. Further, it was noted that the spectrum of reagent grade ZnS (Fig. 1) exhibited a band in essentially the same position and it would seem difficult to attribute any of the ZnS bands to causes other than contamination, because the Zn–S fundamental occurs[19] between 306 and 280 cm^{-1}. Also a natural sphalerite sample that was run failed to show the same band. It was also noted that SO_2 gas has a deep absorption near 4 μm (ref. 20). Finally SO_2 gas has been observed by the Voyager IRIS experiment[21,22].

All these considerations at first led us to suspect that gaseous atmospheric constituents might be contributors to Io's 3.0–5.2 μm spectrum. However, data of Pierson *et al.*[20] show that the production of a 30% absorption near 4 μm requires 1.6 cm amagat of SO_2, which is approximately 10^4 times the limit based on Pioneer 10 occultation results[23,24] and what is known of the ionising plasma flux near Io[25]. Hydrogen sulphide has bands at 2.6 and 3.8 μm, near possible weak features in Io's spectrum, but, again, even a 5% atmospheric absorption at either of these wavelengths requires >2 cm amagat[20]. Note that, based on Io's 0.8–2.7 μm spectrum, Fink *et al.*[25] set limits for NH_3, CH_4, N_2O and H_2S in Io's atmosphere of 0.12, 0.12, 0.4 and 24 cm amagat, respectively. Furthermore, the gaseous SO_2 absorption band occurs at 4.03 μm, and after careful reexamination of our calibration procedures, we concluded that the difference between 4.03 and 4.08 μm, where the salient major band centre is observed on Io, is significant.

The spectrum of H_2S frost has already been reported by Kieffer *et al.*[17], and it exhibits a deep absorption somewhat shortward of 4.0 μm and several other major absorptions. We therefore obtained an IR reflectance spectrum of SO_2 frost. The 2.4–5 μm portion of the SO_2 frost spectrum was obtained from a sample grown on a flat metal surface attached to a LN_2-filled cold finger in an evacuated chamber with a sapphire window. The gas was slowly admitted from a lecture bottle via a line that was cold-trapped to remove any residual water vapour. During observations of the spectrum, the chamber was kept in a vacuum with the mechanical pump isolated by a cold trap. The frost was maintained at <100 K. The sulphur reflectance standard was obtained in the same conditions. The 0.65–2.5 μm portion of the spectrum was obtained by manufacturing SO_2 gas by reacting concentrated sulphuric acid and sodium sulphite and growing the frost on a cold (<160 K) metal surface. The SO_2 gas was dried by bubbling the gas through concentrated sulphuric acid, calcium carbonate, and a cold trap. In both methods of frost growth, the resultant samples were very fine grained (≤30 μm).

The SO_2 frost spectrum (Fig. 1) was found to exhibit a major absorption at 4.08 μm, corresponding to the SO_2 gas band at 4.03 μm with the expected shift to longer wavelength in the solid state. Other bands observed are minor, attributed to minor H_2O contamination, or compatible with the 0.7–5.0 μm spectrum of Io. Although Io exhibits a comparably pronounced absorption to that provided by our frost sample, the fact that we observed a deep broad absorption in the ground, altered sulphur suggests that SO_2 might strongly affect the major absorption as occluded or dissolved SO_2 as well as free frost. It is crucial, nonetheless, to assess the compatibility of free frost *per se* with Io's atmospheric properties. It is difficult to assess whether SO_2 could survive in the rough competition between surface renewal and destruction which must occur on Io[14], but easy to show that SO_2 frost might be physically stable in equilibrium. At 100 K, a reasonable cold-trapping temperature for Io, the equilibrium vapour pressure of SO_2 is 3×10^{-9} torr. This is equivalent to a surface number density of 4×10^8 cm^{-3}, in accord with current atmospheric models[24]. Thus, given higher SO_2 concentrations at certain sites, it would seem reasonable to expect frost formation. Conversely, if SO_2 frost were widespread, it might, together with the occultation limit, imply that SO_2 is the main atmospheric constituent. If so, this would in turn imply higher atmospheric temperatures than transient H_2S or other constituents[25]. The H_2S vapour pressure is much higher than that of SO_2 and violates the occultation limit by three orders of magnitude at 95 K. Remembering also that H_2S gas must be present at the level of >2 cm amagat to influence strongly the spectrum, we conclude that only H_2S trapped in surface material could strongly affect the 3.0–5.0 μm spectrum. Such occluded H_2S could, however, help to explain the ancillary features at 3.8 and 2.6 μm since its gaseous spectrum shows absorptions there, and not elsewhere in the region of interest, as reported by Pierson *et al.*[20].

With most of the identifiable features accounted for, it is not necessary for any compounds on Io's surface which may contain metallic cations to be spectrally active. In contrast, any abundant

phases must be spectrally bland in the region 1.0–5.0 μm (except possibly in the noisy ambiguous region 4.2–5.2 μm). With extensive volcanic activity, release of volatile metals may be expected. Regardless of whether Na and other constituents are sputtered from Io's surface or added directly to space from volcanoes, some metallic cations are liable to find their way to the surface. At low oxygen partial pressures, terrestrial volcanic sublimates are often dominated by alkali metals in the form of halides[26–28]. It is common knowledge that halides are spectrally bland in the near IR and would satisfy our criteria, now that the major features are explained, but Io may have 'run out' of halogens by this point in its active devolatisation history. Other common sublimate phases include sulphates, often but not always, as the result of oxidation by air[27–29]. On Io sulphides may be more likely. As Fig. 1 shows, sulphates have a huge band at 4.2–4.5 μm and it is questionable how much 'room' there is for such a band in that region of Io's spectrum. Sulphides and hydrosulphides are spectrally bland in the 3.0–5.0 μm region and very compatible with Io's spectrum otherwise (Fig. 1). Therefore they may be more likely candidates to harbour the cations.

The great depth of the 4.1-μm band suggests that SO_2 in some form in solid surface material must be almost ubiquitous. The concentration of the brightest material near the equator puzzles us, but may be related to a greater ion bombardment flux near the poles or proximity to gas sources. If SO_2 could condense anywhere on Io's surface as a free frost, then it may prove worthwhile to reconsider Binder and Cruikshank's[30] and Sinton's[31] suggestions that post-eclipse brightening could be caused by a frost. A straightforward experimental test would be to look for a sharp correlation of both rotational and post-eclipse darkening at 4.08 μm with visual brightening. Other peculiar properties of Io such as the discrepancy between its 10-μm and 20-μm temperatures may also be related to frost phenomena. Thus, many of Io's peculiar properties may be a result not only of the occurrence of widespread current volcanism, but also of the specific physical properties of the volatiles that are issuing from the volcanoes. Finally, as the resemblances between the visible and near UV spectra of the 'non-sulphur' spectrum of Io and Saturn's rings and Europa has been considered, the possibility that the presence of non-H_2O frosts or dissolved gases may affect the properties of the latter two or Ganymede or Callisto should be investigated.

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Spectral evidence for sublimates and adsorbates on Io

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The reflectance spectrum of Io can be explained by a surface layer consisting of fine-grained particles of sublimated alkali sulphides and sulphur on which H_2S and SO_2 are adsorbed.

THE discovery by Voyager 1 of Io volcanoes, and thus active degassing of Io's interior, suggests that deposition from a vapour phase may be the dominant process producing the composition and texture of Io's optical surface. We report here our first results from laboratory studies regarding sublimates and adsorbates, materials not seriously considered in previous attempts to explain Io's spectral reflectance[1–4]. We believe that sublimation and adsorption considerations open a new and fruitful area of study for the interpretation of Io's optical properties. We conclude that Io's spectrum from 0.25 to 5.0 μm can be explained by an optical surface layer consisting of fine-grained particles of sublimated alkali sulphides and free sulphur on which is adsorbed a substantial component of H_2S and/or SO_2.

Io's spectral geometric albedo (Fig. 1) is unusual in that it is strongly absorbing in the UV and very reflecting in the IR[5]. The important features in Io's spectral geometric albedo are (from UV to IR) a downturn shortward of 0.34 μm which when combined with spacecraft UV data suggests a band at 0.29 μm, a sharp upturn longward of 0.38 μm on the leading side and 0.40 μm on the trailing side, an absorption feature at 0.56 μm, a possible broad band at 1.35 μm, weak bands at 2.55, 3.45, 3.8, 3.9 μm and a strong band at 4.1 μm[4,6–13].

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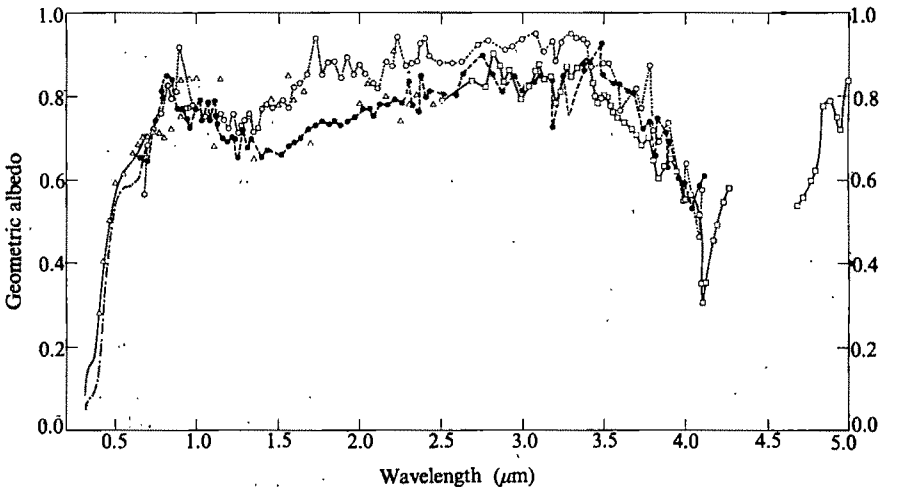


Fig. 1 Spectral geometric albedo of Jupiter's satellite. Io, 0.26–5.0 μm based on previous studies $\theta = 135°$ (solid line) ref. 4; $\theta = 307°$ (chain line) ref. 4; △, refs 6, 7, data below 0.35 μm not included due to filter problems; ○, ref. 8, leading side; ●, ref. 8, trailing side; □, ref. 9 mixed. The leading side albedos ($0° < \theta < 180°$) have been normalised to 0.622 at $\lambda = 0.56$ μm, the trailing side albedos ($180° < \theta < 360°$) have been normalised to 0.58 at 0.56 μm following the discussion in refs 10–12.

To interpret these spectral features we measured the hemispherical and bidirectional spectral reflectance of typical sublimate phases in varied temperature and particle (proton beam) irradiation conditions. We used phases containing elements that are found in Io's extended exosphere and thus which may originate from Io's surface[14]. The materials studied were Na_2S, NaHS, K_2S, and mixtures thereof with free sulphur. We used reagent grade materials and, because of their extreme hygroscopic nature, prepared them in dry-nitrogen filled glove bags fitted to all the instruments used. The only exception to this procedure was in the 2.5–5.0 μm spectrophotometer runs where the samples were briefly (~10 s) exposed to low-humidity room air during transfer. This exposure was not considered significant because of subsequent sample drying by purge air in the instrument.

The hemispherical spectral reflectance relative to MgO, in the range 0.24–2.5 μm, was measured for the powdered samples (≤100 μm particle size) with a Beckman DK-2A spectrophotometer. The bidirectional reflectance 2.5–5.0 μm for all three samples was measured relative to an aluminium mirror using a Perkin-Elmer model 180 spectrophotometer modified to take horizontal upright samples and purged with air filtered of substantially all H_2O and CO_2.

Because of the very strong water bands in this spectral region, we repeated the 2.5–5.0 μm spectra after the powders were heated in a vacuum oven at 170 °C for 24 h.

The results of the 2.5–5.0 μm spectrophotometry are shown in Fig. 2 for the freshly powdered samples and Fig. 3 for the vacuum oven dried powdered samples. The upper portions of Figs 2 and 3 show the ordinate displaced spectral geometric albedos of Io. Note the apparent disagreement in wavelength of the band centres of the reported Io spectra[8,9].

We find that Na_2S K_2S, and NaHS all have features in the 3.9–4.1 μm region at the location of the strong Io bands. The band centres for the laboratory samples are at 3.96 and 4.05 μm for Na_2S, 3.93 and 4.11 μm for K_2S, and 3.93 and 4.05 μm for NaHS. Furthermore, both K_2S and Na_2S have a feature at ~2.55 μm, and NaHS has features at 3.4 and 3.8 μm. Io has band minima at all of these wavelengths.

The drying procedure did little to diminish the depth of the adsorbed water band at 3.0 μm; however, it did alter dramatically the depth of the non-water related bands at ~4.0-μm. We suspect that this may represent surface phase alterations in addition to desorption of water.

A possible explanation of the observed ~4.0-μm band structure in these sulphides is the presence of H_2S or SO_2 as an adsorbate phase. As clearly shown in Fig. 3, all three sulphides yield IR bands near 4.0 μm. The S–H stretching bond frequencies are well known to produce bands near this wavelength[15]. The $\nu_1 + \nu_3$ band of SO_2 gas is also at 4.0 μm (ref. 15). The precise positions of these bands depends on the details of the molecular structure[16]. We found that an oderific gas was evolved when these materials were dried, either by vacuum oven heat or dry purge air in the spectrophotometer. As the H_2O adsorbate was driven off, the 4.0-μm bands became stronger. We interpret this effect as being due to formation of H_2S or SO_2 by chemical reaction with the matrix alkali sulphide and the retention of the gas on the surface of the sulphide particles as a chemisorbed phase.

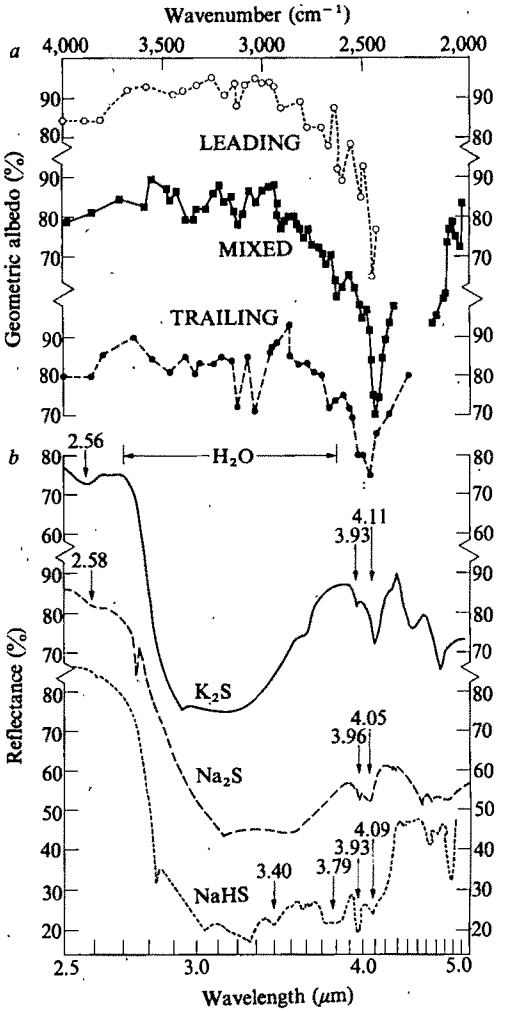


Fig. 2 *a*, The ordinate-displaced spectral geometric albedos of Io from ref. 8 (○, ●) and ref. 9 (■). Note the apparent disagreement in the positions of the band centres between the two studies. *b*, The bidirectional reflectances of the selected sublimates at a phase angle of ~20° at room temperature.

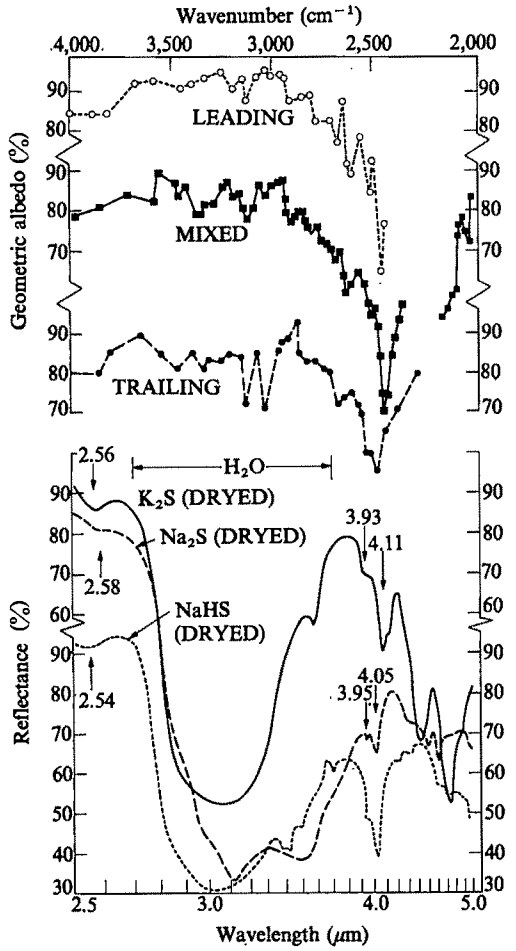


Fig. 3 *a*, The ordinate displaced spectral geometric albedos of Io from ref. 8 (○, ●) and ref. 9 (■). *b*, The bidirectional reflectances of the selected sublimates at room temperature after 24 h in a vacuum oven at 170 °C.

Because of the strong similarity of this band structure to Io's 4.0-μm band, we suggest that adsorbed gas may be its cause on Io. H_2S and SO_2 are strongly polarised molecules as is H_2O which produces strong IR bands in terrestrial powders even though present only as an adsorbed component (see ref. 17). H_2S is unstable as a frost on Io[18] but can reasonably be expected as an adsorbate phase on the fine-grained, and thus high-specific-surface, sublimate particles on Io's surface. We are conducting more detailed experiments to test this hypothesis.

To examine possible effects of jovian charged particle flux at Io on alkali sulphides, the powdered samples of Na_2S and NaHS were introduced into a vacuum system at room temperature (see ref. 19) where they were subjected to a dose (2×10^{15} H^+ cm^{-2}) of 5 keV protons. Bidirectional reflectance relative to MgO was measured (0.28–0.70 μm) before and during irradiation. This irradiation treatment was repeated for Na_2S at 170 K.

Before irradiation the NaHS had a shoulder at 0.4–0.6 μm and a downturn at 0.35 μm. The Na_2S had bands at 0.30 μm and an increase in reflectance between 0.36 to 0.55 μm. The spectra of Na_2S and NaHS before and during irradiation are shown in Fig. 4. Both samples were darkened by the proton irradiation, causing the NaHS to more closely match Io's spectrum and albedo in this wavelength range. The irradiation also caused a band in Na_2S to form at 0.56 μm, where Io has a distinct feature. The proton irradiation did not cause any spectral changes in the IR spectra of Na_2S or NaHS.

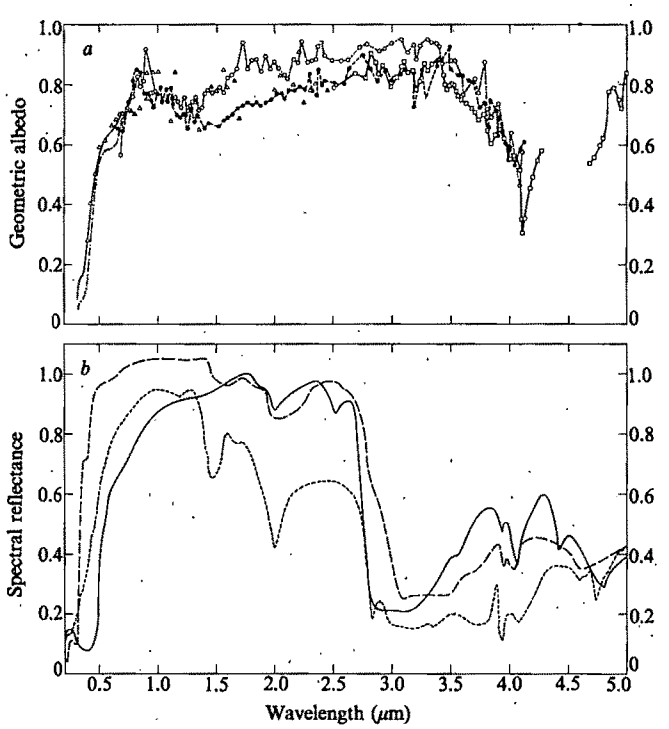


Fig. 5 *a*, Spectral geometric albedo of Io 0.28–5.0 μm. $\theta = 135°$ (solid line) ref. 4; $\theta = 307°$ (chain line) ref. 4; △, refs 6, 7 data below 0.35 μm not included due to filter problems; ○, ref. 8, leading side; ●, ref. 8, trailing side; □, ref. 9, mixed. *b*, Spectral reflectance of the selected sublimates 0.24–5.0 μm. The deep bands at 1.5, 2.0 and 3.3 μm are due to water in the laboratory samples. Na_2S (dashed line); K_2S (solid line); NaHS (dotted line).

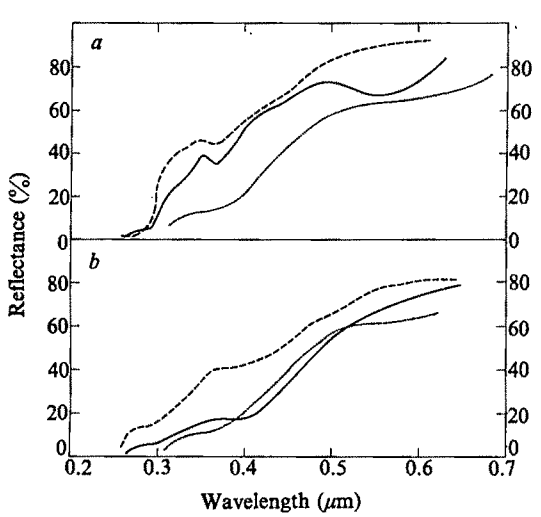


Fig. 4 *a*, Spectrum of Na_2S before (dashed line) and during (solid line) low-energy proton irradiation at $T = 170$ K. *b*, Spectrum of NaHS before (dashed curve) and during (solid line) low energy proton irradiation $T = 300$ K. The Io spectrum[4] is shown for comparison (dotted line) in both *a* and *b*.

Figure 5 shows the spectra of the three sublimates for the complete wavelength range 0.25–5.0 μm. The Io spectral geometric albedo from Fig. 1 is shown for comparison. In addition to matching the principal absorption features in Io's spectrum quite well, all three sublimates (when water free) have the general continuum shape of Io's spectrum.

Conclusions

We conclude that sublimates and adsorbates are important, if not the dominant, phases causing Io's observed spectral reflectance in the range 0.25–5.0 μm. In particular, the compounds of Na_2S, K_2S and NaHS have absorption bands at all the key wavelengths in the UV and visible range. Mixtures of sulphides and free sulphur are being studied in the laboratory in efforts to produce a refined compositional analysis, especially to fit the overall continuum of Io's spectrum. Furthermore, we believe that adsorbed gases if not frosts are important phases affecting the IR spectrum of Io's surface. The adsorptivity of

various sulphur and other compounds and the physical and optical effects of adsorbed gases such as H_2S and SO_2 thereon need to be studied as possible strong contributors to Io's spectral reflectance and other surface properties.

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Spectral evidence for SO_2 frost or adsorbate on Io's surface

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The infrared reflection spectrum of Io matches that from an SO_2 frost grown in the laboratory.

IT has been recently suggested that adsorbed SO_2 and/or H_2S on the surface of a spectroscopically benign substrate can explain the IR spectrum of Jupiter's satellite, Io[1]. SO_2 gas has been identified as a major constituent of the volcanic gas plumes recently discovered by the Voyager 1 spacecraft[2]. We have measured the reflection spectrum of SO_2 frost grown under low pressure at 140 K in the laboratory and find that it provides an excellent match to Io's spectrum in the 1.0–4.5 μm range.

The apparatus used and experimental methods have been described by Kieffer[3] and Smythe[4] respectively. Figure 1 compares the spectral geometric albedo of Io[5,6] and our laboratory spectrum. Clearly SO_2 frost can explain the Io absorption bands at 1.35, 2.55, 2.80, 3.80, 3.90, 3.95, and 4.08 μm. Other candidate materials such as sulphates, nitrates and carbonates[5] do not provide such a unique match to these bands.

Although SO_2 frost may be unstable on Io, except at the poles or nightside (see ref. 7), adsorbed SO_2 should be present on the surface particulates due to the extensive volcanic outgassing. The absorption bands of adsorbed and condensed species are known to be similar in wavelength[8]. Slight shifts in band positions and depths may reflect areal or temporal variation in frost/adsorbate or condensate/substrate contribution ratios to a multicomponent spectrum. Mixtures of alkali sulphides, free sulphur and adsorbates of SO_2 or H_2S have been suggested to explain Io's overall UV-VIS-IR reflectance[1]. We conclude here that SO_2 as a frost or an adsorbate is the primary contributor to Io's IR spectral reflectance.

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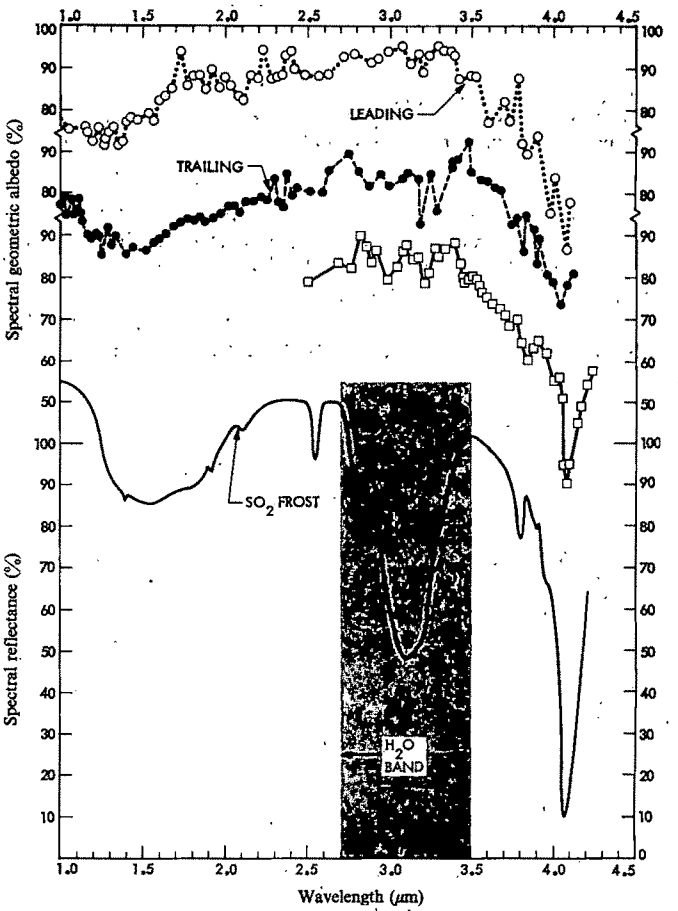


Fig. 1 *a*, Spectral geometric albedo of Jupiter's satellite Io, 1.0–4.5 μm based on previous studies[5,6], normalised as in ref. 1. ○, ●, Data from ref. 5; □, data from ref. 6. *b*, Spectral reflectance of SO_2 frost grown in the laboratory. The 3.1 μm H_2O band (stippled area) is due to contaminant water.

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Auroral hiss observed near the Io plasma torus

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A band of whistler-mode noise identified as auroral hiss has been observed on the inner edge of the Io plasma torus. This noise provides evidence for the existence of aurora-like charged particle beams on magnetic field lines through the inner edge of the torus. These beams probably consist of low-energy electrons and may be associated with field-aligned currents linking the plasma torus to the jovian ionosphere.

DURING the Voyager 1 flyby of Jupiter on 5 March 1979 an unusual band of electric field noise was detected by the plasma wave instrument near the Io plasma torus. This band of noise has spectral characteristics very similar to a type of whistler-mode emission called 'auroral hiss'[4] which is commonly observed in the Earth's auroral regions[1-5]. Auroral hiss is believed to be produced by low-energy (10 eV to 1 keV) beams of auroral electrons by a Cerenkov radiation mechanism[6,7]. Because the presence of auroral hiss near the Io torus may indicate the occurrence of charged particle acceleration processes similar to the Earth's auroral zones, we have undertaken a detailed analysis of these noise bands. This article describes the characteristics of the auroral hiss emissions detected at Jupiter, compares these characteristics with similar emissions detected in the Earth's magnetosphere, and comments on the implications regarding the interaction of the plasma torus with the jovian magnetosphere. The plasma wave instrumentation on Voyager 1 and an initial survey of the Voyager 1 plasma wave observations at Jupiter are given elsewhere[8,9].

Observations

The noise bands of interest were observed near the inner edge of the Io plasma torus on both the inbound and outbound portions of the Voyager 1 pass by Jupiter. For a discussion of the plasma torus formed by material escaping from Jupiter's satellite Io see refs 10 and 11. The plasma wave electric field intensities detected near the closest approach to Jupiter are shown in Fig. 1*b*. A sketch of the spacecraft trajectory in relation to the plasma torus is shown in Fig. 1*a* together with the electron plasma frequency, f_p^-, obtained from the planetary radio astronomy experiment[11] and the electron gyrofrequency, f_g^-, obtained from the magnetometer experiment[12].

The emissions identified as auroral hiss are indicated by the cross-hatched areas in Fig. 1*b*. The distinguishing characteristic of these emissions is the systematic change in the emission frequency with spatial position, increasing with increasing time for the event at 09.30 UT, and decreasing with increasing time for the event at 14.00 UT. Because of the limited frequency–time resolution of the intensity measurements in Fig. 1 it is not possible to identify these emissions as auroral hiss on the basis of these data alone. Fortunately, for the event at 09.30 UT, a series of wideband waveform measurements was available, providing much better frequency–time resolution. The wideband waveform mode of operation uses the 115 kbit s^{-1} telemetry system normally used for pictures to transmit a 12 kHz bandwidth of electric field waveforms to the ground. Because the 115 kbit s^{-1} telemetry link must be time shared with the imaging system, the waveform measurements are not obtained continuously. During the period around 09.30 UT, one 48-s burst of waveform data was being obtained every 192 s. No wideband measurements were obtained for the period around 14.00 UT. Three consecutive 48-s spectrograms of the wideband waveform measurements are shown in Fig. 2 for the event at 09.30 UT. These spectrograms show a broadband emission with a very sharp low frequency cutoff which increases linearly with increasing time. The broadband character of the emission and the smoothly varying low frequency cutoff provide an almost unmistakable identification of this emission as a V-shaped auroral hiss event of the type described by Gurnett[2], McEwen and Barrington[3] and others. As will be discussed, the low frequency cutoff is a whistler-mode propagation effect. At the Earth the spatial variation of the cutoff frequency produces a characteristic V shape on a frequency–time spectrogram with the centre of the V located in a region of intense low-energy electron precipitation[4].

The auroral hiss emissions detected by Voyager are unusual in that only one branch of the V-shaped low frequency cutoff is evident. Such unsymmetrical V-shaped auroral hiss events are also occasionally seen in the Earth's auroral zone, usually in association with a large density gradient. A similar relationship is evident for the events detected by Voyager. As Fig. 1 shows both of the auroral hiss events occur near a very large density gradient at the inner edge of the plasma torus. Whether the missing branch of the V is a propagation effect related to the density gradient or is simply obscured by the more intense chorus and hiss emissions[9] inside the plasma torus is not known.

Analysis

For auroral hiss at the Earth it is generally believed[7,13] that the V-shaped low-frequency cutoff is a spatial propagation effect which occurs for short wavelength waves near the resonance cone. As will be shown the beamwidth of whistler-mode radiation near the resonance cone is frequency dependent, with the highest frequencies having the largest beamwidth. As the spacecraft approaches the source the highest frequencies are detected first, because these ray paths can propagate at the largest angle to the magnetic field. Progressively lower frequencies are detected as the spacecraft comes closer to the source.

The spatial dependence of the cutoff frequency is best analysed using the index of refraction construction shown in Fig. 3*b*. The ray path at a given wave normal direction is perpendicular to the index of refraction surface at that wave normal angle[14]. Figure 3*b* shows that the angle between the limiting ray path direction and the magnetic field, ψ_{max}, is given by $\psi_{max} = \pi/2 - \theta_{Res}$, where θ_{Res} is the resonance cone angle. Using Stix's notation[14], the angle ψ_{max} is given by:

$$\cot^2 \psi_{max} = \tan^2 \theta_{Res} = -\left(\frac{P}{S}\right) \qquad (1)$$

All waves near the resonance cone will have ray path directions less than this limiting angle. For the conditions applicable to the Voyager 1 observations, $f_g^- \ll f_p^-$, and $f_g^+ \ll f \ll f_g^-$, the terms S and P can be approximated by

$$P = -\left(\frac{f_p}{f}\right)^2 \quad \text{and} \quad S = \left(\frac{f_p}{f_g^-}\right)^2\left[1 - \frac{f_{LHR}^2}{f^2}\right] \qquad (2)$$

where $f_{LHR} = (f_g^- f_g^+)^{1/2}$ is the lower-hybrid resonance frequency.

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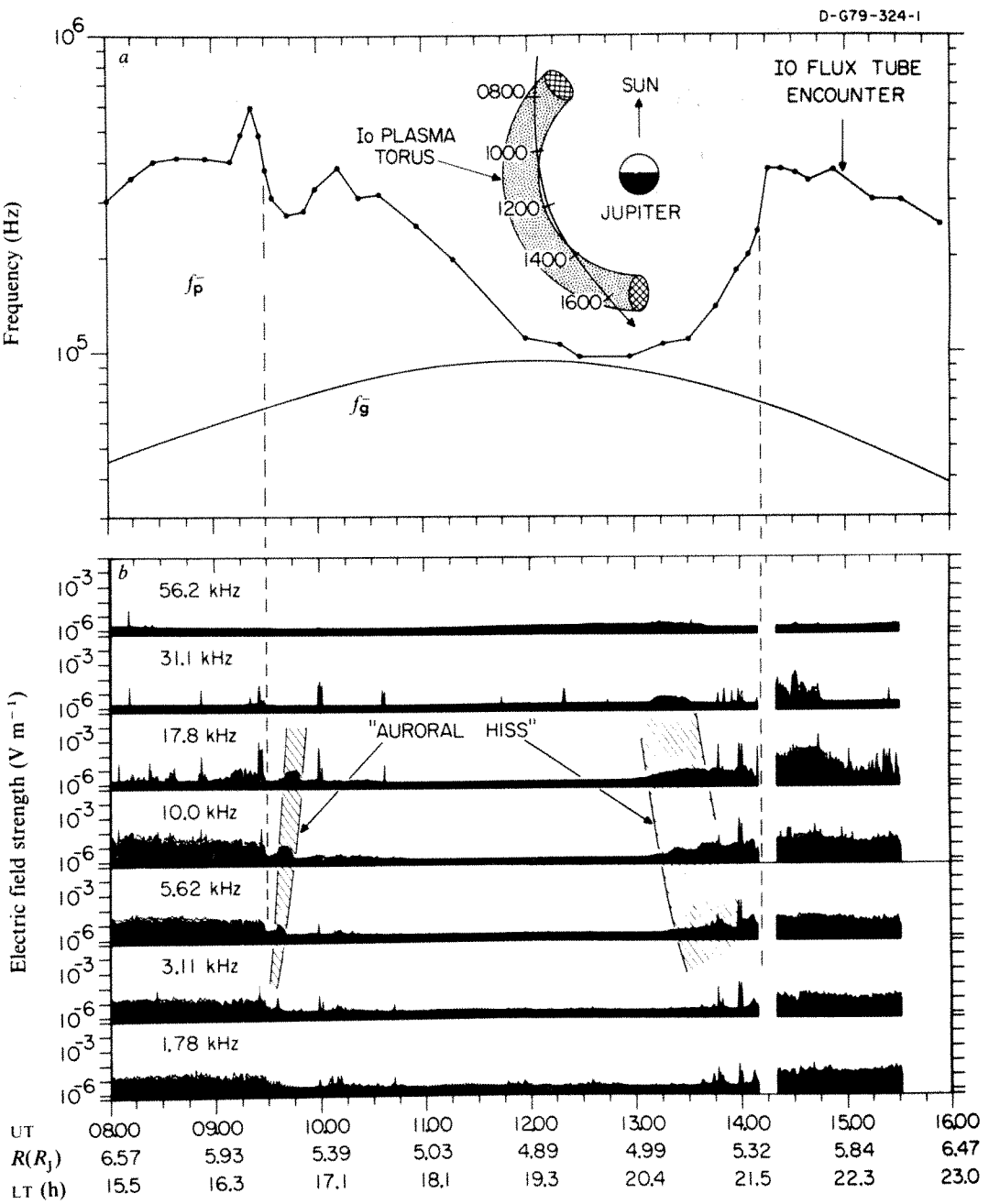


Fig. 1 The plasma wave electric field measurements for the Voyager 1 pass through the Io plasma torus. The emissions identified as whistler-mode auroral hiss are identified by the cross-hatched areas. In both cases the auroral hiss emissions were observed near an abrupt gradient in the plasma density (see the vertical dashed line) near the inner edge of the torus.

Combining these terms gives the following equation for the limiting ray path angle as a function of frequency,

$$\tan^2 \psi_{max} = \frac{f^2 - f_{LHR}^2}{(f_g^-)^2} \qquad (3)$$

Equation (3) shows that the limiting ray path angle increases monotonically with increasing frequency for $f^2 \gg f_{LHR}^2$, and goes to zero at $f = f_{LHR}$. If we now consider an idealised point source emitting all frequencies the resulting radiation will illuminate a conical region along the magnetic field with a beamwidth which increases with increasing frequency and goes to zero at f_{LHR}. This frequency-dependent beamwidth is illustrated in Fig. 3*a*. A spacecraft passing through the illumination region first detects the highest frequency, f_2, then progressively lower frequencies, f_1, until the lowest frequency f_{LHR} occurs when the spacecraft is on the magnetic field line through the source. The frequency-dependent beamwidth explains the V-shaped spatial variation of the low frequency cutoff.

For the Voyager auroral hiss observations, as for the terrestrial auroral hiss, it seems more appropriate to consider a line source extending azimuthally along the torus rather than a point source. This configuration is supported by the fact that somewhat similar symmetrical auroral hiss emissions were observed on both the inbound and outbound crossings at substantially different local times. Ignoring the curvature of the magnetic field lines, the ray path problem can be reduced to a simplified two-dimensional geometry by introducing a coordinate x, which is the perpendicular distance from the spacecraft to the magnetic field line through the source, and a distance h, which is the height of the spacecraft above the source (see Fig. 3). Simple trigonometry shows that for the limiting ray path $x/h = \tan \psi_{max}$. Substituting into equation (3) and simplifying gives the following equation for the cutoff frequency as a function of x,

$$f^2 = f_{LHR}^2 + \left(\frac{x}{h}\right)^2 (f_g^-)^2 \qquad (4)$$

This equation is a hyperbola. Even when the magnetic field curvature is considered, equation (4) is still valid to second order provided x is measured perpendicular from the magnetic field through the source.

To obtain the approximate location of the source, equation (4) has been adjusted to fit the measured cutoff frequencies. The cutoff frequencies, which can be measured with very good accuracy (~ 100 Hz), are shown by dots in Fig. 3*c* along with the best fit hyperbola representing the propagation cutoff. Only three free parameters occur in equation (4), the lower-hybrid

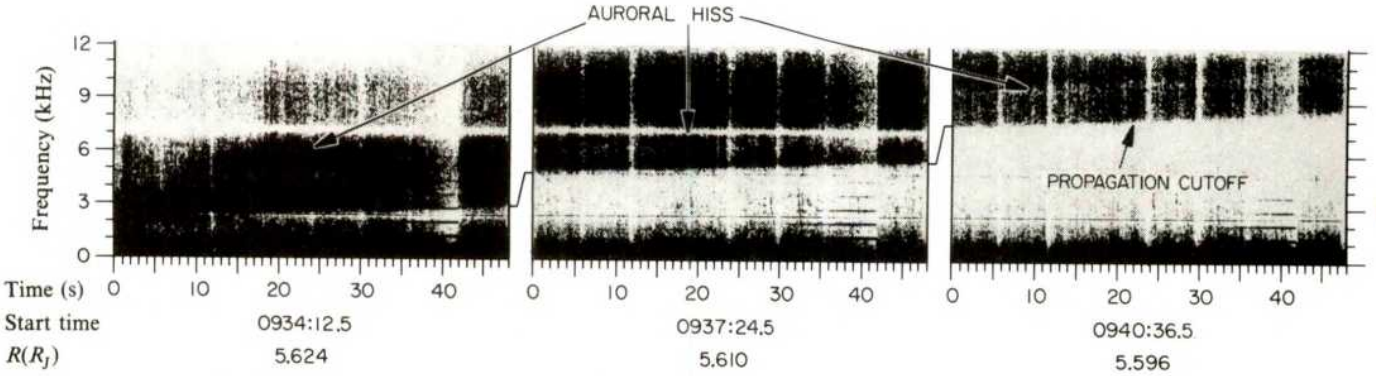


Fig. 2 A sequence of wideband spectrograms for the event near 09.30 UT in Fig. 1. The broadband character of the emission and the sharp low frequency propagation cutoff increasing linearly with time provide a clear identification of this emission as auroral hiss.

resonance frequency, the location of the magnetic field line through the source, and the height h of the spacecraft above the source. In principle, the lower-hybrid resonance frequency can be determined from the plasma parameters and need not be considered a free parameter. However, the lower-hybrid resonance frequency depends on the ion composition which varies considerably. As the ion composition is not well known along the ray path, f_{LHR} is considered an unknown parameter. The best fit parameters indicate that the magnetic field line through the source was crossed at 09.32 UT, that the distance h to the source was 0.65 R_J, and that the lower-hybrid resonance frequency was 1.56 kHz. At the time of the closest approach to the source, Voyager was 0.51 R_J north of the magnetic equator. Two possible source positions must be considered, either northward along the magnetic field line towards Jupiter, or southward along the magnetic field towards the magnetic equator. The northward position would place the source approximately $0.65\,R_J + 0.51\,R_J = 1.16.R_J$ north of the equator on the $L \simeq 5.6$ field line. The southward position would place the source approximately $0.65\,R_J - 0.51\,R_J = 0.14\,R_J$ south of the magnetic equator. These two source positions are illustrated in Fig. 4*a* and *b*. Since the plasma wave instrument does not have any capability for determining the direction of propagation, it is not possible to distinguish experimentally these two source locations.

Although the north–south ambiguity cannot be resolved, certain indirect arguments can be made favouring each of the two source positions shown in Fig. 4. The equatorward source is favoured by the fact that this location, only 0.14 R_J south of the magnetic equator, coincides with an obvious symmetry point where a source could reasonably be expected. The best fit lower-hybrid resonance frequency is not, however, in good agreement with the lower-hybrid resonance frequency expected in this region. Since the inner region of the Io torus is thought to

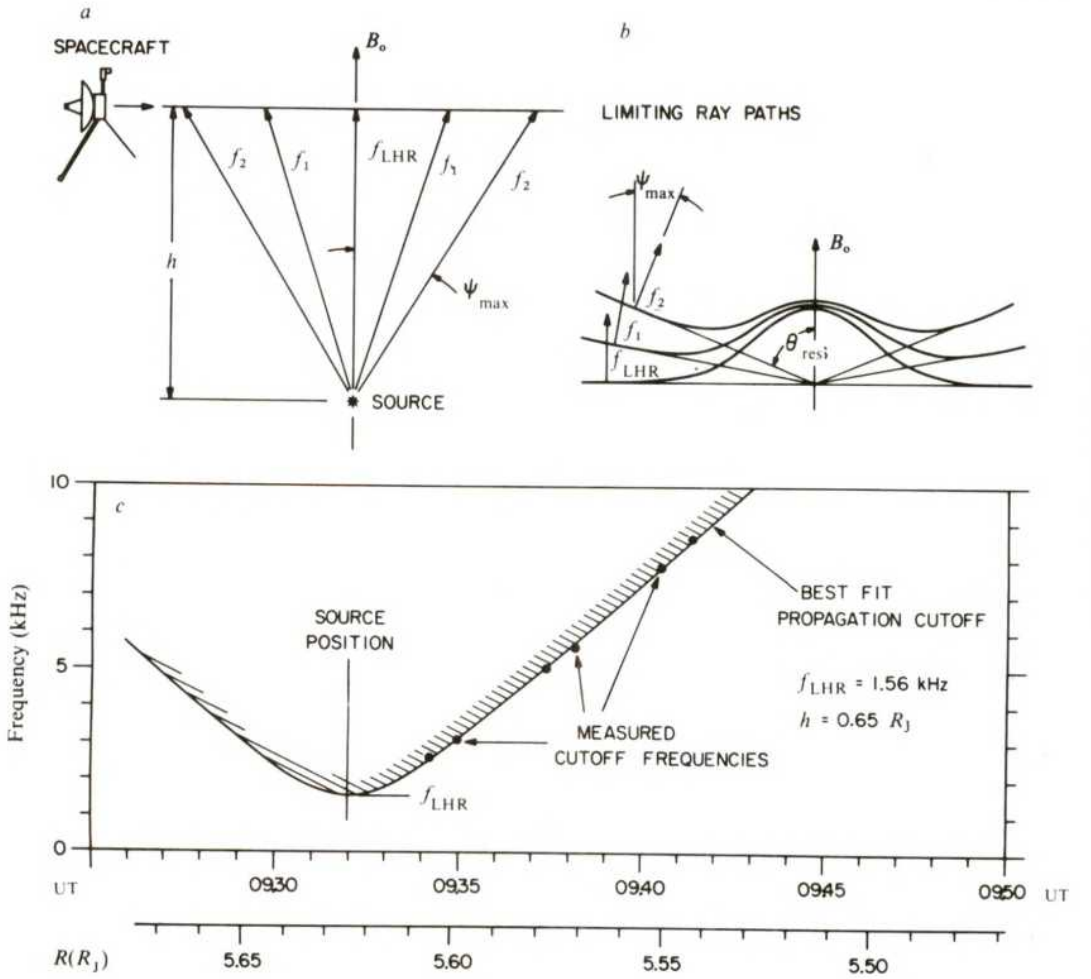


Fig. 3 *a* And *b* show that the beamwidth of whistler-mode emissions near the resonance cone increases with increasing frequency. A spacecraft approaching a point source, therefore, detects the highest frequencies first, forming a V-shaped low frequency cutoff. *c*, The best fit propagation cutoff for the auroral hiss emission near 09.30 UT. Only one branch of the V-shaped cutoff was observed.

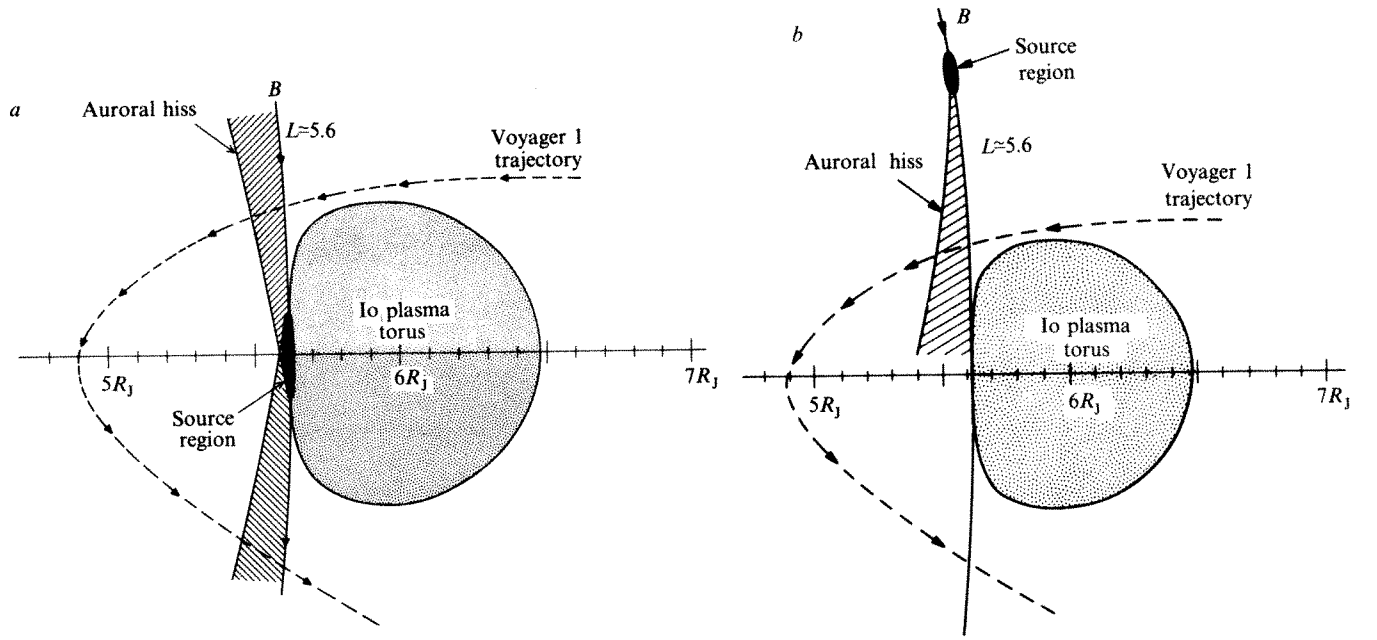


Fig. 4 The two possible source positions obtained from the best fit analysis in Fig. 3. *a*, The equatorward source position. *b*, The northward source position which has a very close analogy to auroral hiss emissions at the Earth, with the Io plasma torus playing a role comparable to the ionosphere at the Earth. The auroral hiss emissions strongly suggest the occurrence of low-energy electron beams and field line currents associated with the large density gradient near the inner edge of the plasma torus.

be dominated by heavy ions[10,15], the lower-hybrid resonance frequency is expected to be of the order of 300–500 Hz inside the torus. The best fit lower-hybrid resonance frequency, 1.56 kHz, seems to be more consistent with a plasma in which protons are the dominant ion species. As protons are expected to be a more important constituent outside the torus, this fact would tend to favour a source well away from the equator, such as in Fig. 4*b*. Note, however, that the lower-hybrid resonance frequency has the largest uncertainty of all the free parameters in equation (4). This difficulty occurs mainly because no wide-band data is available around the time, 09.32 UT, when the cutoff frequency is near the apex of the hyperbola. The uncertainty in f_{LHR}, together with the absence of suitable information on the ion composition away from the torus, makes it very difficult to distinguish between the models in Fig. 4 on the basis of the lower-hybrid cutoff frequency.

Discussion

These observations of auroral hiss provide strong evidence of auroral-like charged particle beams originating from within, or near, the Io torus. As the intensity and spectral characteristics of the auroral hiss detected at Jupiter are very similar to the auroral hiss observed at the Earth, it is expected that the radiation should be generated by comparable particle intensities, namely electrons with energies from about 10 eV to 1 keV and fluxes in the range 10^8 to 10^{10} electrons cm^{-2}. The existence of comparable low-energy electron precipitation from the torus is already implied by the UV spectrometer observations of aurora at the foot of the magnetic field lines through the torus. The fact that the auroral hiss is observed over a very small spatial region with very sharply defined cutoff frequencies implies that the electron beam generating the radiation must be very narrow, not extending over a range of L-values more than about 0.02. The source position of the auroral hiss shows that the electron beam occurs on an L-shell which coincides almost exactly with the abrupt decrease in plasma density at the inner edge of the plasma torus. Again in analogy with the terrestrial auroral zone, it seems likely that the auroral hiss and associated electron beams would be associated with a thin sheet of current which flows along the magnetic field lines linking the inner edge of the plasma torus with the polar ionosphere of Jupiter. At present we are unable to determine the direction of the current in this sheet, as this depends on the direction of propagation of the auroral hiss. For the model in Fig. 4*a*, the auroral hiss and the electrons producing this hiss would be moving away from the equatorial plane, which implies a current directed along the magnetic field lines from Jupiter towards the Io torus. For the model in Fig. 4*b*, the directions would be reserved.

At the present early stage of data analysis, no specific evidence is available, other than the auroral hiss observations, to confirm or disprove the existence of low energy electron beams and field-aligned currents at the inner edge of the Io plasma torus, although such investigations are under way (N. Ness and H. Bridge, personal communication). The likelihood of such field-aligned currents and particle precipitation effects is, however, considered quite high, as several processes can give rise to field-aligned currents at abrupt gradients in the plasma density. Given that a field-aligned current of sufficient magnitude exists, the acceleration of particles to large energies could proceed via the development of parallel electric fields, as in the Earth's auroral zones. Observations of auroral hiss at the Earth have in fact been suggested as being directly related to regions of parallel electric fields and auroral particle acceleration[16].

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Pre-Voyager velocities, accelerations and shrinkage rates of jovian cloud features

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The Voyager 1 data yield detailed information on the latitudinal dependence of velocities in the jovian atmosphere. This enhances the possibility of interpreting the time-dependent behaviour of long-lived jovian cloud features. This article summarises pre-Voyager velocity data, stressing gross differences with the Voyager 1 results. Analytic expressions are given for average drift rates and shrinkage rates for the Great Red Spot and white ovals (located at −23° and −34° latitude, respectively) from 1943 to 1979.

HISTORICAL records of longitudinal positions and size of jovian cloud features include visual transit measurements by Reese from 1943 to 1964[1,2] dovetailed with photographic observations obtained at New Mexico State University (NMSU) from 1963 to the present [3–9]. Because Reese assisted in the development of the measuring technique for the photographic data while continuing to make visual transit measurements, and then continued with the photographic programme through 1976, these data should be self-consistent. Although the visual transits have a large measuring error (standard error, 1–2 degrees), the average values of size and longitudinal position of the centre of each feature near the time of opposition can be used to derive long-term shrinkage rates and average drift rates.

Since 1974, a concentrated effort has been directed towards obtaining longitudinal and latitudinal position measurements of all jovian cloud features which can be consistently identified over periods of a few weeks or more. Figure 1 presents a composite of the velocities obtained between April 1974 and May 1979, where the horizontal bar represents the range of velocities that were observed at a specified latitude. Latitudes are reported as planetographic[2] and velocities are expressed relative to System III longitude[10].

This study provides a finer latitudinal discrimination than that presented by Smith and Hunt[11]. The velocity at a particular latitude cannot always be determined because relatively large features must be present for groundbased detection; however, the verification of the presence of all of the previously measured latitudinal velocities plus the determination of the velocity patterns around the Great Red Spot (GRS) and ovals by Voyager 1 data [12] should provide a large contribution to understanding long-term cloud motions. Voyager data [12,13] yield a velocity pattern that is more symmetric about the equator than the data shown in Fig. 1. Note that large features in the southern hemisphere tend to dominate the groundbased velocity profile, while the measured velocities in the northern hemisphere are obtained by tracking smaller features whose diameters are ~5,000 km and whose albedos contrast strongly with their surroundings. These small, strongly contrasting features are rare in the southern hemisphere at groundbased resolutions; hence, further interpretation of the historical data requires the more complete understanding of the atmospheric velocity structure now being obtained from the Voyager data.

The formation of the three white ovals occurred in 1938, following an obscuration of the south temperate belt[2] by a high-albedo cloud formation. Three dark regions appeared within the belt and gradually the intervening bright areas coalesced into ovals. During their formation they were hazy and diffuse; however, by 1953 they had contracted to 26,000 km in longitudinal extent, or length, and have continued to decrease to a present length of 12,000 km. In 1941, the ovals drifted at a rate of 7.5 m s^{-1} and have since slowed down to 4.0 m s^{-1}. Even

Table 1 Least squares drift rate where longitudinal position in System III equals $C_0 + C\Delta t + C_2\Delta t^2$

Solution	C_0 (deg)	C_1 (deg yr^{-1})	C_2 (deg yr^{-2})	Acceleration (m s^{-2})
GRS	86.16	102.4	−0.1124	1.310×10^{-10}
Forced fit of the three white ovals	−11.14 116.47 231.91	−148.5	−1.3448	-1.442×10^{-9}

Δt is the time (yr) elapsed since 1965.0.

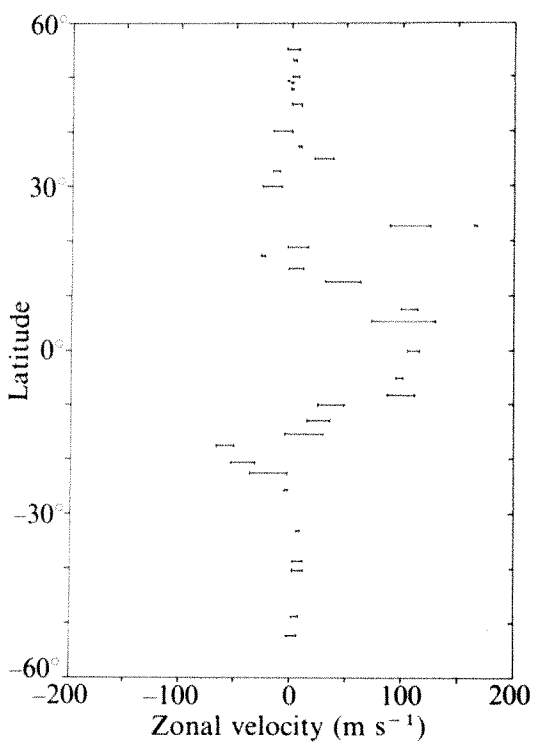


Fig. 1 Zonal velocities derived from groundbased photographic data. Positional measurements have been reduced to System III longitude[10] and drift rates are obtained for features that can be identified from day to day on the photographs. An automated measuring program allows accurate determination of latitude, as well as measurement of a large number of features.

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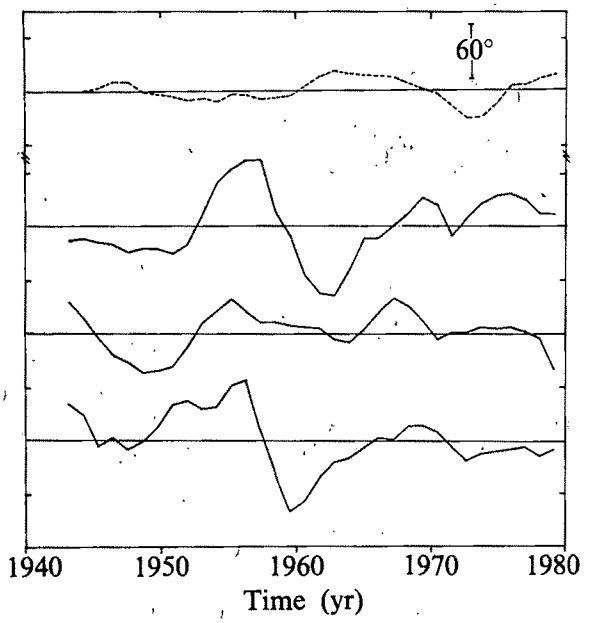


Fig. 2 Residuals of longitudinal positions. These residuals have been obtained assuming that the GRS (dotted line) drifts at constant acceleration throughout the time interval and that the three white ovals (solid line) move together as a system undergoing constant acceleration. The vertical scale is displacement in degrees of longitude with the magnitude of displacement indicated by the vertical bar at the upper left of the chart. Note that displacement below the line represents observed drift rates that are larger than the second-order polynomial fit implies.

though ovals drifting at a rate of 3 m s^{-1} were observed at this latitude from 1915 to 1938[14], there are no recorded observations of their disappearance. These earlier ovals were not observed to dissipate but, instead, became obscured by the general brightening of the south temperate belt in 1938.

Historical records indicate that the GRS has also decreased in length. During the period 1879–82, its dimensions were 40,000 by 13,000 km (ref. 2). Its length has shrunk to 25,000 km at present, while its width has remained approximately 13,000 km (ref. 9). For the past 40 yr there has been only minor observable activity in the south tropical zone, which lies between the white ovals and the GRS, and the drift rate of the GRS has been approximately constant.

To evaluate the rate of acceleration of these features quantitatively, a data set consisting of the longitude of the centre of the feature at the time of opposition for each apparition since 1943 was subjected to a least squares procedure which fit the data to a second-order polynomial. In the case of the three white ovals, the solution required that data from the three ovals have the same time dependence. The coefficients of the second-order fit are shown in Table 1 along with the acceleration of the features, assuming constant acceleration relative to System III longitude throughout the time interval. The residuals of the fit are plotted as a function of time in Fig. 2.

The GRS rotates more slowly than System III longitude (towards increasing longitude), and the white ovals rotate more rapidly; therefore, the coefficients in Table 1 suggest that the drift rates at both latitudes tend towards a common value. A disturbance in the south tropical zone was observed to catch up with and pass the GRS on 23 December 1970, accelerating the GRS as it did so[5]. Dark streaks were also observed in the south tropical zone in 1946–47 and 1955–57 (Reese, personal communication). Inspection of the GRS residuals suggests that the GRS is predictably accelerated by this activity, while the ovals are insensitive to it.

Because the GRS is larger than the ovals, Fig. 2 suggests that the smaller the oval cloud feature the more variable its velocity. A contradiction of this assumption is that observed velocities of small white ovals at −41° latitude are quite constant at 7 m^{-1} and the spacing between these individual ovals does not change as it does for the larger white ovals at −34°.

The rate of decrease in observable areas of the GRS and white ovals is proportional to the length of the features. The width of these four features is apparently determined by the surrounding zonal flow pattern; thus, their width has varied little while their length has decreased as a function of time. Shrinkage rates have followed two patterns. In the case of the GRS, the shrinkage was approximately linear throughout the interval from 1943 to 1979. The white ovals decreased rapidly in length between 1943 and 1966; however, since 1967 the shrinkage rate has been approximately linear. The data for the white ovals indicate that the shrinkage rate during the interval 1943–55 was slower than that predicted by an exponential function; hence, the function $\exp[a(t-t_0)^{1/2}]$ has been used to generate the analytical expressions given in Table 2. The residuals from cases 3 and 4 do not indicate a more convincing fit than case 2; however, observational scatter and the differences in the sizes of the three ovals during their early states of contraction do not recommend additional analysis. Because case 4 is less influenced by early variations in the length of the individual ovals, and because they have been virtually indistinguishable in size since 1948, it is more reliable for predicting future contraction of the ovals. Case 1 predicts that the GRS will span 21° in 1985 (24,500 km compared with the present value of 25,000 km) and, according to case 4, the white ovals will extend 9.1°±0.3 in 1985 (9,700 km compared with 12,000 km, currently); hence, shrinkage rates are small for the GRS, but are significant for the white ovals.

Table 2 Rates of shrinkage of the length of the GRS and white ovals

Case	Feature	Time interval	Formulae	L_0	C	s.e.
1	GRS	1944–79	$L=L_0+C\times$ $\times(t-1944.115)$	25.9	−0.114	2.2
2	White ovals	1944–79	$C\ln L=\ln L_0+$ $+(t-1944.115)^{1/2}$	68.7 75.6 64.6	−0.324	0.111*
3	White ovals	1944–66	$\ln L=\ln L_0+$ $+C(t-1944.15)^{1/2}$	67.1 77.1 62.3	−0.317	0.113*
4	White ovals	1967–79	$L=L_0+C\times$ $\times(t-1967.005)$	13.7 13.9 13.3	−0.249	0.98

L is length of feature in degrees (to convert to kilometres multiply L by 1,160 km per deg of longitude for the GRS and 1,060 km per deg for the white ovals). t is date (yr). s.e. is the standard error.
* These errors correspond to $\Delta\log L$.

Conclusions

Our data provide smoothed values for shrinkage rates and acceleration of the GRS and white ovals for the period from 1943 to 1979. Additional analysis of data from 1963 to the present will be presented later.

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Zonal velocity and texture in the jovian atmosphere inferred from Voyager images

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Measurements of the latitudinal profile of zonal velocity are used to estimate the absolute vorticity gradient. A classification scheme of small features visible at resolutions of 100 km or better is presented

THE first report[1] of the Voyager imaging science team following the 5 March 1979 encounter described Jupiter's changing appearance at resolutions down to 10 km, over intervals as small as 1 h. Examples of small-scale convection, rapid variations of features, and complex interactions of closed vortices were presented. This article extends these results in two ways. First, we present measurements of the latitudinal profile of zonal (eastward) velocity, from which we estimate the absolute vorticity gradient. Second, we present a classification scheme based on texture, by which we mean the patterns of small features visible at resolutions of 100 km or better. In accompanying articles we discuss characteristic structures and flow features of separated regions[2], and equatorial wave phenomena as observed by Voyager[3].

To date, we have not used the full potential of multi-colour imaging coupled with IR observations to infer vertical structure. Below we assume that all observed motion takes place at the same pressure altitude. We have also not fully analysed the high resolution (10–100 km) frames taken during the last few days before encounter. Thus we also assume that the frames that have been analysed are representative of the planet. Finally, we have not yet made detailed comparisons between Voyager data and results of theoretical models. Such comparisons will be made as the empirical basis of understanding matures.

Zonal velocity structure

Figure 1 shows zonal velocities with respect to Jupiter's interior[4] computed from displacements of cloud features that are about 100–200 km in size over time intervals of 10–30 h (1–3 jovian rotations) in late February 1979. We find no systematic differences between these results and those measured from frames taken 2 h apart, although velocities in the latter case are more uncertain. Figure 1 shows the work of several individuals analysing different pairs of frames. The spread of points provides an estimate of our measurement error.

The latitudes in Fig. 1 and elsewhere in this issue are planetographic, with assumed equatorial and polar radii of 71,400 and 66,770 km, respectively. Latitudes in the first Voyager report[1] were planetocentric, but were erroneously reported as planetographic. By definition, planetocentric and planetographic latitudes are the elevation angles with respect to the equatorial plane of a line from the centre of the planet and a line perpendicular to the surface of the planet, respectively.

Comparing these Voyager results (Fig. 1) with 100 yr of Earth-based results[5–8], there is remarkable constancy in latitude of the major zonal currents. Virtually every major current that has ever been seen in 100 yr of Earth-based telescopic observations is detectable in one 10-h sequence of Voyager observations. This is especially striking when one recalls that many major currents (for example at 23° N, 29° S and 44° S) have gone undetected for years at a time[6]. We conclude, therefore, that the currents are quasi-permanent, but a lack of suitable markings sometimes renders them invisible from Earth.

The constancy in latitude of the major currents contrasts with the changing visual appearance (colour and albedo) of Jupiter. For example, during the 4.25 yr between the Pioneer 11 encounter[9] and the Voyager 1 encounter[1,10] the bright white zone initially at 12°–24° S became narrower by a factor of 2, and the zone currently at 18–30° N became wider by a similar factor.

Compared to the visual appearance of Jupiter, the zonal velocity exhibits very little north–south (hemispheric) asymmetry. In the north, eastward velocities tend to be stronger, and westward velocities weaker than in the south. The local velocity minimum near the equator apparently lies at 1° S (ref. 6). But the velocity profile in Fig. 1 exhibits greater symmetry about the equator than a map based on structural features (see below and ref. 2) or a colour image of the planet[1].

The maximum speeds of the zonal jets measured by Voyager tend to be greater than those measured from Earth[5–8]. Earth-based observers tend to measure the larger, longer-lived spots, which generally lie between the major currents and do not participate in the high-speed flow. The Earth-based data shown in Fig. 1 are from a very intensive study[8] for the period 1974–79, and show much more of the high speed motion than earlier Earth-based studies[5–7].

The data of Fig. 1 may be used to compute the latitudinal gradient $d\zeta_a/dy$ of absolute vorticity associated with the zonal wind profile $\bar{u}(y)$. By definition,

$$\frac{d\zeta_a}{dy} = \beta - \bar{u}'' = \frac{2\Omega \cos\theta}{r} - \frac{d^2\bar{u}}{dy^2}$$

where y is the northward coordinate, ζ_a is the vertical component of vorticity in inertial coordinates, Ω is the planetary rotation rate, r is the radius, θ is the latitude, and $\bar{u}''$ is the curvature of the velocity profile.

Since β is positive everywhere, the latitudinal average of $\beta - \bar{u}''$ is positive. The latitudes where $\beta - \bar{u}''$ is most likely to be negative are those where $\bar{u}''$ is large and positive. These are the latitudes of the westward jets. Accordingly, Fig. 2 shows curves of $\bar{u}$ for which $\bar{u}'' = \beta$, and these curves are centred on the westward jets and on the equator. Note that the measured points near each westward jet lie to the right of the smooth curve, that is

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the observed velocity profile exhibits more curvature than β. We conclude that $\beta - \bar{u}''$ and hence $d\zeta_a/dy$ changes from positive to negative in the vicinity of the westward jets.

These Voyager data extend an earlier study[11] of Earth-based data which concluded that $\beta - \bar{u}'' \geq 0$ everywhere, with $\beta = \bar{u}''$ at the latitudes of the westward jets. The difference is a result of the tendency of Earth-based observers to underestimate the zonal speeds.

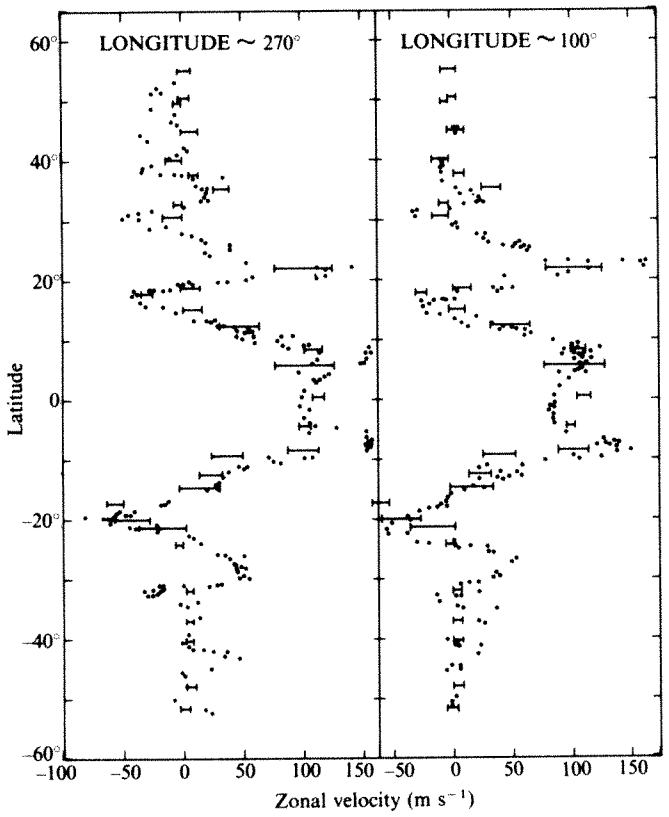


Fig. 1 Zonal velocities derived from Voyager 1 imaging data. Each point is computed from the displacement of a ~200 km cloud feature in frames 10–30 h apart. Earth-based measurements[8] from the period 1974–79 are shown as horizontal bars. Because of frequent lack of suitable markings, not all currents are visible from Earth at the same time or during the same year. The spread of points and the length of horizontal bars are indicators both of measurement error and of variability on Jupiter. Two significant features of the zonal velocity profile are its constancy over periods of many years and its relatively high symmetry with respect to the equator.

According to the simplest two-dimensional models, the fact that $d\zeta_a/dy$ changes sign near the westward jets implies that the profile $\bar{u}(y)$ is unstable at these latitudes[11]. The westward jets, particularly on their equatorward sides, are certainly more active and disturbed than the eastward jets[2,12]. However, this interpretation depends on neglecting the vertical structure of the flow. The condition $\beta - u'' \geq 0$ everywhere is the barotropic stability criterion[13], a sufficient condition for stability of a zonal flow $\bar{u}(y)$. When $\bar{u}$ varies also with depth, this criterion is relevant provided the static stability (thermal stratification) is large enough. The condition $\beta - \bar{u}'' \geq 0$ is also the statistical equilibrium state of planetary turbulence, according to recent numerical studies[14–16]. In these experiments, kinetic energy is introduced as small-scale eddies. Turbulent processes transfer this energy to a predominantly zonal flow whose latitudinal scale, defined by $(\bar{u}/\bar{u}'')^{1/2}$, increases until the condition $\bar{u}'' \leq \beta$ is reached. All of the above results depend on the model of vertical structure and lower boundary conditions[17–18], about which little is known. The observation that $\beta - \bar{u}''$ changes sign may provide useful constraints on these unknown parameters of Jupiter's atmosphere.

Figure 2 also indicates that $\beta - \bar{u}'' > 0$ at the equator, since the measured points lie to the left of the smooth curve $\bar{u}'' = \beta$. This observation, together with the fact that $\bar{u} > 0$, implies that the equatorial zone near the cloud tops has an absolute maximum of angular momentum. Theories of how this 'equatorial acceleration' is maintained against viscous dissipation are summarised elsewhere[19,20]. A significant feature of the observations (Fig. 2) is that angular velocity increases poleward between 9° S and 9° N, although angular momentum decreases.

Texture and morphology

Jupiter at 100 km or better resolution exhibits a variety of textures, close-packed round puffy features, puffy features in linear arrays, short linear features, long curved or folded filaments, and amorphous regions in which only large-scale, regional variations appear. Texture is a regional characteristic that is distinct from brightness or colour, although certain ranges of brightness, colour and texture may tend to occur together. Using an Earth analogy, texture provides clues about processes that are occurring in the visible clouds. We aim here to develop a textural classification of jovian clouds, and to provide a global map based largely on texture.

Plate 8, p. 790 shows a montage of images showing examples of different textural types, together with our cartoon representation of each type. Type *a* includes round puffy clouds with irregular organisation, suggestive of small-scale cumulus convection on Earth. Type *b* includes puffy clouds in linear arrays, suggesting convection with lateral shear in the horizontal wind. Type *c* shows a linear pattern suggesting strong shear and/or waves with small irregular variations along the wave crests. Type *d* is an isolated bright region, usually with puffy clouds visible on the periphery[1]; although this description includes a reference to brightness, these features, which appear suddenly and spread rapidly, are, nevertheless, a morphologically distinct type on Jupiter. Type *e* includes filaments—long, coherent, parallel threads suggesting laminar flow. Type *f* is a filamentary spiral, opening outwards either in a clockwise or counterclockwise direction. Type *g* is an example of folded filaments. Type *h* is a V-shaped pattern that forms where linear features with opposite tilts intersect. Type *i* is a region of low texture. Our cartoon representation of this type is simply a low density of lines, and is left blank.

Plate 9, p. 790 shows one face of Jupiter on 08.45 UT, 27 February 1979. This mosaic was made by projecting nine narrow angle frames in the orange filter onto a cylindrical grid. The resolution of the original frames is 120 km, although the resolution on the printed page is several times poorer. *Plate 10a*, p. 791 shows a morphological map of the same mosaic. The map was made from the nine orange frames and also from frames in other filters taken several days later at better than 100-km resolution. In the context of *Plate 10a*, the textural classification of any feature seems to be independent of the filter used in the images. At 150-km resolution, the texture of broad regions did not change during the several days before closest approach. We assume that the texture at higher resolution also did not change during this time interval. Studies of the highest-resolution (~8 km) frames suggest that the size of Jupiter's convective elements is at least 100 km, and therefore is adequately described in the following analysis.

Texture, like velocity, colour and albedo, is arrayed in a banded pattern around the planet. Bands at adjacent latitudes typically have oppositely tilted linear features, that is, features running north-west/south-east alternating with those running north-east/south-west. The intersections of these oppositely tilted features at adjacent latitudes form the chevron patterns (type h) that were mentioned in the first Voyager report[1].

Streamlines, defined by cloud motions, and streaklines, defined by the linear patterns, usually do not coincide. The latter are often tilted with respect to lines of constant latitude. The

former are rarely tilted; that is, the motion is largely zonal. From the pattern of zonal velocity versus latitude (Fig. 1) it is clear that the extremes of zonal velocity coincide with the vertexes of the chevrons. The vertexes (>or<) point in the flow direction (eastwards or westwards, respectively). This chevron pattern occurs at all latitudes, although it is more common at mid-latitudes. The correlation with velocity structure implies that texture on Jupiter is a strong function of lateral shear in the horizontal wind. (But note that we have almost no information about vertical shear.) The linear patterns could represent either transient features that are being pulled apart by the shear between the jets, or waves that are generated near the jet maxima and propagate in a V-shaped pattern behind the jets.

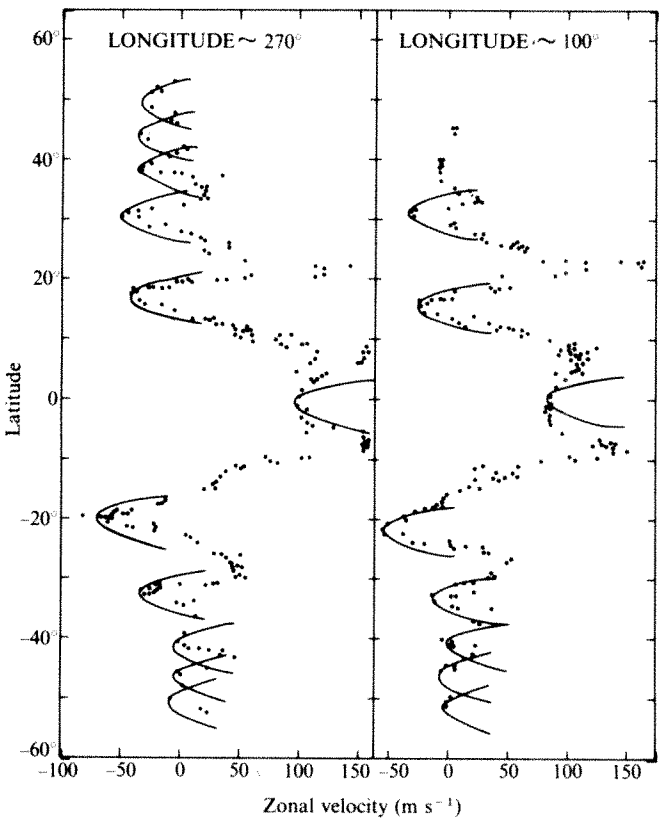


Fig. 2 Curvature $\bar{u}''$ of the zonal velocity profile compared to β. The measured points are the same as in Fig. 1. The parabolic curves are defined by $\bar{u}'' = \beta$, and have been centred on the westward jet maxima. At the equator one observes $\bar{u}'' < \beta$, which implies that angular momentum per unit mass decreases monotonically from equator to pole. At the westward jet maxima one observes $\bar{u}'' \geq 2\beta$, which implies that the absolute vorticity gradient with respect to latitude changes sign.

The Great Red Spot (GRS) and white oval immediately to the south also exhibit chevron patterns in the anticyclonic (counter-clockwise in the southern hemisphere, clockwise in the northern hemisphere) flow around their peripheries. The inner parts of these chevrons give the GRS and similar spots an outward spiral appearance (for counterclockwise flow) at their centres and an inward spiral appearance outside. However, the direction of the spiral does not necessarily indicate convergence or divergence. Rather, measured velocities around the GRS indicate predominantly circumferential motion. In fact, the angular velocity of rotation about the GRS centre seems to be greatest near the periphery[2]. Evidence that convergence or divergence is small also comes from the long lifetime (~60 d) of a prominent white feature that circled in the GRS during the Voyager approach phase[1]. In several cases, however, small spots were seen to enter the GRS peripheral flow from outside. No spots originating inside the GRS were seen to leave. This evidence seems to favour convergence, at cloud-top altitudes.

The chevron pattern also describes the direction of spirals at 34°N, 41°N and 41°S. If the material outside rotates more slowly than that inside, the former gets left behind by the rotation of the latter. This possibility is consistent with the observed direction of the spirals for these anticyclonic spots. Again, there is evidence that the inward spirals (for anticyclonic flow) are not streamlines and therefore should not be regarded as evidence of horizontal convergence.

The folded filament pattern (type *g*) is typical of the most rapidly varying, disorganised, turbulent regions. The lifetime of these features is about one jovian rotation. Oblong patches with this texture occur at 40°S separating the dark-haloed spots from each other. The oblongs typically lie somewhat equatorward of the spot centres. On a larger scale the same folded filament pattern occurs to the west of the white ovals and the GRS. These turbulent, folded filament regions always lie on the equatorward sides of the westward jets and have cyclonic vorticity. In contrast, the stable, well-defined spiral spots always lie on the poleward sides of the westward jets, and have anticyclonic vorticity (see Fig. 1 and ref. 2).

The equatorial region between 9°S and 9°N is generally characterised by low textural contrast. The plume heads are an exception as discussed earlier[1]. In the plume tails, unlike mid-latitudes, the diffuse filaments seem to represent streamlines. The difference with mid-latitudes may reflect the greater deformations undergone by equatorial features during their lifetimes. That is, the plume heads act as concentrated sources of bright material which spreads to fill the much larger area of the plume tails. Any tilt with respect to the velocity vector is eventually eliminated by the shear itself.

Conclusions

The latitudinal profile of zonal velocity is more constant over long periods and more symmetrical about the equator than the albedo and colour profiles. Absolute vorticity does not increase monotonically with latitude; it decreases significantly at the latitudes of the westward jets. The texture at 100-km resolution follows the larger-scale banding. The most prominent textural feature is the chevron pattern centred on the zonal jets and on the high-speed circumferential flow around the GRS and other large anticyclonic ovals. The tilted linear elements of the chevron patterns are not streamlines, but reflect the effect of shear on the visible markings. The spiral markings also reflect the effects of shear, and do not necessarily imply convergent or divergent flow. Finally, there is a significant difference between anticyclonic and cyclonic regions. The latter are more compact and steady, forming well defined spots and vortices. The former are elongated in longitude and variable on time scales of one jovian rotation.

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Jovian cloud structure and velocity fields

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Regional views of the jovian cloud structures and associated flows demonstrate the asymmetry between the northern and southern hemispheres.

A GLOBAL view of jovian small-scale morphologies and zonal velocity profiles has been presented and discussed in an accompanying article[1]. Here we give a regional comparison of the structures and velocity fields (meridional as well as zonal velocities) in the jovian atmosphere (scales >200 km) as observed by the Voyager 1 imaging system. In our descriptions we hardly mention their theoretical implications. All velocities are referenced relative to System III longitude. Cloud structures described are fairly independent of the visual wavelength at which the observation was made.

Although both hemispheres of Jupiter show similar patterns of diminishing and alternating eastward and westward jets as one progresses polewards, there is a pronounced asymmetry in the structural appearance of the two hemispheres. The southern hemisphere is dominated by bands of longitudinally alternating elliptical anticyclones and more elongated cyclones. The northern Hemisphere shows fewer longitude-dependent structures with the notable exceptions being the dark oblong features along the northern edge of the North Equatorial Belt (NEB) and a region of folded filaments in the NEB itself. The two hemispheres are separated by a structurally unique Equatorial Zone (EZ).

Northern hemisphere

Jupiter's northern hemisphere is characterised by a lack of large-scale (scales $\geq 10^3$ km) longitudinally dependent structures similar to those observed in the southern hemisphere. As seen by Voyager 1 the smaller northern features are, nevertheless, often similar in morphology and velocity structure to their larger, ubiquitous southern counterparts.

Cloud patterns in the NEB (latitudes from 9° to 18° N) vary considerably as a function of longitude. Diffuse equatorial clouds give way to a more clearly defined and organised structure at about 9° N. At this latitude a band of long filamentary clouds is present in a region of very strong shear between the equatorial current and a westward current at 18° N. Strong longitudinal differences in cloud structures also exist in the shear region. Between 40° and 100° W is a wedge-shape pattern of highly folded light and dark filaments rolling cyclonically (*Plate 11*, p. 792). This pattern is similar in morphology to the area west of the Great Red Spot (GRS) but it is not associated with any visible feature to the east or west. The northern pattern drifts independently of the similar southern pattern and has been observed many times before[2]. Within this region small areas of bright puffy clouds about 500–2,000 km in diameter are observed to form and dissipate on time scales of a few jovian rotations. Note that this variable region extends over only 60° of longitude. It is probably significant that such regions are observed only on the equatorward side of westward jets. Between 140° and 280° W at 17° N elongated dark brown features are present. These structures have generally sharp boundaries with adjacent lighter regions and show a pattern of linear striations inside. The vorticity of these features is, as expected from the profile of zonal velocity, cyclonic. Their association with 5 μm warm regions as observed from Earth leads to the suggestion that these are clearer regions through which the darker lower level clouds may be seen (see Fig. 5 in ref. 3).

From 20° to 23° N, the clouds take on a tilted linear pattern, associated with the opposing currents at 18° and 23° N. Tilting north-east, these features form a chevron shape with north-west tilted rows of puffy clouds to the north. The apex of the chevron pattern lies in the strongest part of the eastward jet. These features from about 19° to 30° N are longitudinally periodic with a wavelength of about 500 km (Fig. 5 in ref. 3). So similar and rapidly varying are the linear features that they are difficult to identify from one rotation to the next (over a 10-h time interval).

At about 31° N a westward current exists within which are clouds of folded ribbon-like features. Farther north at about 35° N a distinct albedo change exists across a transition to an eastward current with darker clouds appearing to the north. Within this transitional area are small circular features about 3,000 km in diameter (34° N, 40° W; see *Plate 11*). These features are comprised of dark rings with white cores and exhibit anticyclonic rotation. Occasional spot interactions have been observed when one of these features overtakes another[3]. These features are not distributed uniformly in longitude. They are found spaced about 10°–15° apart in a longitude range of 350–260°W. They do not appear in the remaining 90° of longitude (*Plate 11*) but seem to be obscured by a higher level of diffuse clouds. Comparing *Plates 11 and 12*, p. 792 there is a similarity in the appearance of this band of spots and those at 41° S with regard to size and spacing of the individual spots. Interestingly, some of the spots at 34° N have been observed from Earth to be bright (hot) at 5 μm, as have some of their southern counterparts. However, the spots at 34° N, which are anticyclones as are those at 41° S, do not have an alternating pattern of oblong cyclones associated with them as do the spots at 41° S.

The region from about 36° N to the northern limb (at about 80° N) is characterised by several white circular features rotating anticyclonically (40° N, 60° W; see *Plate 11*). In some cases faint spiral patterns can be observed in the interiors of the features. Surrounding these features are large areas of puffy unorganised clouds and other regions of folded ribbon-like clouds. A zonally elongated cyclonic flow stretching over tens of degrees of longitude (40° N, 30° W) is observed within these regions (*Plate 10b and 11*, pp. 791, 792).

Long currents are observed around these regions flowing eastwards along the southern edge, turning northwards, 'recirculating' in a westward flow, and then returning southwards. These recirculating cyclonic currents are similar to some of the ribbon-like flow patterns in the southern hemisphere, although

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it is interesting that they are not associated with alternate anticyclonic spots as in the south. At least one small (2,000 km diameter) feature was observed at about 45° N, 55° W (*Plate 11*) to exhibit very rapid cyclonic rotation. Morphologically this feature appears similar to the recirculating currents except for its very compact size.

Equatorial zone

The uniqueness of the broad equatorial eastward jet and the characteristic structures which lie within is well known. At the time of the Voyager 1 encounter we found the EZ to lie between approximately 9° N and 9° S. Earth-based observations have shown that changes in the cloud structure of the region may occur rapidly, with the morphology of a specific feature changing radically within a single rotation of the planet.

At the time of the Voyager 1 encounter, the northern edge of the equatorial region was characterised by a train of 13 plumes encircling the planet[3]. The apparent regular spacing of these features strongly suggests wave propagation in the equatorial zone. In spite of this apparent longitudinal regularity of these features, there are some important differences in their individual morphologies. Only four of the 13 plume features were observed to have bright nuclei during the period covered by the Voyager 1 observations. It seems that there is no specific System III longitude at which changes were observed to occur within the plume heads. The high resolution images of the plume heads suggest that these albedo changes are associated with localised (scales $\leq$200 km) convective activity. Generally, the plume heads are uniformly bright over scales of 2,000 km, though smaller scale (100–200 km) bright features are occasionally observed on the edge of the plume head.

The plumes are observed to move eastwards with the strong equatorial jet with velocities ranging from roughly 100 to 150 m s^{-1}. We have, however, observed some longitudinal dependence in the zonal velocity profile across the EZ. Thus, not all plumes are observed to move eastwards at the same speed. A profile of mean zonal velocities (Fig. 1 in ref. 4) indicates that there is a relative maximum in the eastward jet at both the northern and southern boundaries of the region, while a relative minimum occurs at approximately the equator. The observed range in eastward velocities is from 90 to 150 m s^{-1}.

Note that the plume heads are found only along the northern boundary of the zone. A fundamental problem seems to be to explain this north/south asymmetry in the zone's morphology in light of the symmetrical zonal velocity profiles through the equatorial jet. It may be that activity within the South Equatorial Belt (SEB) influences the southern boundary of the EZ. Indeed, we have seen material pass from the SEB northwards into the equatorial jet. Small waves (scales 70–650 km) have been observed within the EZ and are discussed elsewhere[4]. Some of these waves are observed to persist as long as 14 jovian rotations. The appearance of these waves suggests some dependence on the camera filter used, though further processing is needed to confirm this suspicion. Groups of these waves are observed to move with eastward velocities typical of other features at the same latitude.

Southern hemisphere

In the southern hemisphere both the zonal velocity profiles and the observed cloud structures (compare *Plate 12*, p. 792 with Fig. 1 in ref. 1) are remarkably cyclic as one proceeds from equator to pole. As in the northern hemisphere the zonal velocity profile consists of alternating eastward and westward jets of decreasing magnitude and width as one proceeds poleward. In the south such an alternating jet profile was not previously observed from Earth because Earth-based observers must use the easily identified elliptical spots, which lie at latitudes not associated with the high-speed jets, to deduce drift rates. Indeed, the presence of the large elliptical spots in the southern hemisphere seems to inhibit the formation of small, high-contrast features such as those used by Earth-based observers to deduce velocities in the northern hemisphere[5]. At three different latitudes (14° S, 31° S and 40° S) oblong cyclones with folded filament morphology are found to alternate longitudinally with the more clearly defined elliptical anticyclones. The cyclonic nature of these regions was not anticipated from Earth-based observations. *Plate 10b* depicts the observed flow near the longitude of the GRS. The observed flow in the region of the elliptical spots and the meridional profiles of zonal velocity at longitudes away from the spots are correlated on a variety of spatial scales. The three sets of elliptical spots (the GRS, white ovals and spots at 41° S) are found to lie at latitudes corresponding to the southside of strong westward jets (regions of anticyclonic shear), whereas the associated oblong cyclones lie at latitudes corresponding to the northside of these same jets (regions of cyclonic shear). Interestingly, the size of each of the elliptical spots seems to scale roughly as the speed of the associated jets between which it sits. The ellipticity of the elliptical spots remains fairly constant at each scale, although the spots seem to become rounder as one progresses polewards and as the jet speed diminishes. The same basic statements seem to apply to the oblong cyclones and the associated cyclonic shears which lie at the same latitudes.

The smaller scale morphologies within the elliptical spots are remarkably similar. One is struck by the general lack of organised structure in the larger spots' interiors, such as spiral cloud patterns typical of terrestrial tropical storms. The cloud texture in the interiors form chains of puffy clouds. In the case of the highest resolution mosaics of the GRS a limited number of what may be groups of convective cumulus cells are seen in several areas. Chevron morphologies around the outer edges of the GRS and white ovals suggest higher angular velocities around the spots' outer edges than in their interiors. Indeed, higher velocity jets around the spots' peripheries have been observed in time-series of Voyager images. Regions around the elliptical spots are for the most part darker than the spots themselves and are of a more filament-like texture (see Fig. 7 in ref. 3). In the case of the GRS and some of the spots at 41° S, the peripheries of the ellipse are particularly well defined by very dark collars of filamentary clouds located on the outer (cyclonic) side of the strong peripheral jets.

The morphologies associated with the oblong cyclones are quite different from those of the elliptical spots. When looking near the longitude of the GRS, one sees what resembles turbulent wakes west of the GRS, oval BC, and several of the spots at 41° S (*Plate 12*). Even smaller eddies along the northern and southern edges of the supposed wake can be seen ; however, the notion of a wake is eliminated when the observer notes that the curl of these eddies does not change sign across the purported wake. As we have previously noted, velocity measurements immediately confirm that instead these are regions of cyclonic circulation.

At longitudes away from the GRS, the cloud texture within the SEB itself (latitudes between 9° and 23° S; see *Plate 12*) is generally rather diffuse with the strong eastward velocities of the equatorial jet slowly giving way to a much narrower westward jet along the south edge of the belt. However, on either side of the GRS the flows within the SEB are cyclonic. Why the cyclonic region in the SEB west of the GRS seems more turbulent than the analogous region to the east remains to be seen. It is along the southern edge of the SEB west of the GRS that the bright anticyclonic swirls often observed to interact with the GRS are formed.

The characteristic differences of the oblong cyclones between the three white ovals are interesting. We use here the classical nomenclature of Peek[6]. In *Plate 12* the ovals BC, DE and FA are located at longitudes of roughly 85°, 170° and 5° W, respectively. From oval BC to FA the cyclonic circulation is unmistakably outlined by a dark scalloped ribbon of clouds around a brighter, soft-textured interior. The analogous region between oval FA and DE is not nearly so pronounced, perhaps because of the vast 195° longitude spacing between these two ovals, while the cloud structures take on a folded filament appearance. The

appearance of this region and the vast gap between ovals FA and DE suggest a 'missing' white oval near 270° longitude where a peculiar isolated region of folded filaments is found (*Plate 12*). The western section of the region between ovals DE and BC is marked by a dark scalloped ribbon along the northern edge of a darker central axis. The eastern portion of the region is of a rather chaotic appearance with chevron-like filaments along an axis slightly south of the darker eastern section.

Note that the cyclonic regions associated with both the white ovals and the spots at 41° S are generally of two types: Type I, which resembles folded ribbon-like filaments (much like the region west of oval BC) and Type II, which shows lack of internal detail and a single dark outer loop of cloud filament (for example, the region between ovals BC and FA). Type I regions are seen at even higher latitudes in both hemispheres. A reasonable suggestion seems to be that the Type II cyclonic regions are just Type I regions veiled by a higher deck of white cirrus.

The band at about 41° S displays this same pattern of alternating elliptical spots just south of the oblong cyclones which lie equatorwards of the westward jet. In this case, however, the elliptical spots and associated oblong cyclones occur with approximately a wavenumber 12 periodicity. The elliptical spots themselves seem to be of two general types: the dark-haloed type, displaying a bright interior with little apparent structure and surrounded by a dark relatively broad peripheral collar, and the spiral type, displaying a spiral arm structure with arms originating near the spot's centre and extending for about 180° around the spot. A few of the dark-haloed variety have, in the past, been identified with 5-μm bright (hot) regions from ground-based observations, leading some to suggest convective upwelling in the bright centre with downward flow in the dark halo[7].

At more southerly latitudes there are suggestions of a continuation of the alternating jet pattern, though definitive measurements are yet to be made; however, one still observes something like the alternating pattern of oblong cyclones (mostly of Type I) and smaller elliptical spots.

Conclusions

The most striking characteristic of the planet as a whole is the asymmetry in cloud structures between the northern and southern hemispheres. Such a marked asymmetry is particularly odd in light of the similarity of the observed zonal velocity profiles in both hemispheres. We have observed that the northern hemisphere possesses some regions of strong lateral shear which do not give rise to any large-scale disturbances, whereas other regions of shear, such as the section of the NEB from 40° to 100° W, possess instabilities which have lasted over several years. In the southern hemisphere we have found that all elliptical spots seem to be anticyclonic (as indeed they are in the northern hemisphere) and are associated with longitudinally alternating regions of cyclonic flow lying slightly equatorward. The structures in the southern hemisphere are so regular as to suggest that the dynamics of the GRS, the larger white ovals, and even smaller spots observed further south are quite similar, though seen at differing scales. An obvious question posed by this observation is why should the GRS be red while other anticyclonic spots are generally white? We believe that the EZ is a region of localised intense convective activity. Again, we note a curious asymmetry in that convective regions all seem to lie along the northern edge of the EZ, though the zonal velocity profile is more nearly symmetric about the equator.

Historically, we recognise that the jovian cloud structures are capable of undergoing large-scale changes. We hope that the extended time scale offered by the Voyager 2 encounter in early July 1979 will provide some insight into the longer term climatic changes of Jupiter's atmosphere.

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Voyager observations of small-scale waves in the equatorial region of the jovian atmosphere

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Wave-like disturbances in the equatorial region of the jovian atmosphere are observed to fall into two categories.

THE Voyager 1 imaging system has provided the first glimpse of a wide range of spatial scales of motion, over a period of four months, of the jovian atmosphere[1]. These images have helped to characterise better the general and small-scale features of the atmospheric circulation. Historically, bright spots have been observed in the equatorial zone (EZ) for over 60 yr in Earth-based observations[2,3]. Voyager 1 images show these bright spots to be similar to the features observed by the Pioneer missions[4]. They resemble plume-like objects (on a scale of 10,000 km), whose trails are parallel to the shear[5]. Voyager 1 images show a train of 13 plume features moving differentially, in an eastwards direction, along the northern edge of the EZ, between 9 and 1.7°N, four of which have high albedo nuclei (defined as active) in all visible wavelength filters. Figure 1 shows one of these brighter plumes at high resolution, through a violet filter, taken seven days before Voyager 1's closest approach to Jupiter. The two boxes in the Fig. 1 outline the regions where small-scale (~600 km) periodic cloud structures[6] were observed. The upper box shows these periodic cloud structures, which we believe are waves, with their axes perpendicular to the plume trails at a distance of 10,000 km from the plume nucleus. The lower box shows these waves with their axes perpendicular to the latitude circle of 1°S. We have studied 27 images which display these waves, and due to different contrasts[6] through the filters, they have varying degrees of visibility. We classify these waves into two categories: the plume-tail (PT) waves which appear in the upper box of Fig. 1 have a 'horizontal' extent of up to 3,000 km and the cross-equatorial (CE) waves which appear in the lower box of Fig. 1 have a 'horizontal' extent of up to 20,000 km. These PT waves have been observed with varying intensity, to be associated with the same plume for some 14 jovian rotation periods[6]. During this time, no PT waves

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appeared with any other plume and this particular plume became inactive after the plume nucleus separated and dissipated in the north equatorial belt (NEB). The PT waves appeared clearest in short-wavelength filters and were more pronounced when the plume nucleus was expanding. The CE waves appear at several locations south of the plume nucleus and in Fig. 1 are approximately 8,000 km due south of the nucleus. Figure 2 shows a wide angle violet filter image where the shadow of Io's disk appears near the CE waves. However, the passing of this shadow is unlikely to have any relationship to these waves as

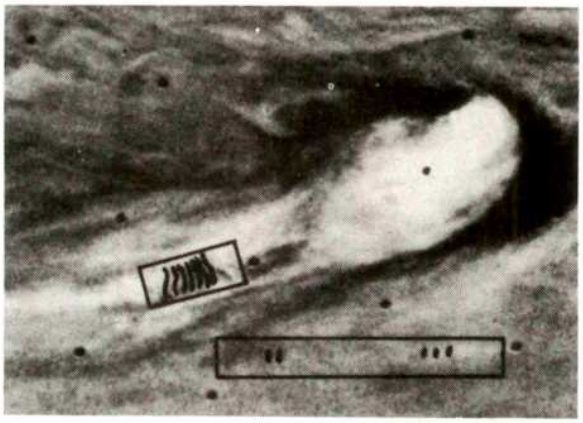


Fig. 1 A violet filter narrow angle of the area centred at 2.2°N and 89.98°W (System III) with a subspacecraft resolution of 136 km per television line pair. A train of waves is seen in the tail of the plume with a spacing of ~600 km. The cross-equatorial waves are only barely visible and their extent has been taken from the nearest orange filter frame. In all figures north is at the top.

the waves are visible when the shadow is not present. The CE waves have been observed in several forms for some 13 rotation periods in the same region of the EZ, though moving differentially to the plume drift rate.

The PT waves appear as alternate bright and dark lines with a spacing lying between 300 and 650 km (ref. 6) and appear to move at drift rates between 100 and 120 ms^{-1}, comparable to other trackable features in their vicinity including the plume nucleus (see Fig. 3). The CE waves appear in several forms including dark, isolated arcs and dark lines superimposed on a smaller-scale bright-and-dark pattern (see Fig. 4 which is a narrow angle enlargement of the area outlined in Fig. 2). The spacing between these lines varies between 70 and 650 km (ref. 6) and they appear to drift at rates between 90 and 110 ms^{-1} which is comparable to the few other trackable features in this area (see Fig. 1[4,7] and, for comparison, Fig. 3).

The PT waves appear to be the clearest when the plume nucleus is brightest. The variation in brightness of the plume nucleus has been interpreted to be consistent with terrestrial-

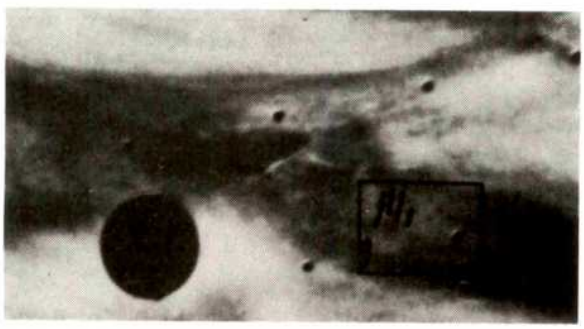


Fig. 2 A violet filter wide angle image of the same area as Fig. 1 taken some 13 rotation periods later, centred at 2.27°S and 166.60°W (System III) with a subspacecraft resolution of 139 km per television line pair. The cross-equatorial waves are visible inside the box with a spacing of ~600 km. The dark circular feature is the shadow of Io's disk.

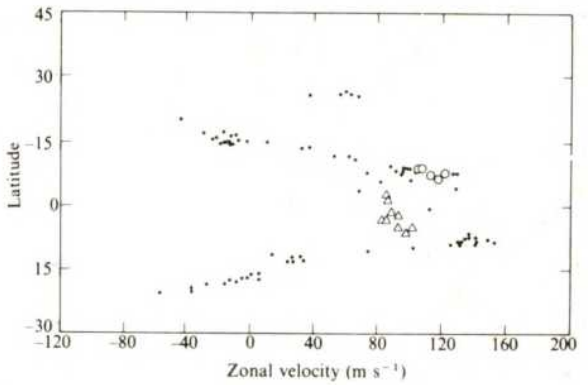


Fig. 3 The zonal velocity profile for the region occupied by both types of waves has been derived by tracking features from identical filter pairs with the image shown in Fig. 1 being the first of the image pair. The positions of the two types of waves have been shown. Zonal velocity has been derived with respect to System III longitude. ○, Plume tail waves; △, cross-equatorial waves.

type mesoscale convection[1]. However, this phenomenon has also been observed in the turbulent regions of the NEB and south equatorial belt (SEB)[6] though there is no evidence of any waves on this scale in these regions. The PT waves appear to be consistent with the interpretation that they are gravity waves initiated approximately 10,000 km upstream by the rapid, fluctuating convection in the plume nucleus. Disturbances of this kind have been observed in the Earth's atmosphere following severe thunderstorms[8]. Gravity waves have also been found to be precursors of strong convective activity in the terrestrial atmosphere[9]. This suggests that the PT waves may be directly related to the renewed brightenings of the plume nucleus in the jovian atmosphere and they may be an example of nondispersive gravity waves. Furthermore, type 'a' texture[5] has been observed in the vicinity of the plume tail, supporting the contention that small-scale convection is present in this region. The CE



Fig. 4 A green filter narrow angle image taken two minutes before Fig. 2 and showing an enlargement of the box outlined in Fig. 2. It is centred at 1.4° S and 163.85° W (System III) with a subspacecraft resolution of 20 km per television line pair. The bright waves have a spacing of ~70 km and the dark waves of ~300 km.

waves, however, do not appear to have an obvious source mechanism.

Wave-like disturbances in the equatorial region of the jovian atmosphere have been shown to fall into two distinct categories differentiated by their 'horizontal' extent, morphology, spacing, drift rate and association with other visible features. It is hoped that further evidence to understand their dynamical significance will be gained from Voyager 2 observations in progress.

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A lower limit on the top of Jupiter's haze layer

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Remote sensing observations of the atmosphere at wavelengths from the UV to the IR are affected by the presence of haze layers above the visible clouds. Such layers are difficult to detect as they generally contain small particles ($\leq 1\,\mu m$). An imaging observation of high-altitude haze is presented that extends through the jovian stratosphere into the mesosphere.

THE observation was taken by the wide-angle camera of Voyager 1 at 1979 March, 4 d 17 h 52 min 36 s UT, to search for aurorae, lightning, and fireballs (Fig. 10, ref. 1). A star was occulted by Jupiter during the initial slew of the scan platform to the pointing direction for the first of a pair of images used of that field. Two other stars appear with sufficient brightness to leave trails during the slew. These trails, along with the measured positions of all of the stars, offer the possibility of looking for atmospheric refraction of the occulted star. The detailed image pattern of the stars is found both for the images that drifted slowly during the first pointing of the double exposure and of the streak left during the slew. The fit to the occulted stars is perfect, indicating that the double horizontal refraction at the top of the layer of haze did not exceed 14 arc s. We shall show that this corresponds to an upper limit of 3.5 mbar for the atmospheric pressure at the top of the occulting haze layers if the scale height is 30 km. This upper limit is proportional to the 3/2th power of the scale height.

Thirteen stars, ranging in visual mag from 6.8 to 9.0, were used to predict the location of the 7th mag star which was occulted. A photogrammetric approach was used with raw image locations (sample and line number) of stars and reseaux as the observations. Reseaux image locations were used to estimate local TV geometric distortions (up to 3 pixels) for the 14 stars. Star image locations were used to estimate TV camera pointing, orientation, and focal length. Inertial positions of the stars were obtained from the AGK3 catalogue.

Star image locations were measured manually from computer listings of imaging data surrounding each star. Star images, rather than being point sources, had been smeared about 10 samples and 20 lines due to camera slewing and spacecraft limit-cycle motion during the 3-min and 12-s exposure. Image smearing limited the measurement accuracy to about 1 pixel (14.0 arc s).

Using all the parameters estimated from the reseaux and 13 star image locations provided a prediction for the image location of the occulted star. It was found that the observed image location of the star up to the point of occultation agreed with the predicted position within the measurement uncertainty (Table 1).

The difference between the observed and computed image locations (sample and line number) of 13 stars plus the occulted star is listed in Table 1. This difference for the occulted star is within the measurement accuracy (~ 0.8 pixels) of the 13 other stars.

Figure 1 shows the fit of an outline of the slew and drift pattern taken from the brightest other star and superimposed on the computer pixel listing for the occulted star. The outline of the limb is also displayed. Clearly even 1 pixel of refraction would have visibly distorted the slew. Furthermore, failure to recognise such a distortion and a corresponding forced fit would lead to an enlarged systematic error in the deduced position of the star, which did not occur. One pixel corresponds to 14 arc s at the scale of the wide-angle camera and we thus arrive at an upper limit for the double horizontal refraction at the top of the jovian haze layer, 14 arc s.

The double horizontal refraction, θ, can be related for an isothermal atmosphere to the refractivity, ν_1, at the minimum height of the ray and the scale height, H (ref. 2) by

$$\theta = \nu_1 (2\pi a/H)^{1/2} \tag{1}$$

where a is the radius of the planet.

The refractivity is proportional to the density and we let the constant of proportionality be denoted by κ. Application of the perfect gas law enables us to find the pressure, P, at the minimum height of the ray:

$$P = \left(\frac{H^3}{2\pi a}\right)^{1/2} \frac{g\mu m_A}{\kappa k}\,\theta \tag{2}$$

where g is the acceleration due to gravity, μ the mean molecular weight, m_A the atomic mass, and k the Boltzmann's constant. The definition of a scale height is also used:

$$H = \frac{kT}{\mu m_A} \tag{3}$$

Table 1 Predicted star location errors

AGK3 no.	Visual mag	Spectral class	Predicted sample	Location error line
105363	8.9	K2	−0.6	0.0
205764	8.2	G5	0.1	0.1
105365	8.6	F0	0.5	−0.1
105370	8.4	K2	1.6	−1.7
205785	8.9	F8	−0.6	−0.1
205789	8.4	K0	−0.6	−0.0
205792	8.7	F0	−0.1	0.2
205796	9.0	G0	−1.6	1.4
205795	7.9	K0	−0.0	0.5
105378	9.0	K2	0.2	1.0
205814	8.8	A2	0.2	−0.0
205815	8.7	G5	0.4	−0.5
205818	6.8	G5	0.3	0.1
205766*	7.0	A0	0.5	−1.0

* Occulted star

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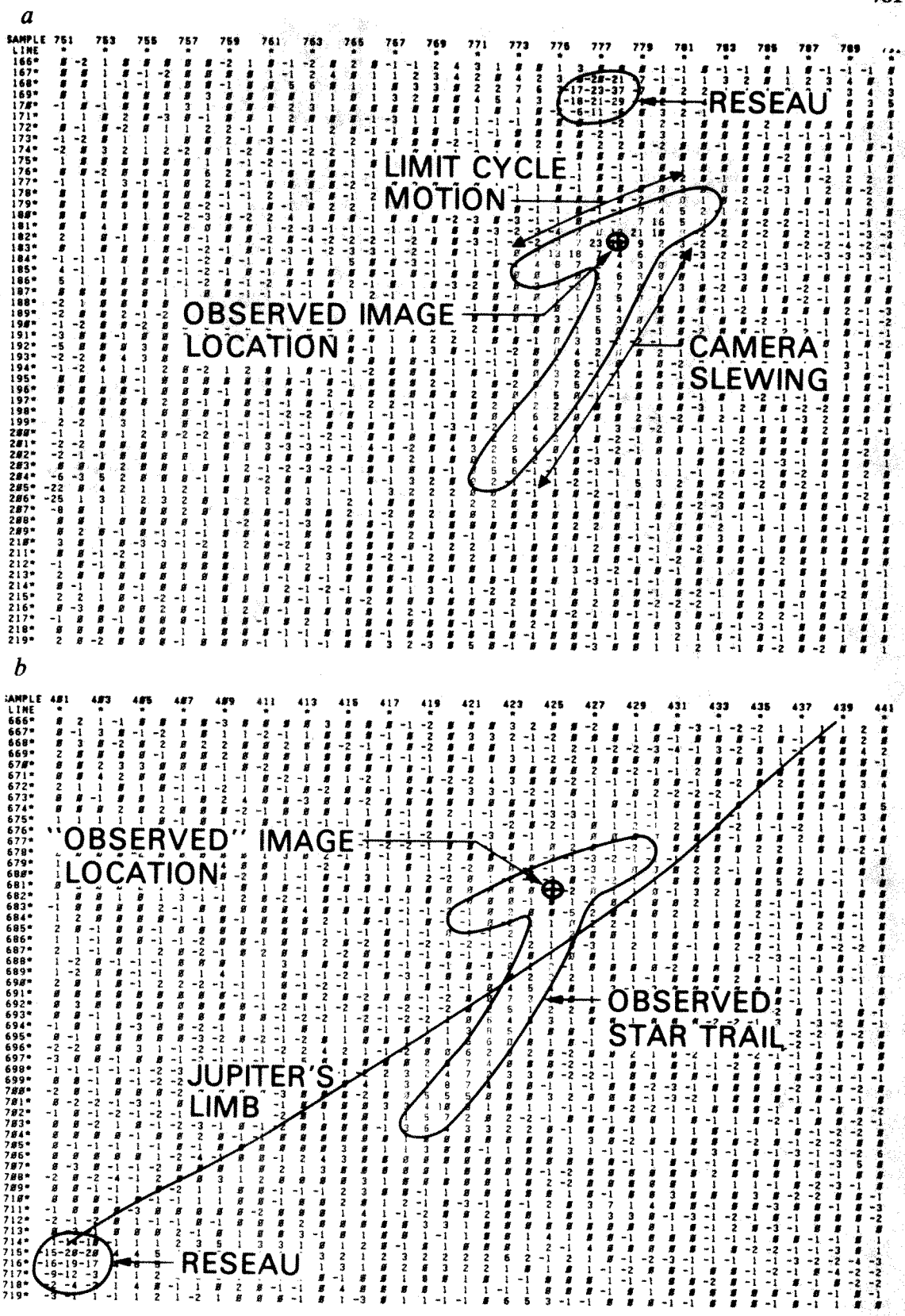


Fig. 1 Fit to the image of the occulted star. The outline of stellar images generated by slew of the scan platform and subsequent drift within the limit cycle. *a*, Reference image shape from 6.8 visual mag star; *b*, observed location of occulated 7.0 visual mag star.

where T is the absolute temperature. At a scale height of 30 km we find an upper limit on the pressure of 3.5 mbar. This very low value applies at jovicentric longitude +35° and latitude +80° and indicates that the haze layer extends far above the visible clouds of Jupiter into the mesosphere. It is therefore important to determine the structure and opacity of the haze in order to accurately interpret spectroscopic observations of Jupiter at wavelengths from the UV to the infrared.

These results were made possible by all those who have worked in the Voyager Project, many of whom are individually acknowledged in ref. 1. We thank especially C. C. Avis, G. W. Garneau, P. L. Jepsen, J. J. Lorre, J. A. Mosher, G. M. Yagi, J. T. Harwood, L. Pieri, and C. Reslock of JPL, and also S. A. Collins as experiment representative at JPL. This paper reports one phase of research supported by NASA at JPL under contract NAS7-100 and supported by JPL at the Smithsonian Astrophysical Observatory under that NASA contract. G.E.H. was supported by the SRC.

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Plate 1

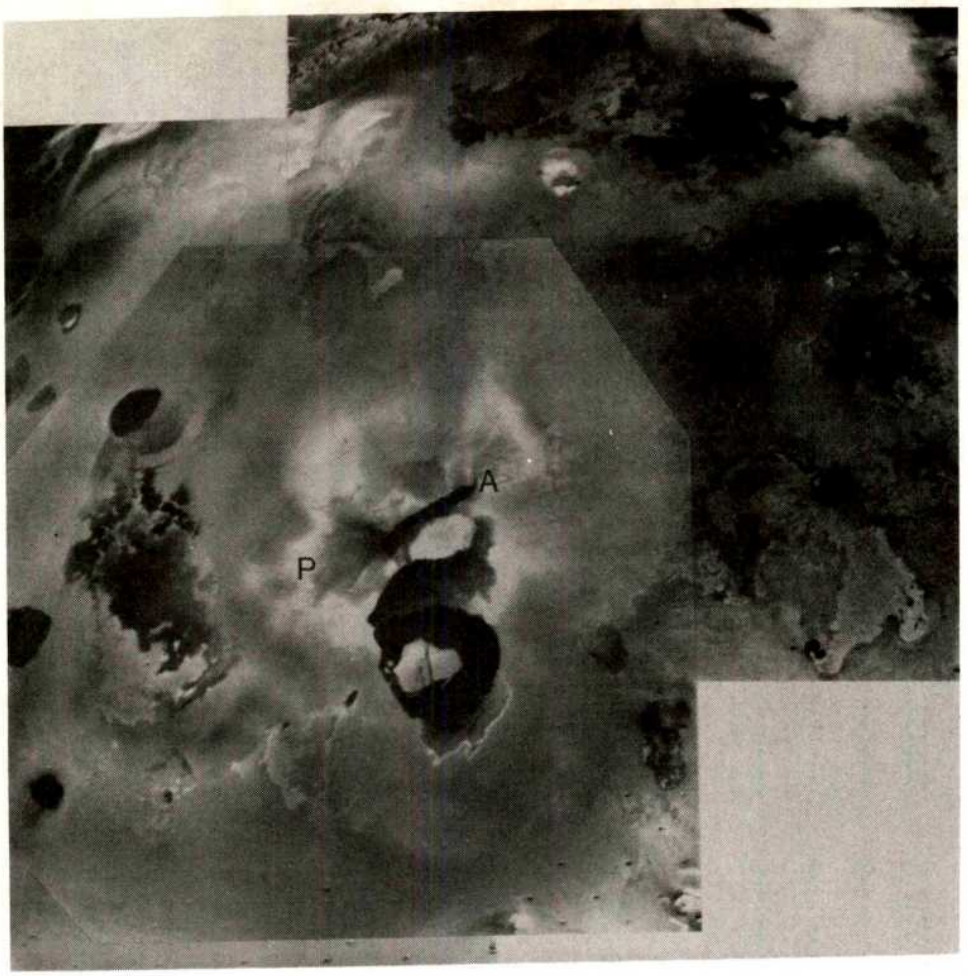


Plate 1 High resolution (2.7 km per television line pair) picture of the eruption site of Plume 2. The plume is probably a fissure eruption occurring along the length of the black strip A. The principal activity seems to be concentrated at the west end of the black area where enormous jets of pyroclastics (P) are being ejected. (FDS 16389.38).

Plate 2 *a*, Flow patterns in the region of 15 °S, 325 °W. At right centre several long, narrow dark flows originate at dark caldera. Several of the flows apparently have bright margins. In the lower left a dark-floored caldera is near the centre of some radial bright markings that could also be flows. Diffuse bright and dark markings are associated with most other calderas. The entire scene is ~900 km across (Frame 0043J1 + 000). *b*, Bright flow in middle right originating in small dark caldera, about 250 km long. It apparently has inundated at least one other caldera. Also shown are several discrete dark calderas, about 100 km in diameter. Near the centre of the frame is a mottled area surrounded by at least 10 broadly lobate flow-like markings of low albedo described in the text (Frame 0105J1 + 000).

Plate 3 The layered terrains of the south polar region. The uppermost layer in the stack at the upper left corner of the picture shows a complex erosion pattern. The layer immediately below is even more eroded and in the upper centre of the picture breaks up into both flat and smooth topped remnants. This area also contains long sinuous erosion troughs. In the lower centre of the picture a complex array of caldera-related bulbous flows overlies a stack of flat-topped layers that show the same type of erosional pattern observed in the south polar region. Bright streaks occur along the lowermost erosional scarp. Emanating from beneath another eroded scarp is a 100 km dark, digitate flow, the distal end of which is surrounded by a bright aureole. The scarp-related bright plumes are believed to be SO_2, the exhalation of which is driving the scarp retreat process. The long dark digitate flow may consist of sulphur released in liquid from the base of the stack.

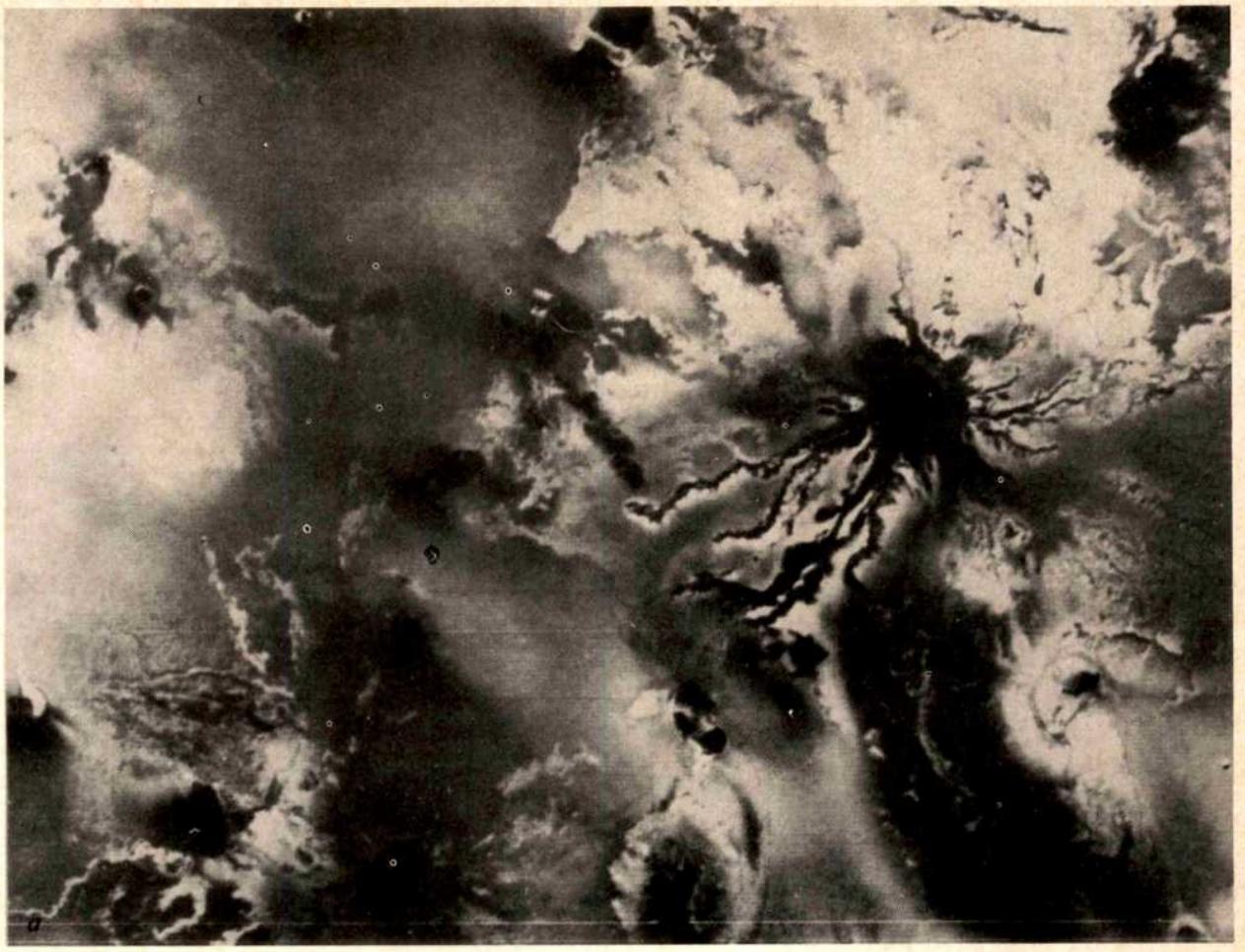
Plate 4 *a*, Picture of irregular mountainous terrain that may be cut by faults and marked by craters, probably of internal origin. The mountains are surrounded by layered deposits that may have been deposited as ash flow tuffs. The arcuate scarps may define a caldera ringed by old mountains and layered plains deposits. *b*, Picture of volcanic vent surrounded by dark ejecta that resembles lunar dark halo craters. This crater lies on the flank of the source vent of Plume 1. Mountainous terrain that may be broken by faults is surrounded by layered deposits. This area, covered by young volcaniclastic deposits, shows simple geologic patterns in contrast to the complex patterns in *Plate 2*.

Plate 5 *a*, Voyager 1 image of Io (left) showing volcanic vents, flows and domes, and geologic map (right) showing interrelationships among terrain units. The image was acquired from a range of 133,000 km and covers an area approximately 1,000 km on a side. The geologic symbols are the same as those in Fig. 1 of Masursky *et al.* (this issue). md, Dark mantle material interpreted as volcanic debris cast from volcanic centres; c, volcanic cones; l, bright (high-albedo) material perhaps fumerolic deposits of sulphurous compounds. Lava flows or other fluid materials occurring in association with pit craters: d, dark; pcl, bright; pcm, mottled; and pcu, mixed-albedo; pck, knobby material associated with dark pit craters. Flow materials associated with volcanic shield-like forms: sd, dark; sl, light; sm, mottled; and su, undifferentiated subunits; sk/sh, knobby and hummocky materials on or near shields. *b*, Comparison of the eight eruptive plumes photographed by Voyager 1. The bar is 200 km long. The top of Plume 6 is visible to the right of Plume 5 and the top of Plume 5 is visible to the left of Plume 6.

Plate 6 *a*, Voyager 1 photograph (FDS 16377.50) showing the location of the eruption sites associated with Plumes 1, 4, and 7, and Plume 3 seen in profile at the limb. The width of Plume 3 is about 250 km. *b*, High resolution (1.5 km per television line pair) oblique picture of the eruption site of Plume 1. The dark sky has been specially processed to show the filamentary structure at the top of the plume. Measurements of this image indicate the plume filaments are about 280 km above its source, consistent with measurements on other images. The three main eruption sites responsible for the dark radial streaks are near the centre of the picture. A caldera is apparently related to the largest eruptive centre. Smaller diffuse dark areas are eruption sites which may have been active when the picture was taken. All eruption sites are associated with a highly fractured uplift about 200 km wide. (FDS 16391.30).

Plate 7 *a*, The eruption sites of Plumes 3, 4, and 7 are shown on this Voyager 1 photograph (FDS 16375.28). Plume 6 is shown in profile on the limb. Its central fountain is about 60 km wide. Dark jets of pyroclastic material are being ejected on ballistic trajectories at the site of Plume 3. To the right of A is the region where changes have occurred during the 5.5 h interval since *b* was taken. Many of the dark spots and bands seen in *b* have almost completely disappeared in this picture. *b*, Full disk picture (FDS 16368.28) of Io showing the locations of five eruption sites associated with Plumes 3, 4, 5, 6, and 7 (arrowed numbers). Unnumbered arrows indicate the probable sites of recent eruptions. The appearance of the region to the right of A has changed significantly since this picture was taken. Many of the dark bands and spots have disappeared in *a* taken 5.5 h later. The outer diameter of the bright ring surrounding site 3 is about 400 km.

Plate 8 Textural types observed on Jupiter. Each image is approximately 7,000 × 7,000 km, and was taken through the violet filter. The inset shows the cartoon representation of each textural type, as used in *Plate 10a*. These types are discussed individually in the text.



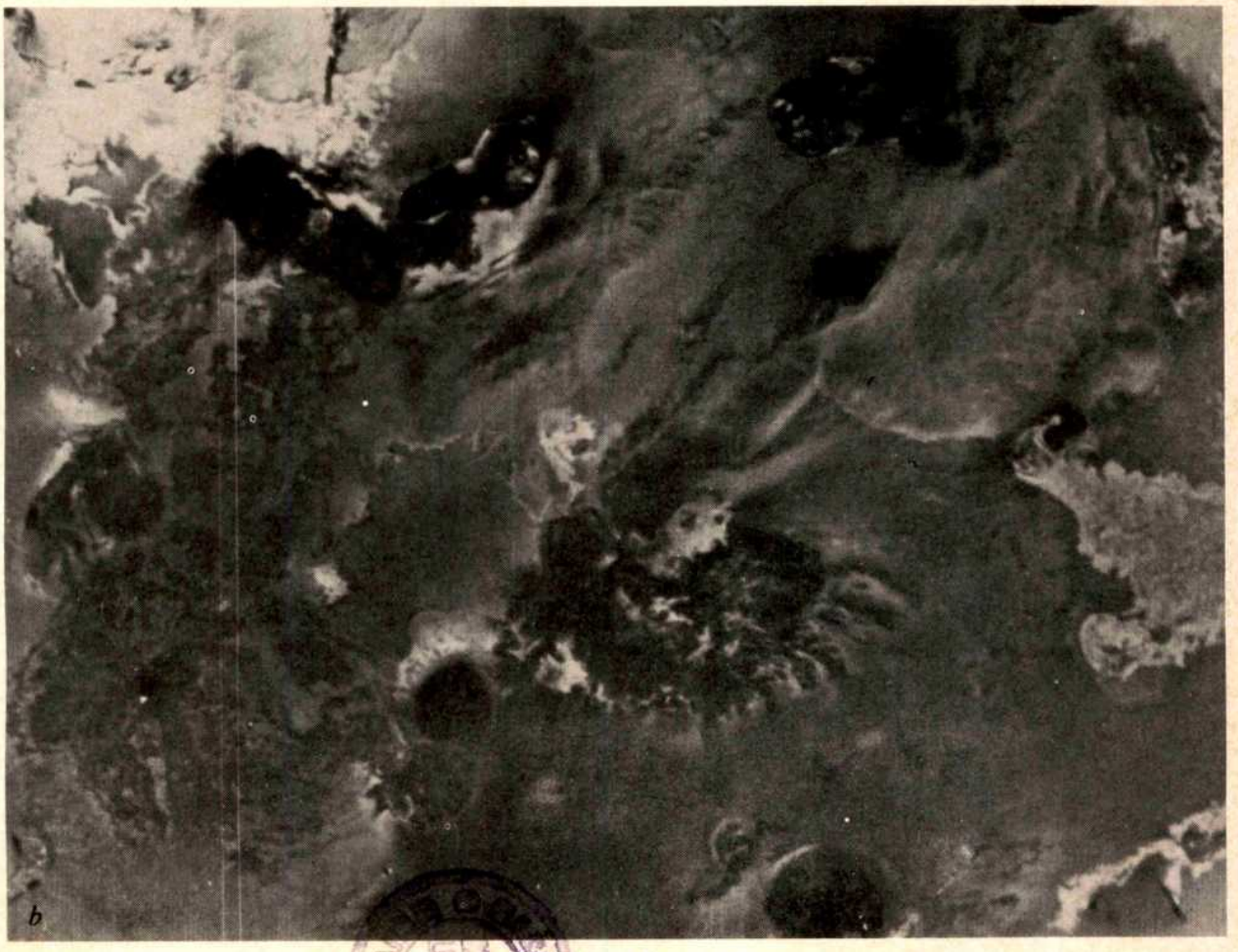


Plate 2

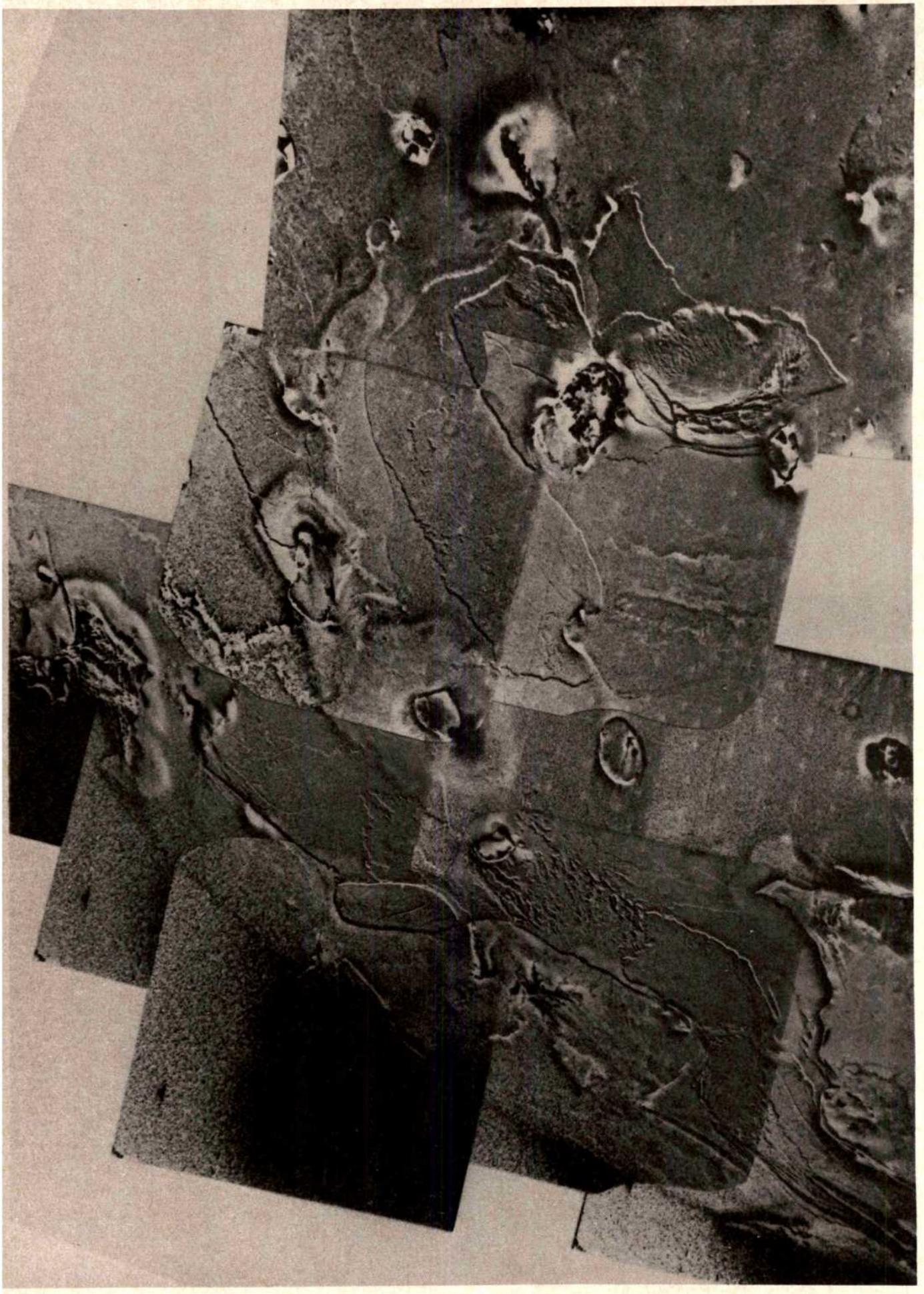
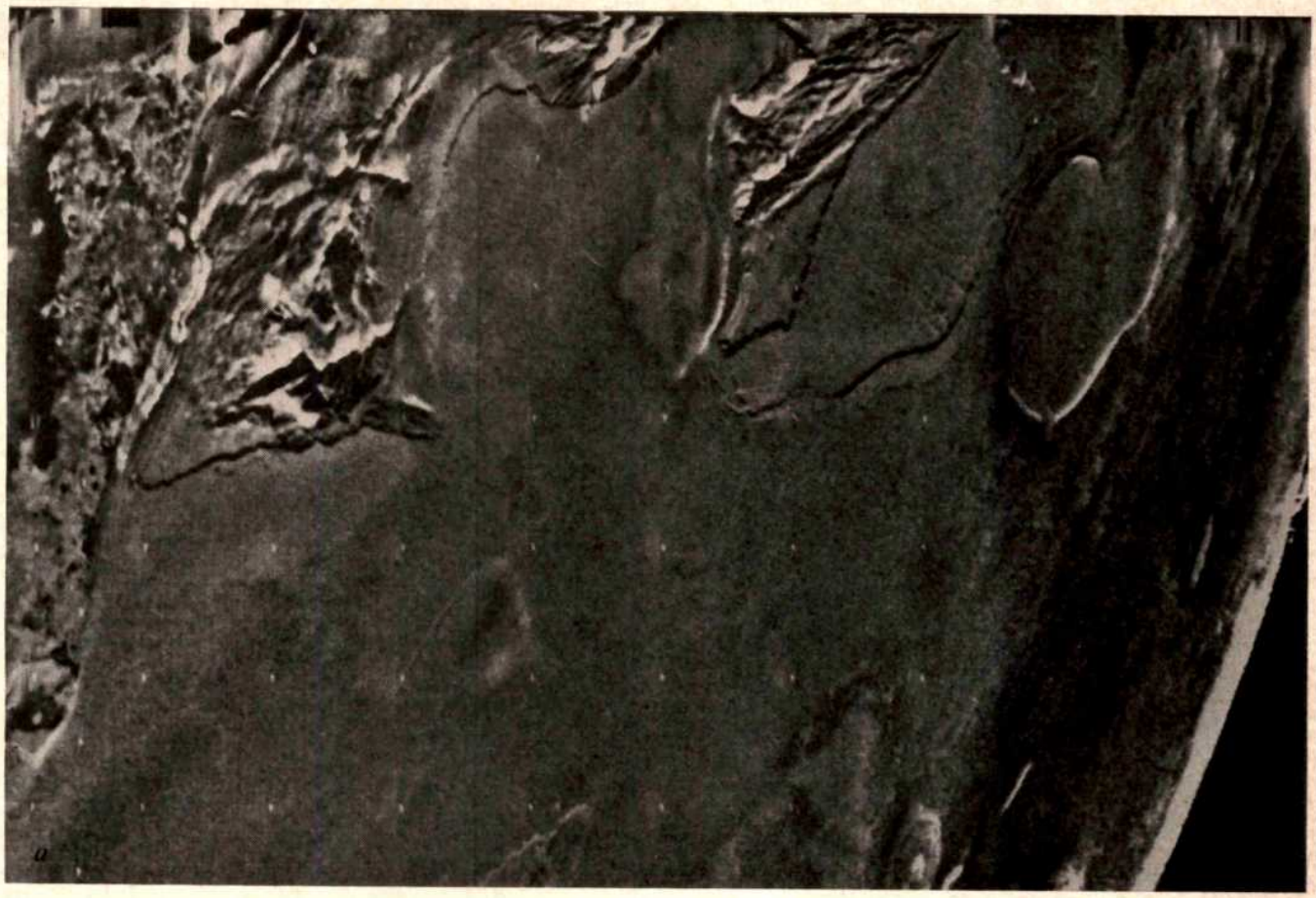


Plate 3



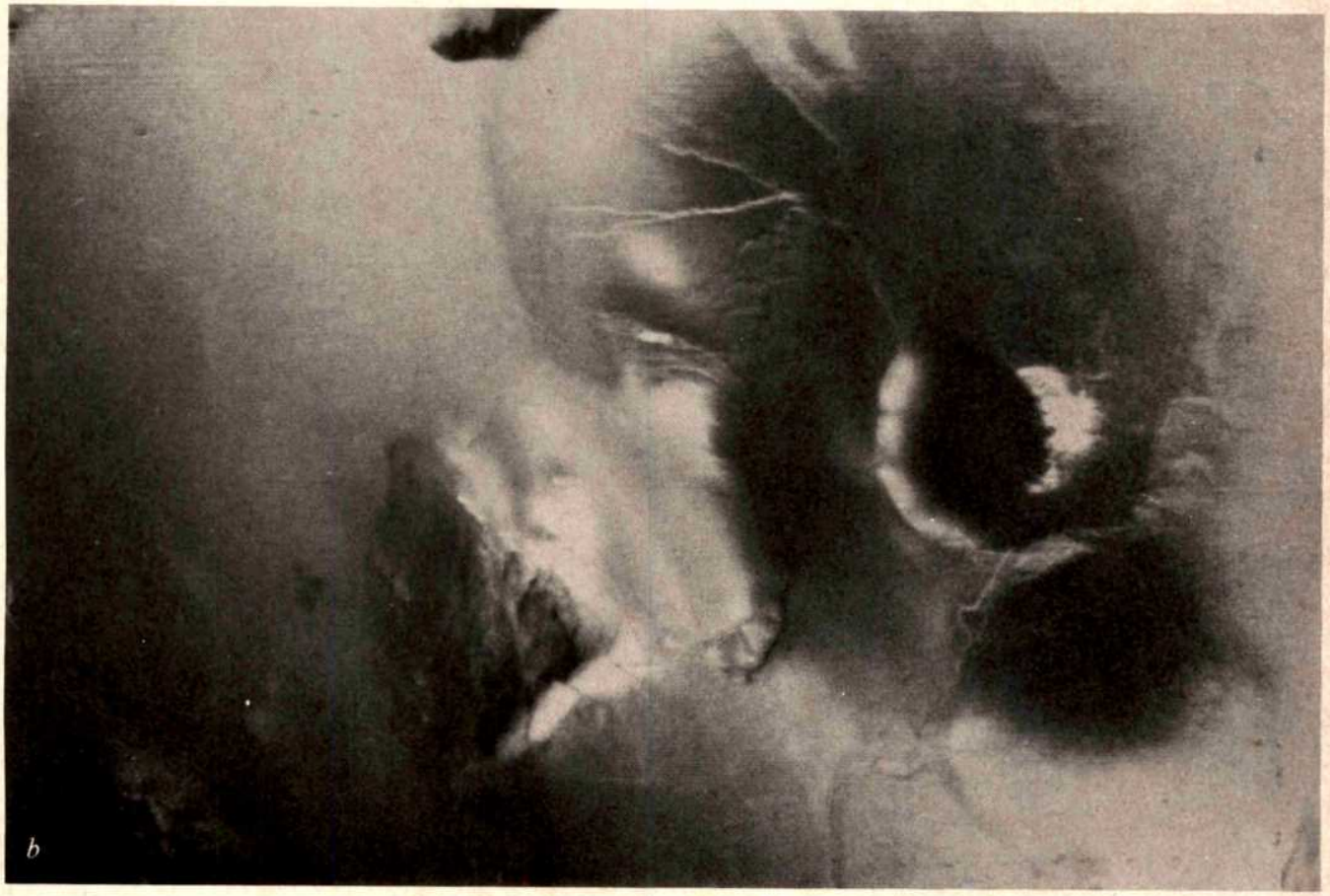
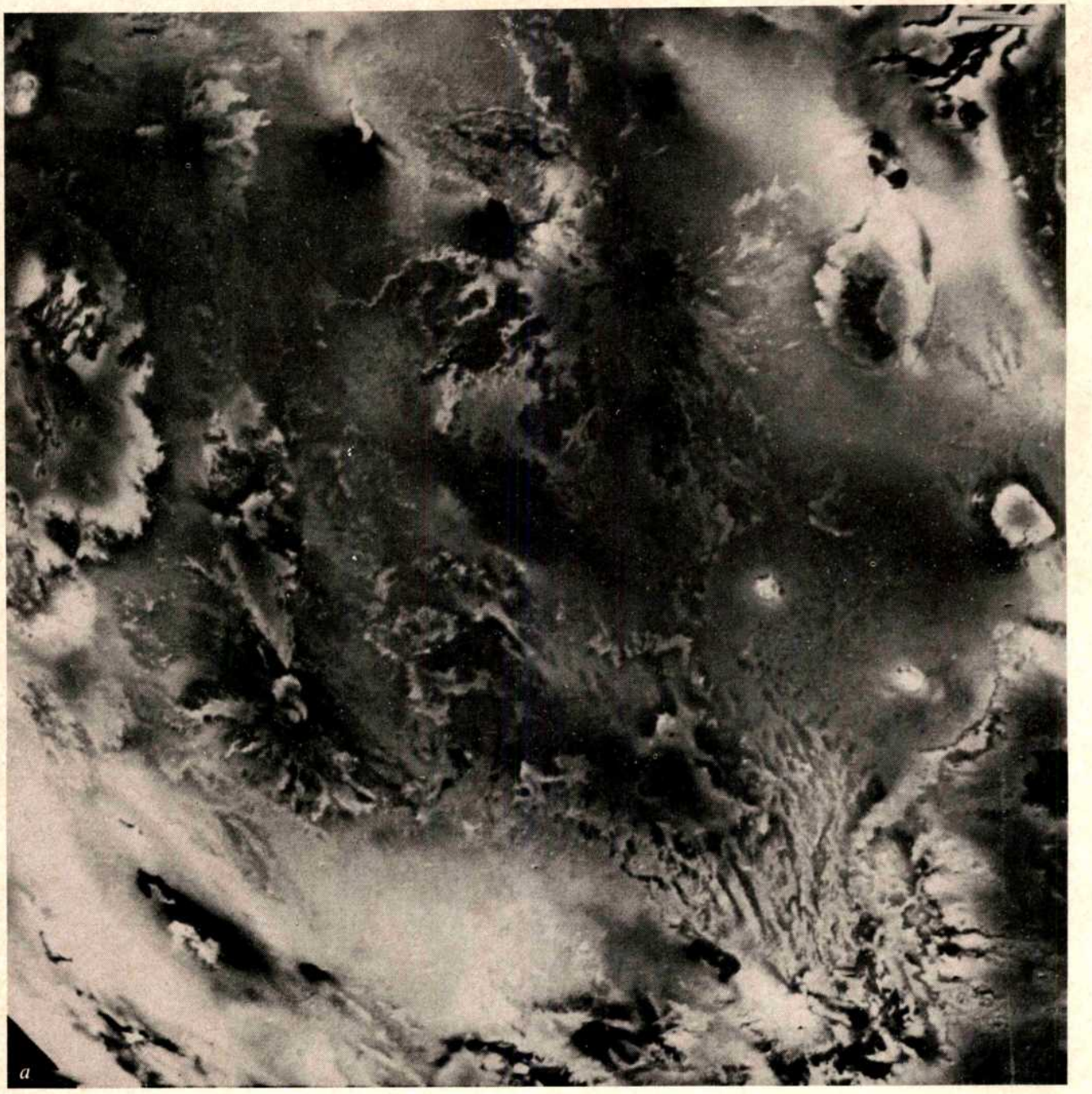


Plate 4



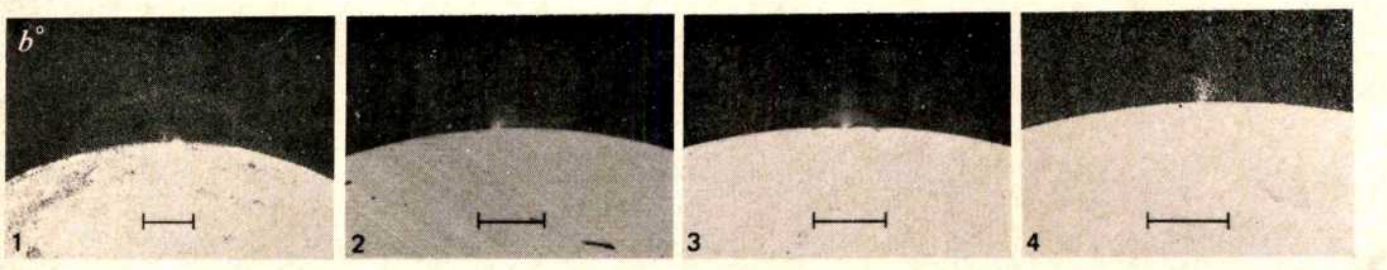
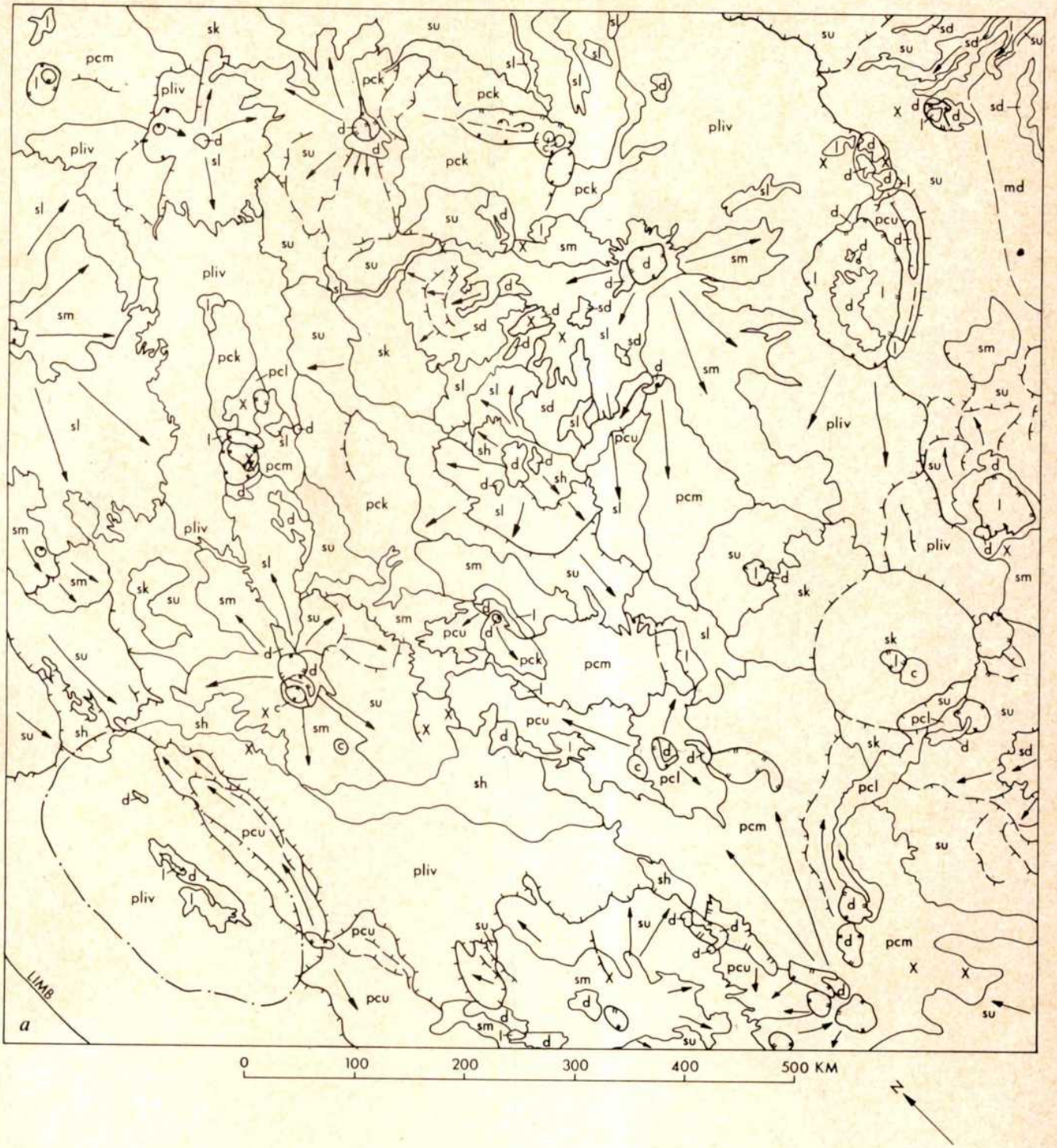


Plate 5


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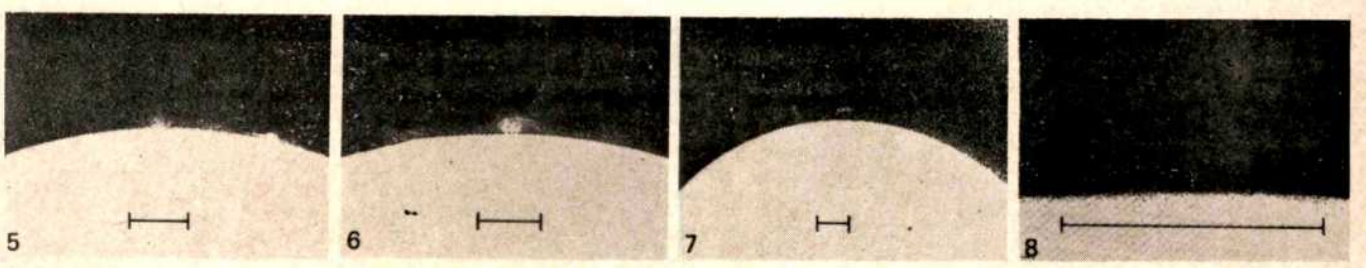

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Plate 5



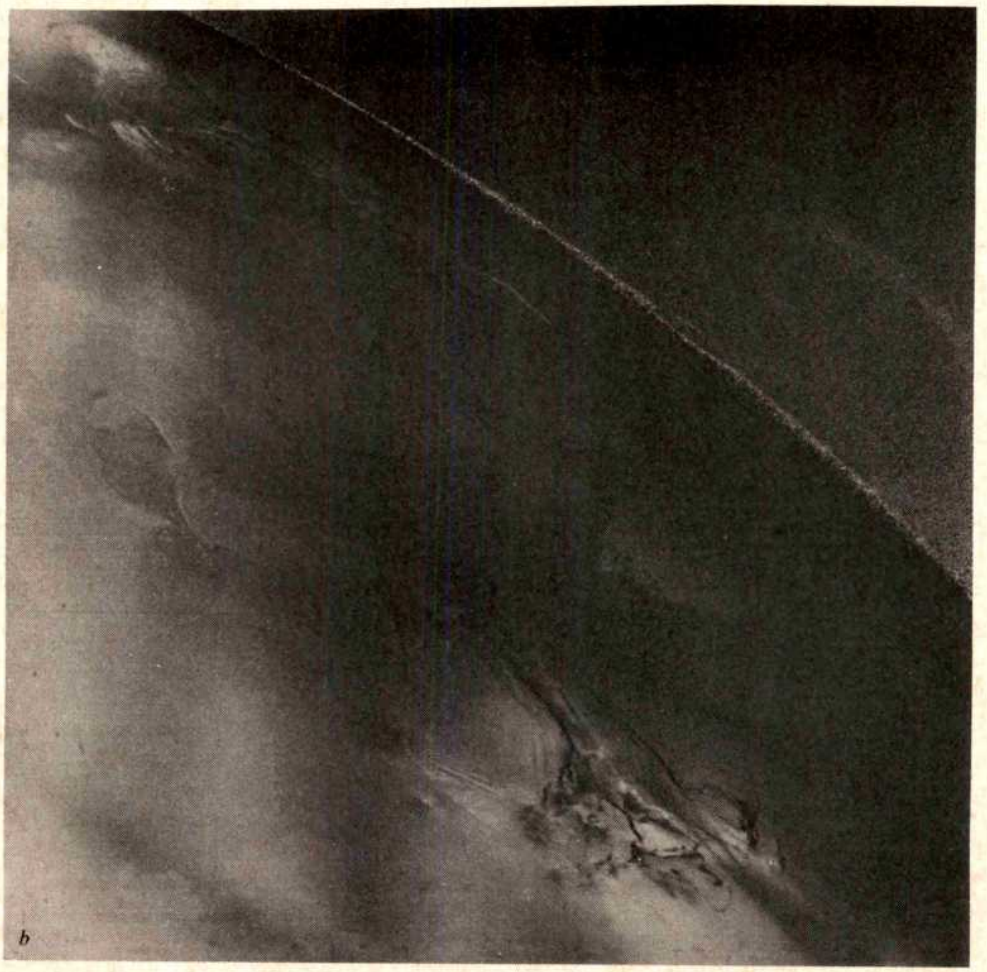
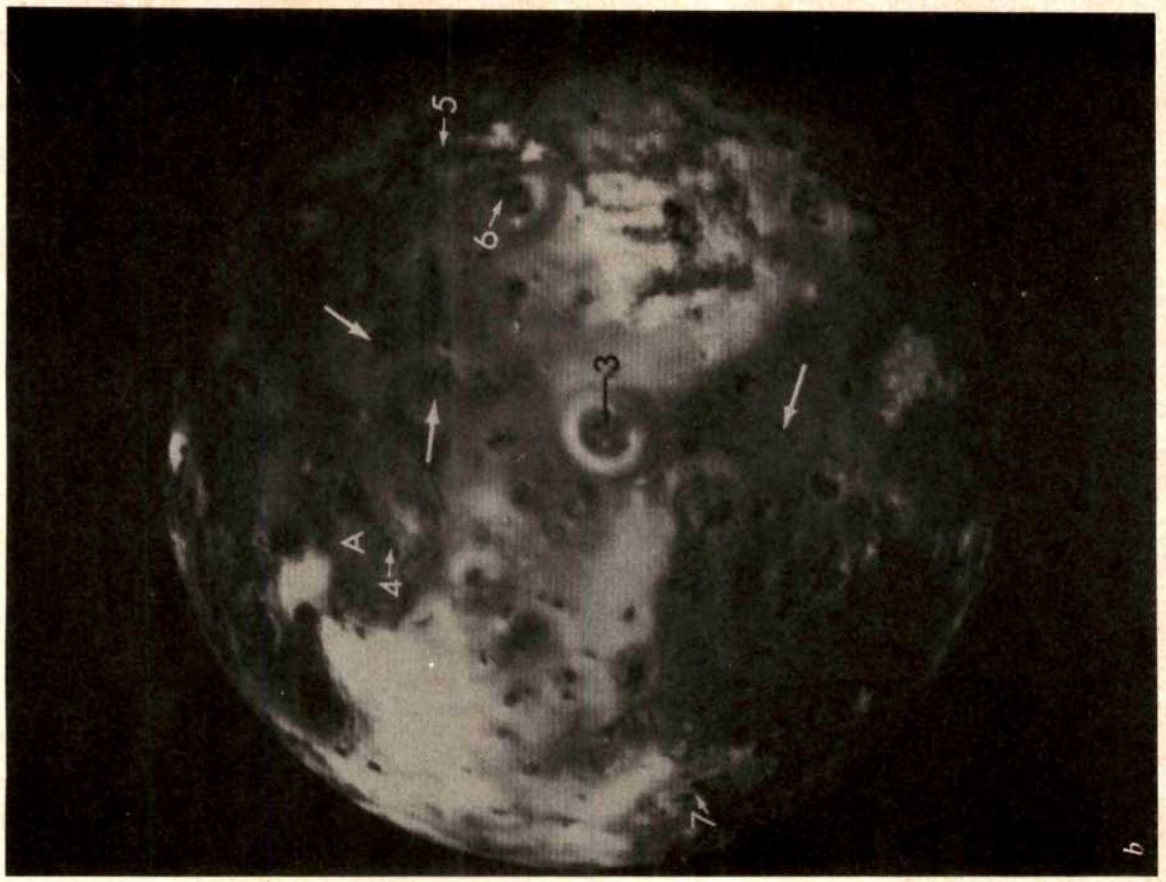


Plate 6



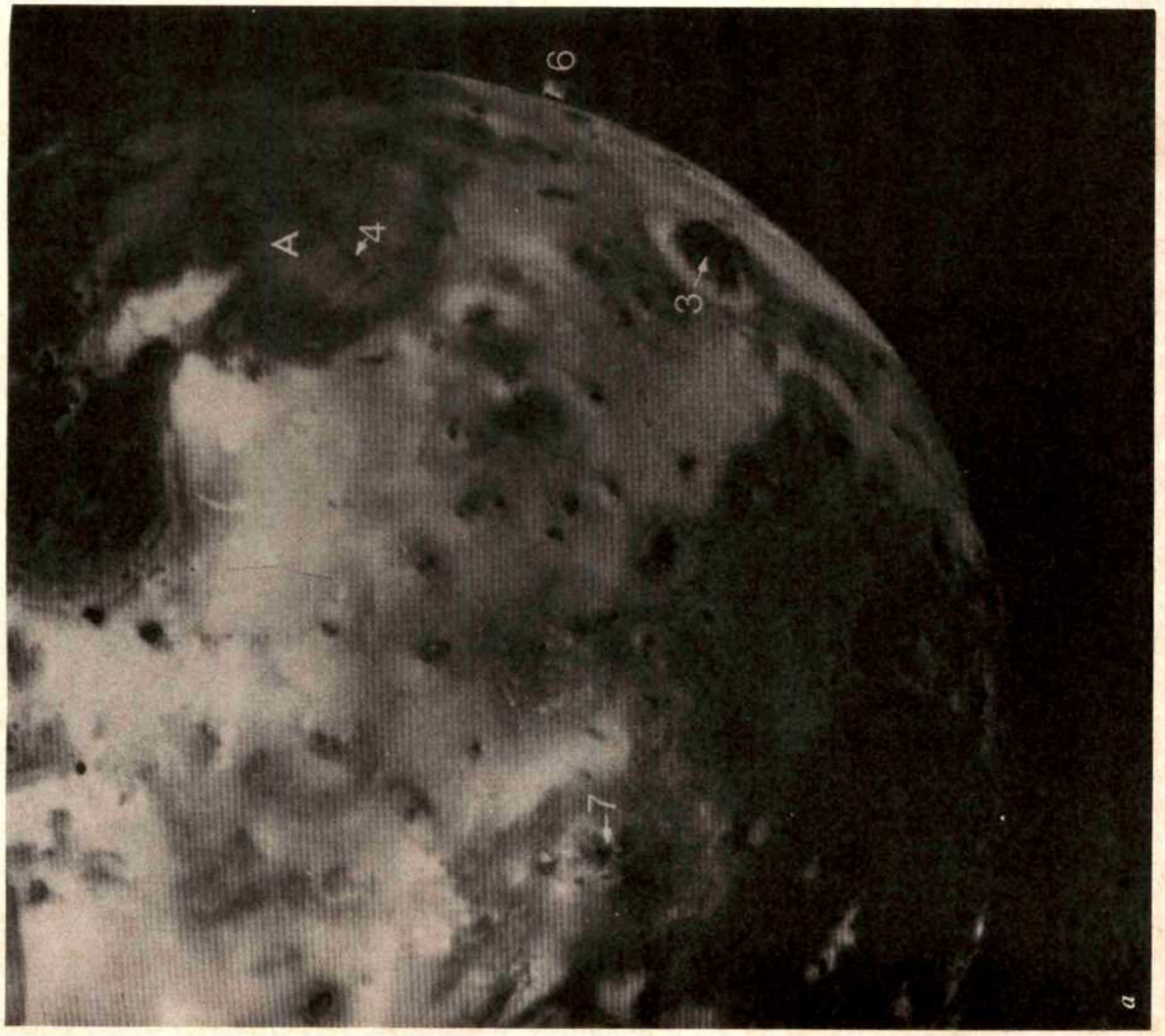


Plate 7

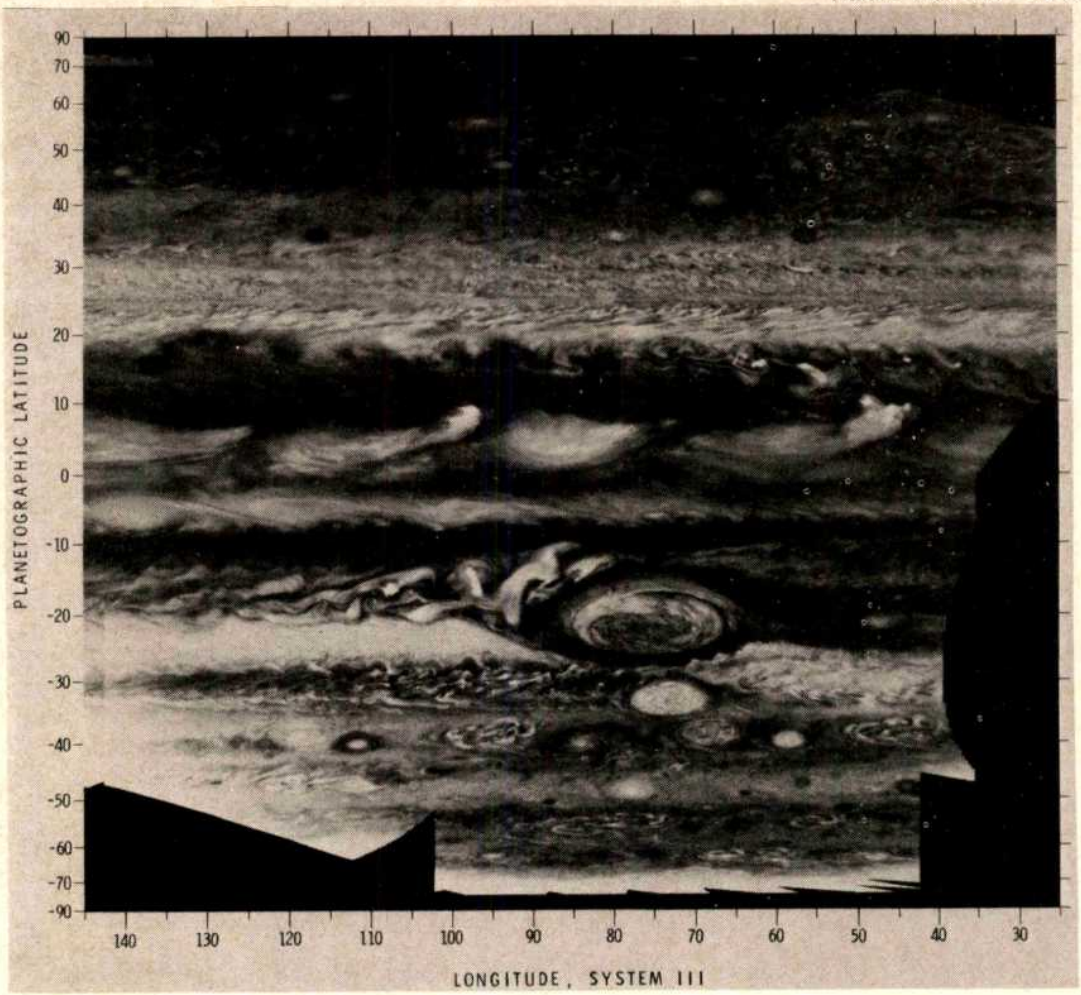


Plate 9

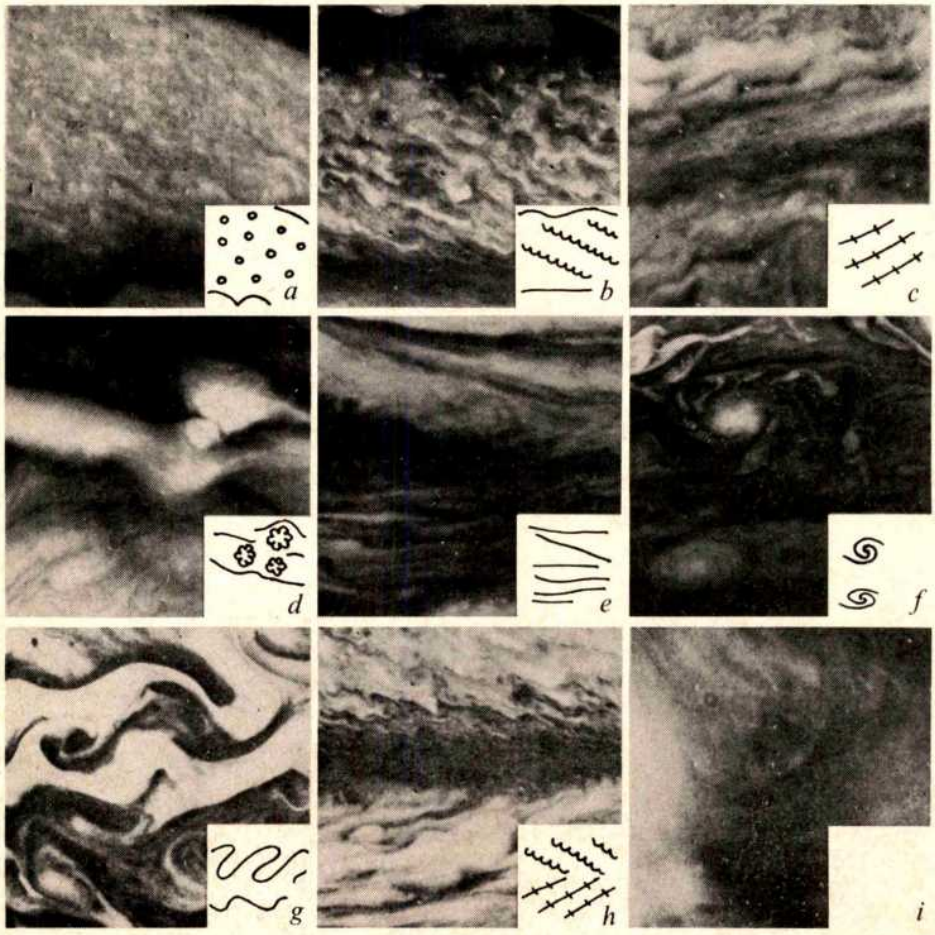


Plate 8

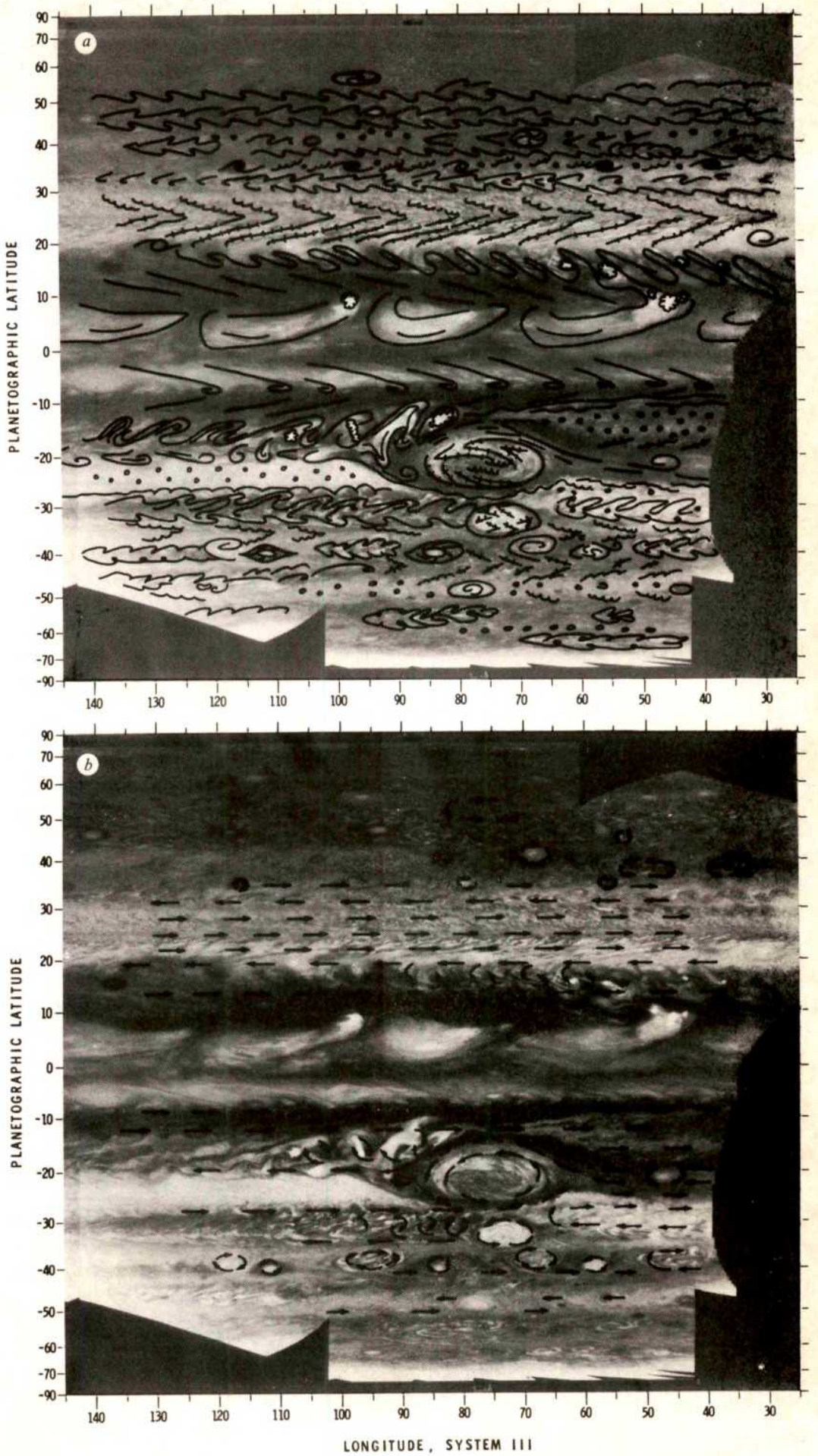

a
b
PLANETOGRAPHIC LATITUDE
PLANETOGRAPHIC LATITUDE
90
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-90
140
130
120
110
100
90
80
70
60
50
40
30
LONGITUDE, SYSTEM III

Plate 10

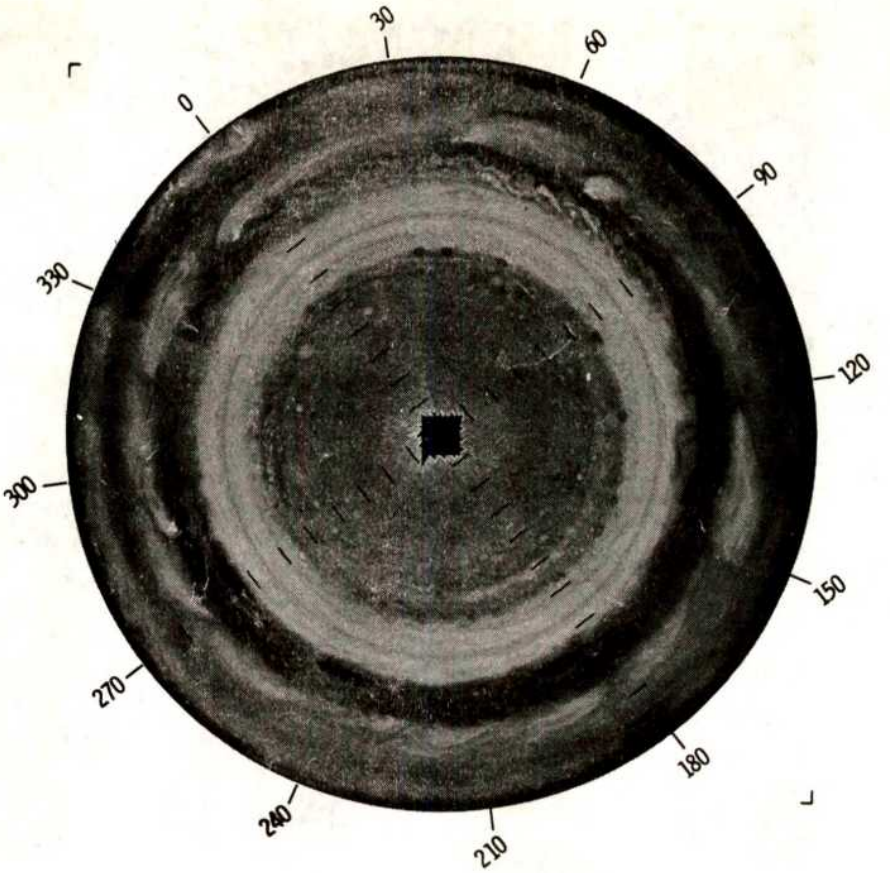


Plate 11

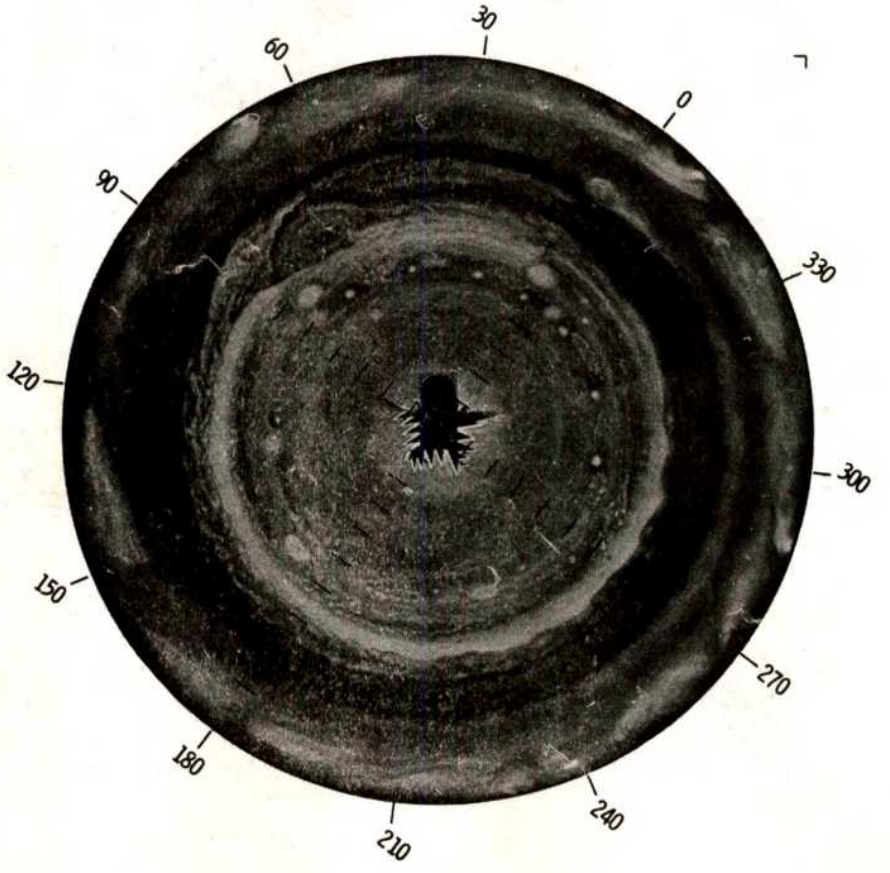


Plate 12

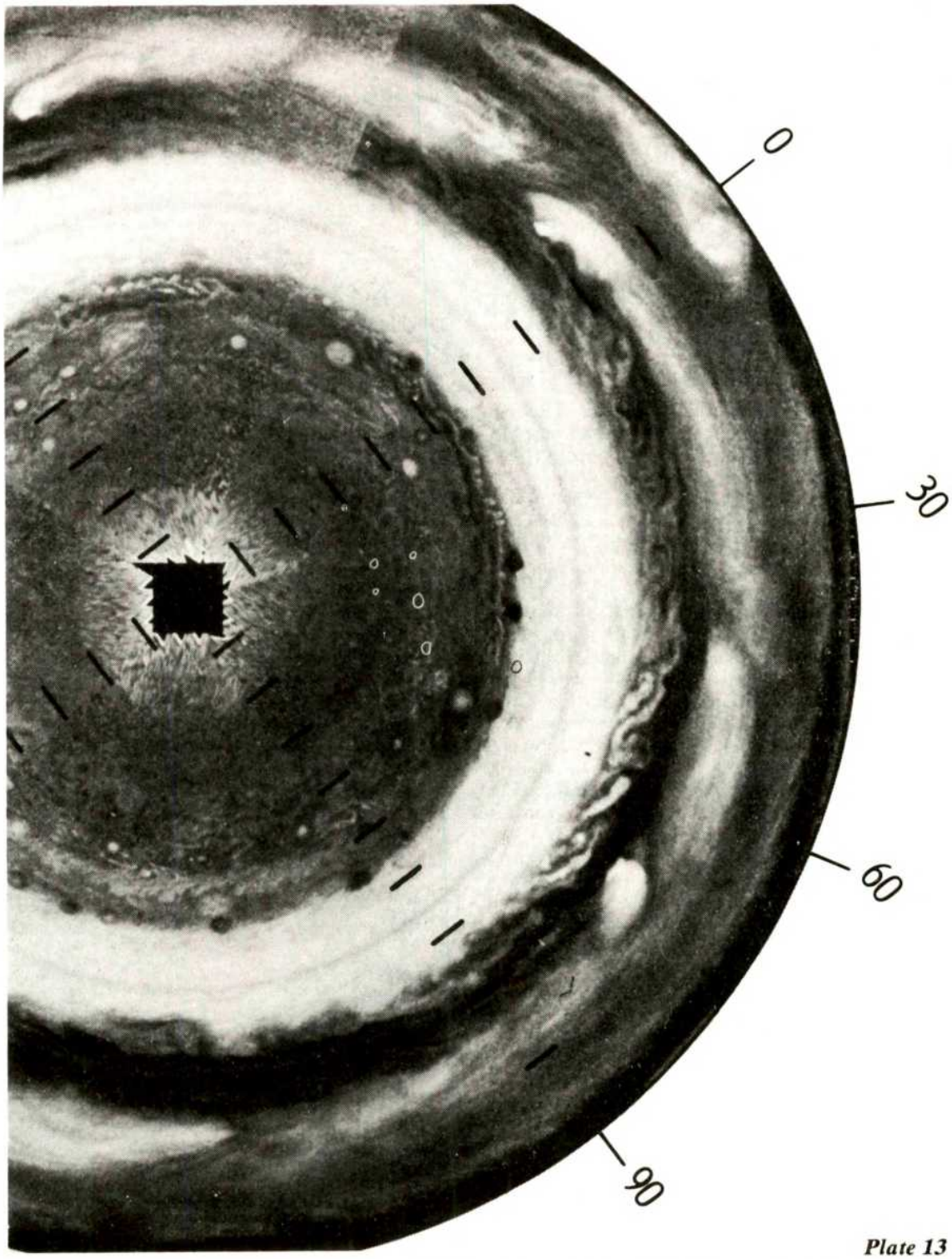


Plate 13

Plate 9 Portion of Jupiter from 25 ° to 145 °W at 08.45 UT, 27 February 1979. This mosaic was made by projecting nine narrow angle frames in the orange filter onto a cylindrical grid. Distance along the ordinate is proportional to distance along the axis of rotation. Latitudes are planetographic, as described in the text. Resolution in the original frames is about 120 km, and is several times poorer when printed.

Plate 10 *a*, Textural map of the portion of Jupiter covered in the mosaic of *Plate 9* using the symbols defined in *Plate 8a*. Textural types generally run parallel to the large-scale bands, but are not uniquely defined by the bands. Linear textures tend to form chevron patterns centred on the zonal jet maxima and on the circumferential flow around the major spots. *b*, A flow diagram indicating observed jovian currents. Arrows indicate only the direction of flow, not its magnitude. The velocity field is overlaid on a cylindrically projected mosaic of nine Voyager narrow-angle frames taken through an orange filter on 27 February 1979.

Plate 11 A polar stereographic map of Jupiter's northern hemisphere made from 27 Voyager narrow-angle images taken over one jovian rotation on 1 February 1979. Longitudes are System III (1965.0), while latitudes are planetographic. Note the motion of cloud features relative to each other when compared with the regional mosaics (*Plate 10*) taken at a later date. Latitude tick marks are located at 10° intervals. The outer circle is located at 10 °S. The Voyager 1 trajectory took the spacecraft on a near equatorial pass by the planet, hence data is missing near the pole.

Plate 12 A polar stereographic map of Jupiter's southern hemisphere made from 27 Voyager narrow-angle images taken on 1 February, 1979. Longitudes are System III (1965.0), while latitudes are planetographic. Again, note the differential motions of features when compared with *Plate 10*. Latitude tick marks are located at 10° intervals. The outer circle is located at 10 °N. The south pole itself was unobservable in Voyager 1's equatorial plane encounter with Jupiter.

Plate 13 First Quadrant of a Polar Stereographic Projection of Jupiter's northern hemisphere on 1 February 1979 with the locations of the lightning flashes from Fig. 1 of Cook *et al.* (p. 794) superimposed. Latitudes are jovigraphic.

First results on jovian lightning

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The geometric reduction of the discovery picture of lightning on the dark side of Jupiter relates the positions of the lightning flashes to visible cloud structure.

THE observations of the dark side of Jupiter were taken at 1979 March 4 d 17 h 52 min 30 s UT (time at the spacecraft) [see Fig. 10 in ref. 1]. A total of 13 stars appeared as double images and were used to establish the direction and orientation of the camera. The lower bound of the aurora establishes the position of Jupiter's limb which matches that found from the best ephemeris of the spacecraft. Longitudes and latitudes of the 20 lightning flashes are plotted in Fig. 1, while adjacent flashes are grouped together.

These positions also appear plotted on the first quadrant of a polar stereographic projection centred on Jupiter's north pole in *Plate 13*, Fig. 2. This projection was made from images obtained on 1 February 1979 at the spacecraft. We believe the mechanisms of generation of lightning on Jupiter and Earth may be similar. The most plausible mechanism is convective electrification[2]. Convection in the clouds distorts a pre-existing background charge distribution and thus collects the charges into a distribution which is discharged by lightning strokes. This mechanism requires a conductor some distance beneath the clouds. On Earth this is often the solid planet itself. However, the surface and a sufficiently conducting layer of cloud-free air will serve as well, as in the case of thunderstorms above warm fronts. This situation may also apply to the atmosphere of Venus in which lightning has also been detected[3] and where the atmosphere is clear of clouds in the lowest 35 km. The extension of this concept to Jupiter requires a sufficiently conducting atmosphere below the observed clouds, which may include a cloud-free region.

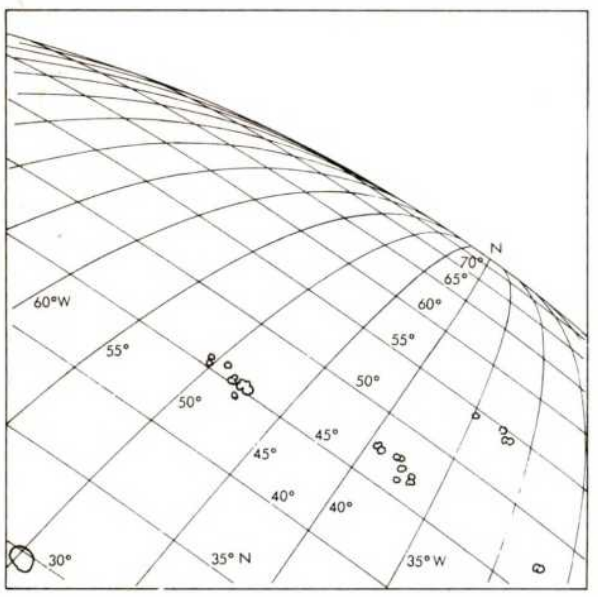


Fig. 1 The arrangement in the television field of Jupiter and the flashes of lightning. Latitudes are jovicentric.

The association of thunderstorms with upwelling in regional circulations is well known on Earth and also occurs on Jupiter. Consequently, observations of lightning may be used as a tracer of upwelling in the jovian circulation. The observations in *Plate 13* indicate that these lightning flashes are concentrated in certain jovian latitudes, and atmospheric features which are located at the two lower latitudes (+30°, +46° jovicentric; +33°, +50° jovigraphic) are cloud structures in the form of bands between herringbone patterns of varying degrees of distinctness. In our case, at the lower latitude the lightning is associated with a northern branch of this band which can be double or triple but rejoined at another longitude. These bands are associated with unstable westward moving jets[4]. In the northern hemisphere (Fig. 2 in ref. 4) these jets occur at latitudes of 18°, 30° (with northward and southward branches) 36°, 45°, 50° and possibly by extrapolation, 54° and 58°N. At the time of these observations, lightning appeared at the second, fourth and sixth of these jets and not at the third and fifth which were also in view. This comparison leads us to suggest that lightning is a tracer of upwelling in the jovian atmosphere. The observation of these lightning flashes at high latitudes is consistent with the concept of internal heating generating convective cloud systems. Further support for this idea must await analysis of images from Voyager 1 and Voyager 2. Note that the detection of whistlers from Jupiter by the plasma wave instrument indicates the presence of lightning in the polar regions[5].

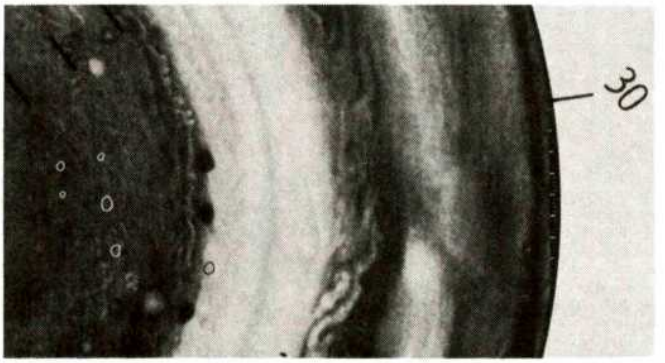


Fig. 2 Locations of lightning flashes in Jupiter's northern hemisphere (from *Plate 13*, p. 793).

These results were made possible by all those who have worked in the Voyager project. Many of these have been individually acknowledged in ref. 1. We especially thank C. C. Avis, G. W. Garneau, P. L. Jepsen, J. J. Lorre, J. A. Mosher, G. M. Yagi, J. T. Harwood, L. Pieri and C. Reslock of JPL, and S. A. Collins as experiment representative at JPL. This paper reports one phase of research supported by NASA at JPL under contract NAS 7-100 and of research supported by JPL at the Smithsonian Astrophysical Observatory under that NASA contract. G.E.H. was supported by the SRC.

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An interpretation of the Voyager measurement of jovian electron density profiles

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The large vertical extent and diurnal variation in the electron density profile of Jupiter observed in radio occultation by Voyager 1 imply a homopause eddy diffusion coefficient of 1–3 × 10^5 cm^2 s^{-1} and an exospheric temperature of about 1,300 K.

THE Voyager 1 radio science experiment (RSS) obtained electron density profiles for the daytime and night-time ionosphere of Jupiter by radio occultation observations on 5 March 1979[1]. The properties of the ionosphere were different from those obtained by the Pioneer 10 and 11 observations[2,3] in December 1973 and December 1974, in several important ways. (1) The vertical extent of the ionosphere in 1979 was about 6,000 km, compared to 3,500–4,000 km at the time of the Pioneer passages. (2) The peak in the daytime electron density occurred about 1,600 km above the reference pressure level (1 mbar) in 1979, compared to ~1,000 km above that level in 1973–74. (3) The scale height of the topside ionosphere in the daytime changes from about 590 km just above the peak to about 960 km at higher altitudes. In the night-time, there is a single topside scale height of 960 km corresponding to the high altitude daytime scale height. (4) Finally, the maximum electron density is a factor of 10 lower on the nightside than it is on the dayside. In the case of the Pioneer observations, only small variations were observed between daytime and night-time ionospheric profiles, and very small differences were evident as a function of latitude (20° N, 26° N, 58° N, and 79° S)[3]. The Pioneer 10 and 11 encounters with Jupiter occurred near solar minimum, while the Voyager 1 encounter was near the solar maximum. Here we attempt to account for the differences between the Pioneer and Voyager observations on the basis of solar cycle changes in solar flux, drastic decrease in the eddy diffusion coefficient in the upper atmosphere and a large increase in the exospheric temperature of Jupiter.

Atreya and Donahue[4] succeeded in accounting for the principal features in the Pioneer 10 and 11 ionospheric profiles by assuming a temperature of 1,000 K for the upper atmosphere and invoking radiative recombination of H^+ as the dominant ion loss mechanism in the principal ionospheric layer. Here we argue, first, that the temperature has increased to 1,300 K in 1979. This neutral temperature corresponds to the plasma scale height of 960 km which was measured at 3,500 km above the reference level on the dayside and at 2,300 km on the nightside. A lower temperature (1,100 K) was quoted by Eshleman *et al.*[1] This was the consequence of using the plasma scale height at 6,000 km. (G. F. Lindal, personal communication). However, photochemical time constants are small compared to diffusion times only below about 3,500 km, and, hence, we have chosen to calculate the temperatures at lower altitudes. Nagy *et al.*[5] have shown that approximate thermal equilibrium between the plasma and neutral gas occurs at this altitude. The difference between neutral, electron and ion temperature should be at most a few hundred degrees and has only a small effect on our results.

We have varied the eddy diffusion coefficient at the homopause from 3 × 10^7 cm^2 s^{-1} which was appropriate for Pioneer 10 and 11 conditions[4] to 8 × 10^4 cm^2 s^{-1} in order to ascertain the effect of a change in that atmospheric parameter on the ion density profiles.

We have also taken into account the variation with temperature of rate constants. The rate constant for radiative recombination of H^+ and electrons varies with electron temperature[6,7] between $T_e^{-0.5}$ and $T_e^{-0.75}$. A reaction discussed by McElroy[8]

$$H^+ + H_2(v' \geq 4) \rightarrow H_2^+ + H \quad (1)$$

becomes much more important at Voyager temperatures than those of Pioneer 10 and 11. Followed by

$$H_2^+ + H_2 \rightarrow H_3^+ + H \quad (2)$$

$$H_3^+ + e \rightarrow H_2 + H \quad (3)$$

Reaction 1 can be a very significant loss process for H^+ ions in the high temperature conditions encountered by Voyager.

Finally, extreme UV solar flux values appropriate to the conditions of the Voyager encounter were taken from those measured by Hinteregger (personal communication).

We have found that it is possible to reproduce the measured electron density profiles by using a model atmosphere appropriate to an exospheric temperature of 1,300 K with temperature varying above the homopause as in our previous calculations[4] and with an eddy diffusion coefficient K of (1–3) × 10^5 cm^2 s^{-1} at the homopause. K varies with altitude inversely as the square root of the atmospheric number density above the tropopause. Its value at the tropopause was adjusted until an electron density profile matching the observed one was obtained. For example, in a model in which the value of the H_2 density at the homopause was 7 × 10^{13} cm^{-3} and K at that altitude was 1.5 × 10^5 cm^2 s^{-1}, the daytime maximum electron density of 2 × 10^5 cm^{-3} occurred where the H_2 density was 3 × 10^{11} cm^{-3}, 1,500 km above the 1-mbar level. (The observed peak was at 1,600 km.) The hydrogen density at the ionospheric maximum is somewhat higher than it was for the conditions of the Pioneer encounters[4] when the thermosphere was cooler and the solar flux was weaker. Given the present state of uncertainty in measured altitudes and plasma profiles, it is probably safe to take K as lying somewhere between 1 and 3 × 10^5 cm^2 s^{-1} at the homopause.

The rate of ionisation during the daytime is 26 protons cm^{-3} s^{-1} at the maximum. This is a full order of magnitude above the rate required to account for the ionosphere observed by the Pioneers. It is partly the consequence of the 2.5-fold increase in ionising solar flux that has occurred since the last solar minimum (H. E. Hinteregger, personal communication). It is also a result of the fact that an increase in atomic hydrogen column abundance in the upper atmosphere of Jupiter by a factor of 100 since 1973–74 is implied by the very large enhancement of jovian Lyα radiation detected by the UVS[9] and IUE (H. W. Moos and S.K.A. unpublished) observations at the time of Voyager encounter.

The time constant for loss of H^+ by radiative recombination is substantially greater than the length of a jovian night. This circumstance led to the small diurnal variations observed by Pioneers 10 and 11[4]. We propose that the diurnal variation now

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observed leading to the tenfold enhancement in the maximum electron density, and the dual topside scale height during the day is a consequence of the enhanced importance of reaction (1) when the temperature in the upper atmosphere is 1,300 K in place of 850–1,000 K. In order to reproduce the observed diurnal variation, the loss frequency for H^+ in charge exchange with H_2 should be $1.3\times10^{-4}\ s^{-1}$, leading to an overall rate constant for reaction (1) of $4.3\times10^{-16}\ cm^3\ s^{-1}$. Reaction (1) is endothermic in the ground vibrational state by 1.8 eV for H^+ at 300 K[10]. The rate constant for H_2 excited to the fourth vibrational level and higher at a temperature of 1,300 K would have to be $4\times10^{-9}\ cm^3\ s^{-1}$ to produce the required overall loss rate. This value would be reduced by about a factor of two if we take into account the high velocity of the H^+ ions at 1,300 K. Because of the rapid variation of the Boltzmann factor with temperature, a small increase in vibrational temperature would also lead to a sizeable relaxation in the value required for this rate constant. On the other hand, if the temperature should be 850 K as it apparently was during the Pioneer 10 and 11 observations, the rate constant for the reaction between H^+ and H_2 excited levels higher than $v=4$ would have to be of the order of $10^{-5}\ cm^3\ s^{-1}$ to produce a similar reduction in ion density.

The photochemical time constants are short compared with a jovian day up to an altitude where the dayside electron density is about $2\times10^4\ cm^{-3}$. Hence, after sunset the electron density near the daytime peak will rapidly decay. The night-time ionospheric maximum will attain a density of $\sim10^4\ cm^{-3}$ and a single topside scale height corresponding to the upper part of the daytime profile. The nightside ionosphere is the dayside ionosphere with its low altitude bulge dissolved.

The ionosphere observed by Voyager 1 seems to imply the existence of a jovian exospheric temperature at 1,300 K and an eddy diffusion coefficient between 1 and $3\times10^5\ cm^2\ s^{-1}$ at the homopause. This high temperature and low value of K compared with Pioneer conditions are also implied by the great enhancement in Lyα radiation observed by the Voyager UVS, and IUE, and the fact that the UVS failed to detect the He 584 Å radiation from the jovian disk. If the volume mixing ratio of He is 0.11 as reported by the IR spectroscopy and radiometry investigation[11], the failure of the helium resonance radiation to be observed by the UVS implies the presence of a large amount of hydrogen above the homopause and a value for K near the homopause of the order of $10^5\ cm^2\ s^{-1}$. Although the rate constant we have calculated for reaction (1) seems reasonable, it will be highly desirable to measure it in the laboratory in jovian conditions.

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Plasma wave turbulence at Jupiter's bow shock

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The first measurements of the wave–particle interactions of Jupiter's bow shock are reported, and some of the wave phenomena detected during the inbound passage are discussed.

THE characteristics of a planetary bow shock are important because the plasma that impacts the magnetosphere of a planet represents solar wind that has been drastically modified by the shock process. Plasma instabilities that develop at a shock front are associated with significant acceleration processes that yield non-maxwellian distributions in the downstream magnetosheath as well as significant fluxes of suprathermal ions and electrons in the upstream region. The Voyager 1 plasma wave investigation has provided the first opportunity to study directly the wave–particle interactions of an outer planet bow shock. Here we discuss in detail some of the wave phenomena detected during the inbound passage.

Measurements

The initial report on the Voyager wave data[1] contains a brief summary of the inbound shock measurements with a display of the 16-channel spectrum analyser data from the first bow shock crossing (detected at 14.34 spacecraft time, 28 February 1979, when the spacecraft was at a range of 85 R_J). Figure 1 shows a corresponding plot of the 16-channel wave data for the third shock crossing of 12.27 UT on 1 March, detected when Voyager 1 was at a range of 72 R_J. We focus attention here on this third inbound crossing for which we have the most comprehensive data records.

Before 12.27, Fig. 1 shows that electrons produced steady oscillations in the 5.62 kHz channel, corresponding to an upstream density of ~0.4 electrons cm^{-3}. The high frequency upstream wave amplitudes were almost an order of magnitude higher than those detected in the corresponding region the day before, but in the low-frequency channels where ion acoustic waves and whistlers are generally detected, the 1 March bow shock exhibited a noteworthy absence of precursor wave activity. (The sporadic signals in the 100-Hz channel are interference tones from the stepper motor of the low energy charged particle instrument.) This third shock crossing was characterised by the detection of intense broadbanded noise enhancements in a well defined time interval with duration of the order of 1 min. Analyses of collisionless shock structures[2] suggest that the minimum shock thickness for a laminar structure should be of the order of $\delta_i = c/(2\pi f_p^e)$ where c is the speed of light, f_p^e is the electron plasma frequency ($f_p^e = 9\sqrt{N_e}$ kHz where N_e is the electron density in cm^{-3}) and $f_p^i = f_p^e/43$ (for protons). For our

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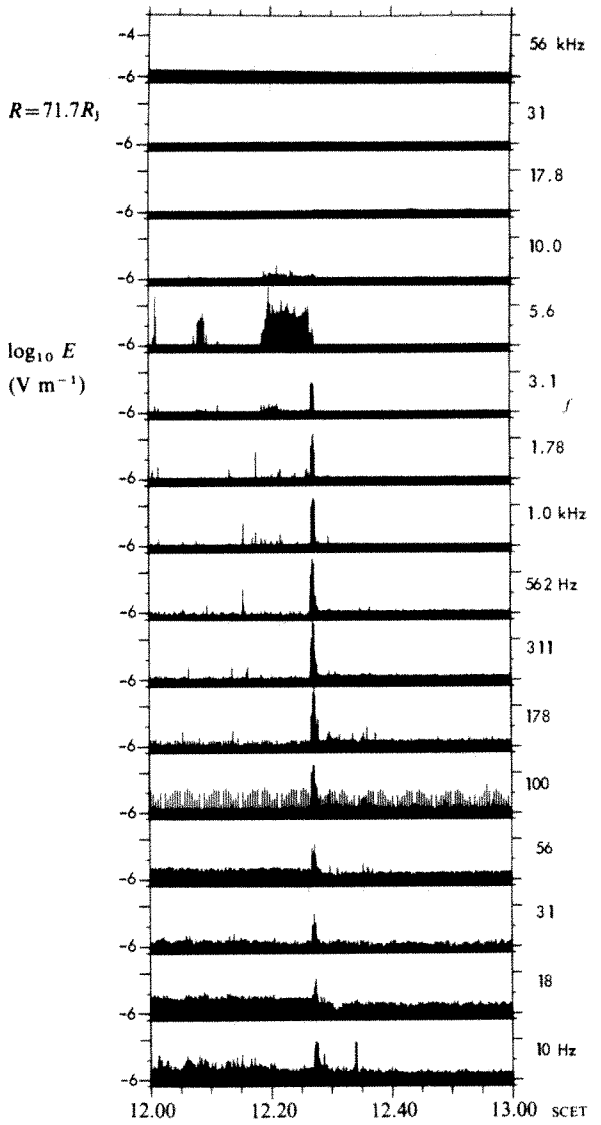


Fig. 1 Measurements of wave amplitudes at the third bow shock crossing. Interference appears in the 100 Hz channel, and the upstream enhancements at 5.6 kHz represents detection of electron plasma oscillations. Note the very low noise levels in the magnetosheath after 12.27.

case we know that $f_p^e = 5.6$ kHz, and hence δ_e ($=\delta_i/43$) should be ~8.5 km while δ_i ~365 km. At this time the Voyager radial speed with respect to the planet centre was ~12.7 km s^{-1}, and hence the spacecraft would have traversed a stationary minimum shock with thickness of 8.5 km in ~2 s. The observed duration of about 20–60 s seems to imply that the thickness corresponded to an ion inertial length.

At the time of this shock crossing we were fortunate to have high rate waveform data available that provided complete spectral information over the range 50 to 12 kHz. Figure 2 shows the frequency–time plot made up using the high rate data from 1226.12 to 1227.48 spacecraft time. (A Voyager-to-Earth data transmission of just over 11 Mbits was used to provide the

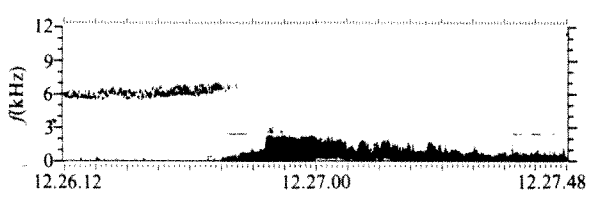


Fig. 2 Frequency–time diagram of bow shock number three made up using the Voyager 1 high rate data link. The upstream electron plasma oscillation line is highly structured just before the shock encounter.

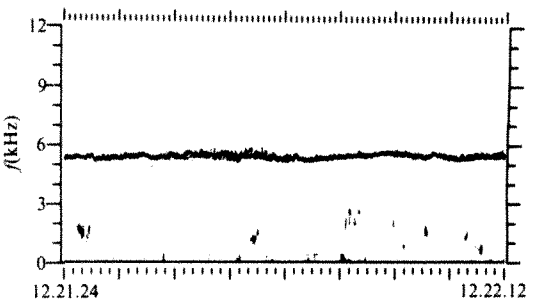


Fig. 3 Frequency–time diagram of regular upstream electron plasma oscillations detected a few minutes before the shock.

plasma wave information in Fig. 2.) The thick structured 'line' with $f \simeq 6$ kHz (before 12.26.45) represents the upstream electron plasma oscillation emission, and we identify the low frequency wave turbulence after 12.26.42 as ion acoustic waves. The upstream electron emission just before this shock has a distinct and unusual structure that may be related to a nonlinear effect operative very near to the shock. Figure 3 shows a frequency–time plot made up just a few minutes earlier with very narrow and unstructured electron plasma oscillation lines, and it is possible that the structure shown in Fig. 2 is related to the coupling of large amplitude electron plasma oscillations and ion acoustic waves[3]. Similar phenomena have occasionally been detected in the region immediately upstream from the Earth's bow shock[4]. However, as Fig. 2 does not show enhanced ion sound wave levels just before 12.26.42, other explanations for the high frequency wave structure may have to be considered. On the other hand, the broadband channel uses an automatic gain control amplifier that responds primarily to the most intense waves, and the bandpass channel data (Fig. 1) show that the upstream ion waves in the Fig. 2 time interval were at least as intense as the ones shown in Fig. 3.

Figure 2 shows several other features of interest. First we note that the plasma wave activity was very low by the end of the record. This result is also indicated in Fig. 1, which shows that the jovian magnetosheath is characterised by a virtual absence of detectable plasma wave turbulence after passage through the shock. (Scarf *et al.*[1] noted that this is true for the entire sheath traversal into the magnetosphere boundary.) Figure 2 also shows a number of impulsive wave structures within the shock with durations as small as 1, 2 or 3 s (see especially the period after 12.27.06) and it is possible that these structures represent regions with scale sizes comparable to $\delta_e = c/(2\pi f_p^e)$.

Clearly the high frequency and time resolution available from the broadband data link will allow us to carry out detailed studies of the thermalisation and acceleration processes that develop at the very strong jovian bow shocks, and future studies will focus attention on the shock structure and on correlations with data from other Voyager instruments. Recent detailed calculations of shock structure based on the use of marginal stability criteria[5] will be applied here when the complete plasma and magnetic field profiles are available. However, it is already apparent that Jupiter's bow shock region provides material for novel and significant plasma physics studies and future research will also be aimed at understanding the absence of magnetosheath noise, as well as the structured electron plasma emissions detected just upstream from the shock.

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In situ identification of various ionic species in Jupiter's magnetosphere

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Positive ions with various mass per charge values up to ~160 have been identified within 20 jovian radii of Jupiter from the analysis of data from the plasma science experiment on Voyager 1.

ONE of the major surprises from the Voyager 1 encounter with Jupiter in March 1979 was the discovery[1] of active volcanoes on Io. This result emphasises the importance of Io as the principal source of the bulk plasma in the jovian magnetosphere[2,3]. The plasma science experiment[4] on Voyager 1 has made the first detailed *in situ* measurements of the plasma (10–5,950 V) near Jupiter[2]. Continuing analysis of these measurements for the ionic composition of this plasma has revealed the existence of further atomic and molecular ions as minor constituents of the plasma. We have now identified ions with mass per charge (A/Z^*) values of 1, 8, 10–2/3, 16, 23, 32, 64, ~104 and ~160 within 20 jovian radii, R_J, of Jupiter in the dayside magnetosphere.

The measurements used in the preliminary analysis reported here were taken in the high resolution mode (M mode) which has ~3.6% resolution in energy per charge between 10 and 5,950 V. The energy per charge scan effectively becomes a mass per charge scan whenever all ionic species have a common component of velocity into any sensor; this common component of velocity as well as the spacecraft potential are parameters of the present analysis. (The instrument contains four independent sensors[4] A, B, C and D, looking in different directions[5].) The measurements are usually displayed as relative distribution function versus energy per charge; the relative distribution function is the measured current divided by the energy per charge width of the particular measurement step. Since the step widths increase with increasing energy per charge, a constant background will result in a smaller value of the distribution function at higher values of energy per charge.

Significant heavy ion abundances are found beyond the inner magnetosphere where ions with $A/Z^* = 8$, 16, 32 and 64 were reported by Bridge *et al.*[2]. For example, at 19.6 R_J, the energy/charge spectrum (Fig. 1) shows several distinct peaks.

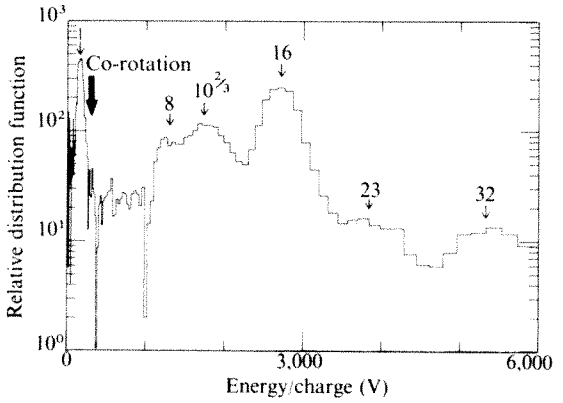


Fig. 1 D-sensor energy per charge spectrum obtained on day 63 at 15.50 UT when the spacecraft was at 19.6 R_J and a magnetic latitude of −8.5°. The heavy arrow labelled co-rotation marks the expected location of a proton peak moving with the geometrically expected co-rotation velocity. The light arrows mark the locations of various A/Z^* values assuming the lowest peak is a proton peak.

This spectrum is typical of the spectra for ~15 min about this time. Each spectrum is analysed with a simultaneous fit to a sum of convected isotropic maxwellian distribution functions with a common component of velocity; the sum is over peaks at $A/Z^* = 1$, 8, 10–2/3, 16, 23 and 32. The analysis of this spectrum gives a common component of velocity of $\simeq$180 km s^{-1} and a spacecraft potential of less than ±5 V. The common component of velocity is not identical to the value of $\simeq$240 km s^{-1} expected geometrically from co-rotation; this difference and related measurements are discussed by McNutt *et al.*[5]. The mass/charge values for the individual peaks obtained from the fit are within half a unit of the indicated numbers. Thus, we can make the following tentative assignments: $A/Z^* = 1$ is H^+; 8 is O^{2+} or S^{4+}; 10–2/3 is S^{3+} or even B^+; 16 is O^+ or S^{2+}; 23 is Na^+; and 32 is S^+ (or $O_2{}^+$?). The peak observed at $\simeq$3.9 kV is most probably sodium and not magnesium or neon. This conclusion is based on the temperatures of the ions derived from the maxwellian fits; within ±20% all ionic species have the same temperature. Under these circumstances, it is possible to produce a peak at $A/Z^* = 23$ by an appropriate combination of Mg^+ and Ne^+. However, the temperature of the 'sodium' peak produced in this way is too high in comparison with temperatures of the other ions. There is a clear absence of a He^+ or He^{2+} peak; a rough upper limit can be put at $n(He^+, He^{2+}) < \frac{1}{15} n(H^+)$. Any signal from $A/Z^* = 64$ would come in at $\simeq$10.8 kV well above our energy per charge scan range. The mass ratio of heavy ions to protons can be computed independently of the actual

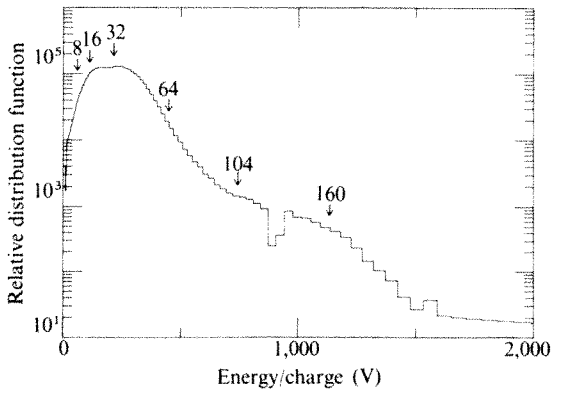


Fig. 2 C sensor energy per charge spectrum obtained on day 64 at 10.00 UT when the spacecraft was at 5.4 R_J and a magnetic latitude of +4.4°. The light arrows mark the locations of various A/Z^* values assuming strict co-rotation. The notch at ≈950 V is caused by interference from another experiment on the spacecraft.

mass and charge of the individual peaks as it depends on $(A/Z^*)^{3/2}$. The heavy ion to proton mass ratio is >95; consequently the mass density is dominated by heavy ions. It is instructive to compare these results with the inner magnetosphere results of ref. 2. First, the $A/Z^* = 23$ peak would have been masked by the prominent $A/Z^* = 32$ peak so that its absence at 5.3 R_J is not significant. Second, the relative abundances of 32 : 16 : 10-2/3 differ markedly; the higher ionisation states are enhanced at the earlier time, greater distance, reported here.

Direct measurement of values of $A/Z^* > 32$ were restricted to regions where the densities are high and the common component of velocity is small. Such is the case in the Io plasma

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torus where the instrument view directions were favourable for the A, B and C sensors[2,5]. Throughout this time protons moving with the geometric co-rotation velocity are below the energy/charge range of the instrument. Typical of the spectra in the torus is the energy/charge spectrum in Fig. 2; this spectrum is from the C sensor but the same results are obtained from the other two sensors (A and B). Compared with Fig. 1, the instrument gain has been reduced by a factor of ~77 in order to avoid saturation. Thus the detection threshold is raised and the curve above ≈1,600 V corresponds to the minimum detectable signal; consequently the graph only extends to 2 kV. The purpose of this figure is to establish the existence of detectable intensities of high mass/charge ions. The signal between ~500 and ~1,400 V shows a broad shoulder well above instrument threshold. We find acceptable fits to this spectrum including peaks at $A/Z^* = 104 \pm 5$ and 160 ± 10 moving with a common component of velocity equal to the co-rotation value; a clean peak at $A/Z^* = 64$ was shown in Fig. 5 of ref. 2, the spectrum at 10.15 UT. The spectrum at 10.15 UT also has a peak at 104 ± 5. These heavy ion peaks are almost certainly molecular ions. The $A/Z^* = 64$ peak could well be either SO_2^+ or S_2^+. The $A/Z^* = 104$ peak is not consistent within the error with ZnS^+ at 96. Speculative assignment for the $A/Z^* = 104 \pm 5$ and $A/Z^* = 160 \pm 10$ peaks may be made based on ions from sulphur salts or polymers which are suggested by observations of the surface of Io.

To conclude: inside 20 R_J: (1) The plasma is composed of ions with mass to charge ratios 1, 8, 10-2/3, 16, 23, 32, 64, ~104, ~160. (2) Wherever both protons and heavy ions are detected, the mass density is dominated by the heavy ions by a factor of ~100. (3) The plasma ions move with a common component of velocity which is not always the value expected geometrically from co-rotation.(4) The ions with $A/Z^* \geq 64$ are probably molecular ions.

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Jupiter's magnetic tail

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Magnetic field observations of the jovian magnetosphere suggest an extended magnetic tail, which has been formed by solar wind interaction with the planetary field.

IN March 1979, Voyager 1 became the third spacecraft to penetrate and study *in situ* the magnetosphere of Jupiter. Earlier observations from Pioneers 10 and 11 in 1973–74 indicated the development of a magnetodisk topology describing the magnetosphere[1,2]. In this model, the combined effects of rapid rotation and a strong planetary magnetic field yield an equatorial region with a considerably enhanced charged particle and plasma population and distended magnetic field lines[3]. Thus, the central region of the magnetodisk effectively carries an azimuthal electrical current and this region is commonly referred to as the current sheet. Controversy arose over whether the current sheet of the magnetodisk was planar and parallel to the magnetic equatorial plane, with a spiraling of the magnetic meridian planes and field lines due to a finite Alfvén speed[4,5], or if the current sheet and magnetodisk were distorted due to the centrifugal forces and deviated or warped so as to become parallel to the jovigraphic equatorial plane at distances greater than 20 R_J (ref. 6). An attempt was also made to interpret the data qualitatively in the framework of a 'magnetic anomaly' model[7], based on the highly asymmetric planetary field[6,8], which did not include an azimuthal current sheet. All these earlier interpretations were based on a planet-centred view of the processes and mechanisms which control the configuration of the outer magnetosphere. We discuss here the Voyager I experimental observations which are most naturally interpreted in terms of a well developed magnetic tail on the nightside of the jovian magnetosphere. This tail, with a 'neutral sheet' separating the upper and lower lobes of opposite field polarity, is formed and controlled by the external forces associated with the solar wind interaction. The inner magnetosphere's current sheet is found to merge with the tail's neutral sheet. This configuration leads to a strong local time control of the outer jovian magnetosphere configuration, in contrast to earlier models which emphasised inner, that is planetary, control.

The preliminary results obtained from the Voyager 1 magnetometer have already been summarised[7]. The Voyager 1 spacecraft entered the jovian magnetosphere at 11.00 LT with magnetopause crossings observed at jovicentric distances between 67 and 47 R_J and left the magnetosphere at 04.00 LT with magnetopause crossings occurring between 153 and 170 R_J. Characteristic current sheet-neutral sheet signatures were observed in the magnetic field data, as the spacecraft passed through the magnetosphere. They showed up as significant decreases in the magnetic field intensity, occurring simultaneous with variations in the direction of the magnetic field. These are interpreted in the inner magnetosphere, $R < 30R_J$, as evidence for a large scale azimuthal current or a current sheet within the magnetosphere.

Within 30 R_J, these magnetically characteristic regions occurred nearly simultaneous with the crossing of the magnetic equatorial plane. Although the field intensity decreased appreciably, it never reached zero, and the variation of field direction was gradual and not consistent with the nearly anti-parallel field directions on opposite sides of the current sheet which were observed at larger radial distances. Beyond 30 R_J, the characteristics of the current sheet crossings were similar to those seen when crossing the neutral sheet in the Earth's magnetic tail: a sharply defined dip in the field intensity, to very small values, while the field direction changed by approximately 180°. Within 80 R_J, there were two sheet crossings every 10 h, although not at a 5 h spacing. Beyond 80 R_J, complete traversals of the current sheet were not observed as evidenced in the field direction. However, very close approaches were made, as seen in the field intensity dips and increased higher frequency fluctuations which occurred with regularity at the 10 h rotation period of the planet. The times of the occurrence of the full traversals merged as the two traversals per rotation period changed to one partial traversal per rotation period.

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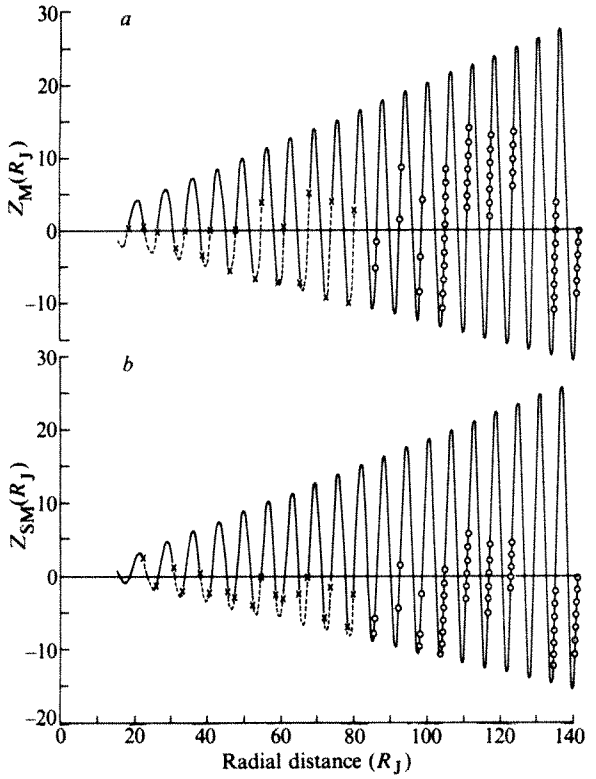


Fig. 1 Position of Voyager 1 spacecraft during exit from Jovian magnetosphere in magnetic dipole (*a*) and solar magnetospheric (SM-*b*) coordinates. SM coordinates are right-handed, non-rotating and defined with x_{SM} axis from planet to sun, z_{sm} axis in plane formed by x_{SM} and M, the magnetic dipole moment of the planetary field. Thus, they rock back and forth about the planet sunline following the magnetic dipole. —— Above NS; - - - below NS; ×, NS; ○, depressed field.

Figure 1 shows the trajectory of the spacecraft outbound from periapsis as observed in both jovian dipole magnetic coordinates (tilt = 9.6° and System III (1965) long = 202°) and solar magnetospheric coordinates. This shows the change from planetocentric control of the occurrence of sheet crossings to solar wind control in the outer magnetosphere. Figure 1*a* shows that within 25 R_J, the current sheet crossings, denoted by crosses, clearly occur nearly coincident with the spacecraft traversal of the magnetic equatorial plane. Contrast this with Fig. 1*b* in which there is no consistency with traversal of the solar magnetospheric equatorial plane. In the outer portion of the Voyager 1 magnetosphere traversal, the magnetic dips, indicated by open circles, are seen to occur during those portions of the trajectory which were near or below the solar magnetospheric equatorial plane. The dips were sometimes observed as extended periods of reduced and variable field strength. Such intervals are shown by sequences of open circles. The conclusion from this figure is that the magnetospheric structure is characterised by a transition from a magnetic equatorial current sheet to the neutral sheet of the tail which is approximately parallel to the solar magnetospheric X–Y plane.

Earlier studies of the Pioneer 10 outbound trajectory had primarily emphasised the times of crossing or penetration of the current sheet[2,3]. In order to understand the magnetic field observation correctly, however, it is also important to consider properly the configuration of the magnetic field. In order to study these Voyager 1 data further, we transform the vector observations into solar magnetosphere coordinates. Figure 2 presents a projection of the hourly averaged X–Y and Y–Z components in both the X–Y and Y–Z planes along the trajectory of Voyager 1. The position of the magnetopause is included in the Fig. 2*a* and was derived by matching the mid points of the inbound and outbound regions of multiple crossings. The length of the field vectors was scaled logarithmically as $K(1+\log B_{ij})$ where $ij = xy$ or yz, with representative values of 1 and 100 nT illustrated. The periodic traversal of the current sheet within 80 R_J is immediately evident in the alternating direction of vectors in the X–Y projection. Those vectors with a positive X component were observed above the current sheet and a negative X component below. Note that the field direction observed in the X–Y plane does not change significantly along the trajectory and indeed approaches a direction, which as the magnetopause is approached, is parallel to the magnetopause.

The X–Z projection shows that close to the planet there is a substantial negative Z component, consistent with the inner magnetosphere being dominated by the planetary dipole term. The field vectors beyond 30 R_J tend to be more parallel to the solar magnetospheric equatorial plane for the most part. However, the field vectors do show an increasingly southwards tilt at larger radial distances and at increasingly smaller and also more negative values of the spacecraft z_{SM} position.

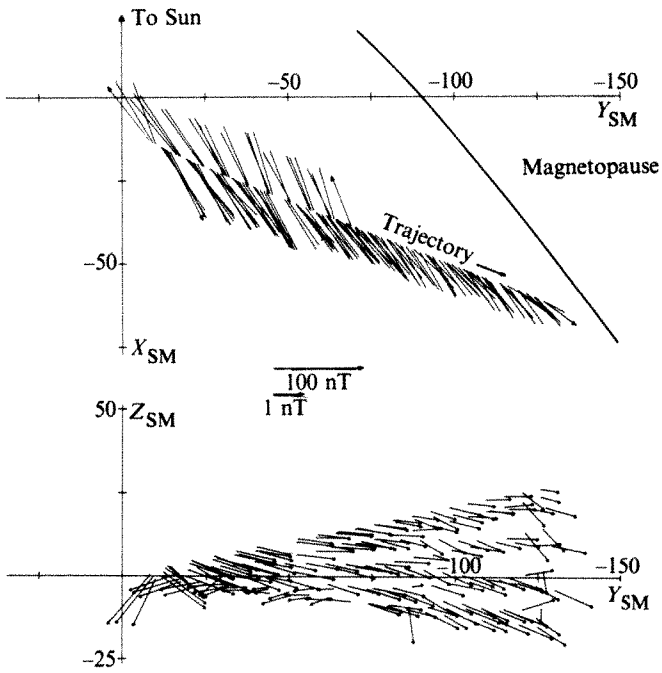


Fig. 2 Projection of hourly average magnetic field components, in solar magnetosphere coordinates, along Voyager 1 trajectory. The observed stratification in the lower panel into four apparently distinct levels is a consequence of the relative phasing between the hourly averages and the very nearly 10 hour rocking periodicity of the coordinate system.

Comparing these data with Fig. 1 in SM coordinates, it is noted that both the orientation of the field at negative SM latitudes and the occurence of incomplete current sheet crossings is consistent with a current sheet merging with a neutral sheet surface which bends southwards at larger distances at this time. Due to the long transit time from periapsis to first outbound magnetopause crossing, 10 days, some of the variations in the data may reflect temporal variations in the magnetosphere structure. These are convolved with large scale spatial variations.

The direction of the field and the position of the current sheet and neutral sheet, as either directly observed or inferred, is reminiscent of observations made near Earth by spacecraft traversing the magnetic tail in the dawn region of the terrestrial magnetosphere. Figure 3 presents magnetic field observations from the IMP 1 satellite which discovered the Earth's magnetic tail[10]. Figure 3 shows that near the dawn terminator, the field is distorted from the magnetic meridian planes and is bent back-

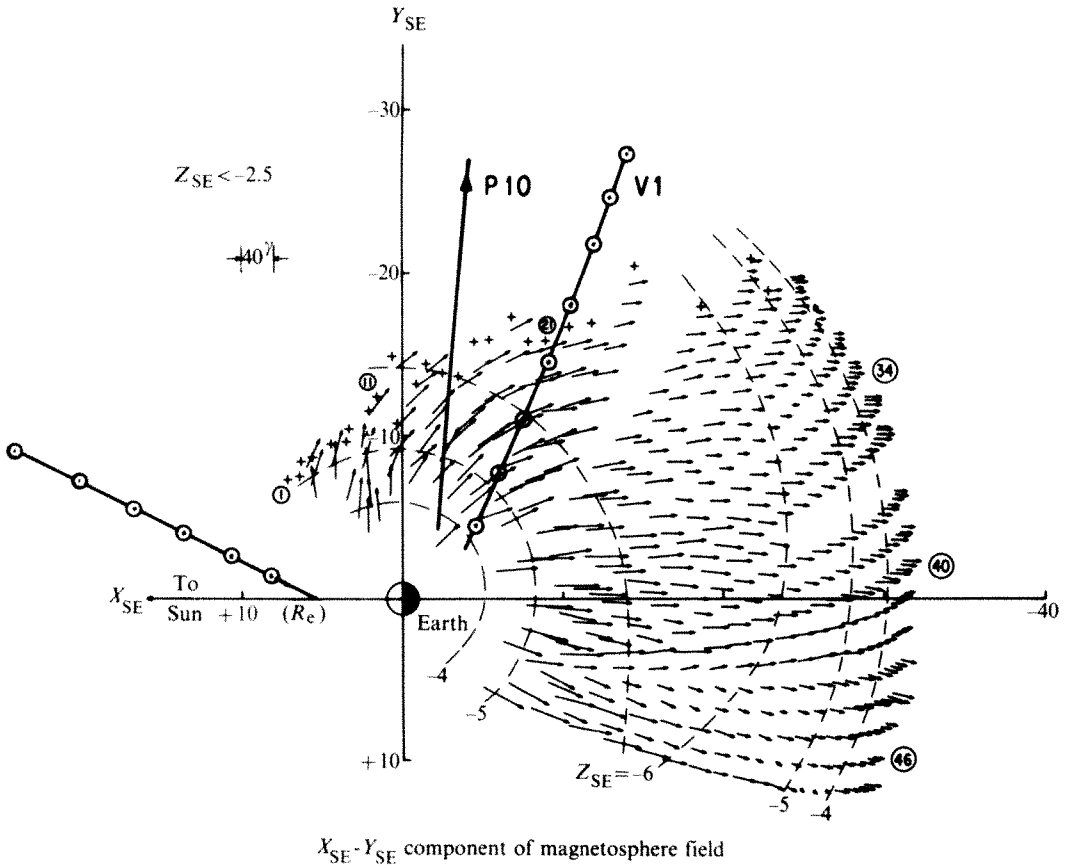


Fig. 3 Relative position of Voyager 1 and Pioneer 10 trajectories superimposed on results obtained by a survey of the Earth's magnetic tail by IMP-1 (Explorer 18). +, Observed magnetopause position.

wards so as to parallel the magnetopause boundary. Superimposed on this diagram are the trajectories of Voyager 1 outbound and Pioneer 10 assuming equal scaling of the positions of the magnetopauses. The Voyager 1 magnetic field results as shown in Fig. 2*a* are consistent with those to be expected from this figure. Figure 3 also shows evidence of a magnetic tail along the Pioneer 10 trajectory less tailwards than Voyager 1. The field orientation would change from the magnetic meridian plane when close to the planet, and become gradually deflected tailwards until an angle of approximately 60° is reached near the dawn terminator, paralleling the magnetopause. This is exactly the behaviour observed by the Pioneer 10 magnetic field experiment[6].

The Pioneer 10 observations just inside the magnetopause also showed several complete traversals of the current sheet[6]. This is most readily understood if solar wind control of the outermost regions of the jovian magnetosphere was the mechanism responsible, rather than an internal process as required by the alternate models. Thus we conclude that, on the basis of Voyager 1 magnetic field observations, a consistent model of the jovian outer magnetosphere should incorporate the development of an extended magnetic tail with an embedded neutral sheet.

The observations of the positions of the outbound magnetopause crossings imply a magnetic tail radius of 300–400 R_J, if the tail is assumed to be roughly circular. Figure 4 portrays the distortion of the magnetic field in the current sheet region in the inner magnetosphere and the merging of the magnetodisk current sheet with the neutral sheet of the magnetic tail in the outer magnetosphere. Figure 4 is based primarily on an analogy with terrestrial magnetosphere studies and is adapted for Jupiter by showing a much larger distension of the magnetic field lines near the current sheet. Jupiter also presents a smaller angle of attack for the solar wind, relative to the magnetic equatorial plane of

Table 1 Predicted system III (1965) coordinates of jovian auroral zone

Northern			Southern		
Lat	Long	|B|	Lat	Long	|B|
+81.2°	+143.4°	10.8	−77.3°	8.4°	9.1
+80.6°	+180.4°	10.3	−78.0°	336.7°	9.8
+73.9°	+190.2°	10.6	−80.5°	306.4°	10.2
+67.0°	+184.1°	11.5	−84.2°	261.5°	10.2
+62.7°	+174.2°	12.5	−84.5°	177.7°	10.0
+61.9°	+163.0°	13.2	−79.9°	128.9°	9.6
+64.4°	+151.3°	13.3	−76.1°	102.6°	9.1
+69.5°	+140.0°	12.8	−75.3°	78.1°	8.6
+75.7°	+132.8°	11.8	−76.6°	45.8°	8.6

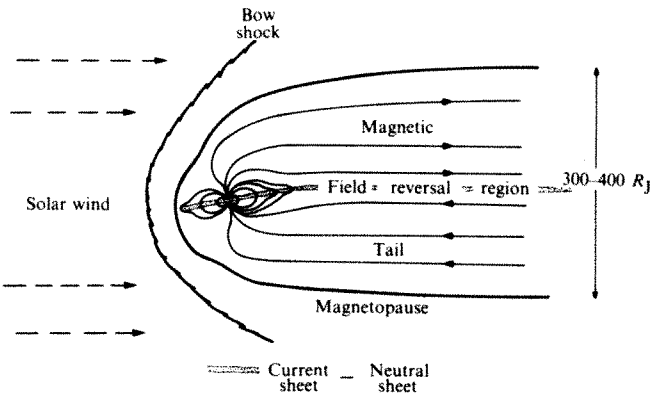


Fig. 4 Sketch of noon–midnight magnetic meridian plane (the x–z_{SM} plane) geometry of jovian field lines and current sheet–neutral sheet.

Jupiter, because of the small obliquity to the ecliptic of the jovian rotation axis (~3°). Nonetheless, the observed curvature of the neutral sheet and current sheet observed by Voyager 1 in the outbound traversal imply a three dimensional geometry so that the tail is not symmetric about the neutral sheet. Voyager 2, which will pass further down the magnetic tail, is expected to provide additional insight on the structure of the jovian magnetosphere, especially the outer portion.

As on Earth, it can be assumed that the magnetic field in the magnetic tail connects to the polar cap regions in order to estimate the size of the polar cap region itself. Figure 5 compares tail field observations near the magnetopause by Voyager 1 and Pioneer 10 with theoretical models which assume a polar cap radius of 8°, 10° and 13°. For this computation, it is assumed that only the planetary magnetic dipole term is responsible for the total flux connecting to the magnetic tail. As seen in Fig. 5, Voyager 1 and Pioneer 10 suggests a polar cap auroral zone of 10°±1°. This is substantially smaller than Earth's, which is approximately 18° to 22°. This is also much smaller than the polar cap region inferred at Mercury by the same analysis, approximately 18°–26°.

The Earth's magnetic tail field is known to decrease significantly in magnitude down the tail and to vary in time due to changing solar wind and internal magnetospheric conditions. Thus, due to probable merging across the neutral sheet and flaring of the magnetopause boundaries, the field intensity in the jovian tail is expected to decrease significantly tailward and indeed, the Voyager 1 (3 nT) and Pioneer 10 (5 nT) observations show this to be true.

The implication of the existence of a magnetic tail, with respect to the polar cap region, can be further studied by determining the location of the polar cap auroral zones more precisely. While this cannot be done for an exact model of the jovian magnetosphere, a reasonable approximation can be made. The equivalent dipole co-latitude has been computed by equating the polar cap flux with the total flux in the magnetic tail. With this value, we trace the field lines from the equator at radial distances corresponding to 8° and 13° using the GSFC O_4 planetary magnetic field representation, including quadrupole and octupole terms, but neglecting external currents. The auroral zones so derived are shown in Fig. 6.

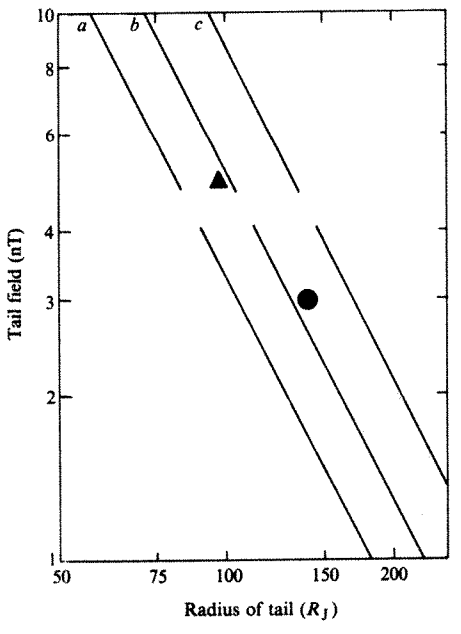


Fig. 5 Relationship between radius of jovian magnetic tail and field intensity, assuming conservation of polar cap magnetic flux in magnetic tail. ▲ Pioneer 10; ● Voyager 1. $B_T = 4B_{0J}(R_J/R_J)^3 \sin^2\theta_{pc}$ where θ_{pc} is the co-latitude: *a*, $\theta_{pc}=8°$; *b*, $\theta_{pc}=10°$; *c*, $\theta_{pc}=13°$.

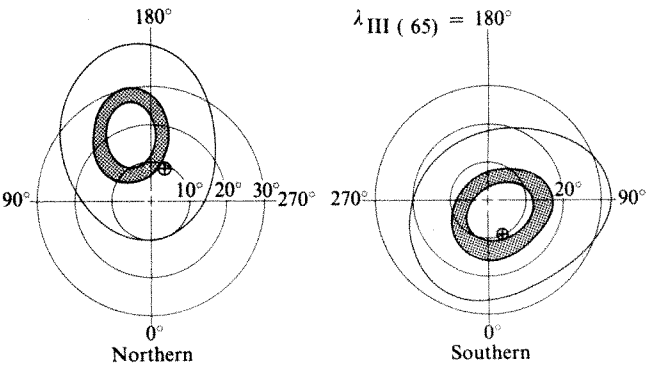


Fig. 6 Jupiter's polar cap auroral zones predicted from the observed magnetic tail and the GSFC O_4 planetary field model. ⊕ Magnetic dipole axis; stippled area indicates auroral zone; solid line indicate Io *L* shell footprint.

The small size of the zones is immediately evident as is a surprisingly large eccentricity of the northern polar cap. Also shown are the footprints of the Io fluxtube, which map out the position of the jovian line of force which threads the satellite Io as its orbits the planet. The asymmetric location of the oval is due to the multipole characteristics of the planetary field while its size is determined by the number of field lines required to connect to the total flux in the magnetic tail. The uniquely eccentric position of the northern polar cap auroral zone of Jupiter is an important and anomalous feature of the jovian magnetospheric configuration. Table 1 presents the coordinates of the polar cap auroral zones, assumed dipole co-latitude of 10°, and field magnitude (in gauss).

These observations show convincing evidence of the development of an extended magnetic tail and imbedded neutral sheet in the nightside magnetosphere of Jupiter, which is formed by the solar wind interaction. This indicates substantial external control of the outer magnetosphere of Jupiter rather than only inner control as postulated by the magnetodisk and magnetic anomaly models. The size of the tail inferred (300–400 R_J diameter), and the field intensity measured (3 nT) imply rather smaller polar cap regions at Jupiter than at Earth. The complex planetary magnetic field model GSFC O_4 yields a highly eccentric northern polar cap region, encircling neither rotation axis nor magnetic axis.

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Departure from rigid co-rotation of plasma in Jupiter's dayside magnetosphere

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The MIT plasma experiment on Voyager 1 shows that the plasma flow is not in strict co-rotation at distances greater than 10 jovian radii.

CO-ROTATION of the jovian magnetospheric plasma with the planet to some radial distance has been the basic assumption in all analyses of plasma dynamics in near Jupiter space[1]. Brice and Ioannidis[2] hypothesised that co-rotation should dominate convective motion in the entire magnetosphere. The MIT plasma experiment on the Voyager 1 spacecraft has made the first detailed *in situ* measurements of the low energy (10 eV to 5.95 keV) component of the jovian magnetospheric plasma[3]. The preliminary analysis of these measurements presented here shows departure of the plasma flow from strict co-rotation at radial distances greater than ≈10 jovian radii (R_J). Data used in this analysis were taken in the dayside jovian magnetosphere before the closest approach of the Voyager 1 spacecraft to the planet.

The experimental package consists of four modulated-grid Faraday cups[4]. Three of these form the main sensor cluster and the fourth, or side sensor, is mounted perpendicular to the symmetry axis of this cluster. During that part of the inbound leg of the Voyager 1 encounter which concerns us here, the cluster symmetry axis looks away from Jupiter and towards the Earth while the side sensor looks roughly into the direction of co-rotating plasma flow. In the positive ion mode, the experiment measures currents in both a low resolution mode (the L mode) with ~29% resolution in energy per charge and a high resolution mode (the M mode) with ~3.6% resolution in energy per charge. One L mode spectrum is obtained every 96 s and one full M mode spectrum every 192 s.

We have analysed low resolution mode spectra inbound from 12.00 UT, 4 March, 1979 (22.6 R_J) to 02.00 UT, 5 March, 1979 (11.4 R_J). By virtue of its wider energy-per-charge channels, the L mode has a higher signal-to-noise ratio than the *M* mode and gives better quality measurements for the protons in the data set considered. The L mode measurements in the side sensor frequently show good separation between the proton peak and that of the heavy ion constituents although the latter are, in general, unresolved (see Fig. 3 of ref. 3). As long as the plasma is cold (greater than mach 2) and flows directly into the side sensor, the response function[5] of the side sensor can be taken as unity to about 5% accuracy. Under this assumption, we have fit two convected, isotropic maxwellians to the side sensor L mode spectra, one to the protons and one to the heavy ions. In general, this provides a good representation of the data. The fit is poor if the mach number becomes so low that the two peaks strongly overlap or sufficiently high that the multi-component nature of the heavy ions begins to appear. Such fits are not presented here.

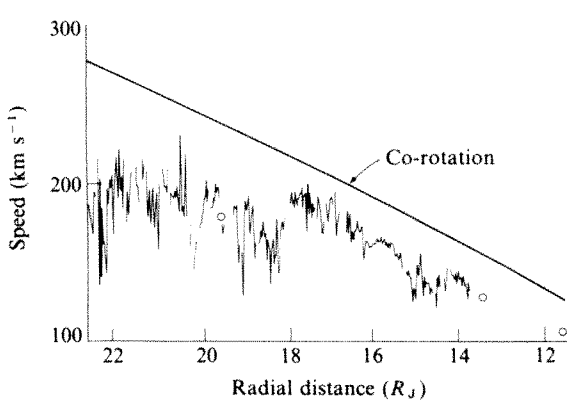


Fig. 1 Upper limit on the component of bulk plasma velocity into the side sensor versus radial distance.

A more realistic response function for the side sensor is (S. Olbert, personal communication)

$$\exp(-\alpha u_t^2/u_p^2)$$

where α is a constant of order unity, u_t is the ion velocity transverse to the cup normal and u_p is the ion velocity parallel to the cup normal. It can be shown by convolving this response function with a convected, isotropic maxwellian that maxwellian fits assuming unit response always overestimate the plasma bulk speed into the side sensor. The error increases with decreasing mach number of the flow and to a lesser extent with increasing component of plasma bulk velocity transverse to the cup normal. Thus, the speeds we derive from such fits should be taken only as upper limits on the component of plasma bulk velocity into the side sensor.

In Fig. 1, the solid line is the predicted component of rigid co-rotation velocity into the side sensor. The time period covered is that mentioned above with the radial distance of the spacecraft from Jupiter shown along the abscissa. We also plot the component of bulk velocity of the protons into the side sensor, as derived from the analysis described above. Gaps in the data trace appear where the fit is poor. The three circles represent proton measurements from three individual M mode spectra. During this time period, these M mode spectra are somewhat anomalous in that the protons exhibited a strong M mode signal. The spectra give proton and heavy ion bulk speeds consistent with the more numerous L mode measurements; they also show conclusively that the observed departure from co-rotation is not a spacecraft charging effect[6].

We stress that the plotted quantity is an upper limit. The present analysis is highly dependent on mach number which varies for these spectra between 1 and 4. Hence, structure in the curve should not be overinterpreted. The derived upper limits on the proton bulk speed into the side sensor lie consistently below the value expected for rigid co-rotation in the range from 11 to 22 R_J. We intend to do a more complete analysis of this data set, incorporating data from the main sensor and using the detailed response functions of all sensors. Construction of the full vector velocity of the plasma may then be possible for this magnetospheric region.

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Voyager 1 photopolarimeter experiment and the phase curve and surface microstructure of Ganymede

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Optical wavelength photometry used to determine the surface microstructure of Ganymede, shows that its surface is more porous than Callisto.

GANYMEDE, Jupiter's largest satellite, has a density of 1.9 g cm^{-3}, suggesting a bulk composition of about 50% water. Earth-based near-IR spectroscopic studies indicate that a half to two-thirds of its surface is covered with water ice[1]. Images of the satellite at a resolution of 2.5 km obtained by Voyager 1 cameras show an astonishing topography of cratered areas and grooved areas[2]. Most of the grooved terrain is a mosaic of systems of sinuous grooves and ridges. Radar echoes of Ganymede show a surprising reversal of the sense of reflected circular polarisation[3], indicating that the echoes arise principally from double reflections, and that the surface of Ganymede is therefore quite rough at the wavelength scale (13 cm). Optical wavelength photopolarimetry can reveal the surface characteristics of an atmosphereless object at a still finer scale, that is, its surface microstructure.

A detailed description of the Voyager photopolarimetry experiment has been published elsewhere[4]. The instrument was used as a single-wavelength photometer for whole-disk observations of Ganymede, made over a 3-day period, beginning 5.5 days before the closest approach to Jupiter. Figure 1 shows the brightness of Ganymede at 590 nm as a function of the range to the satellite. Each dot represents the average of 160, 320, 400, 320 and 160 individual raw data measurements, respectively, while the error bar denotes the standard deviation. The background count was essentially zero at such large distances from the radiation belts. As the range to the satellite decreased, its observed brightness did not increase with the inverse square of the distance (the smooth curve in Fig. 1). The instrument was seeing a smaller and smaller lit surface as the Sun–Ganymede–spacecraft angle (solar phase angle) was increasing. Ground-based photometry and Voyager 1 imaging have shown that the surface albedo of Ganymede is inhomogeneous, so that its integrated disk brightness varies with longitude.

It is not possible to separate the phase effect and the rotational effect using the Voyager 1 data alone. However, the rotational brightness variation of Ganymede has been observed from Earth through *UBV* bandpass filters[5]. Fortunately the spectral reflectance of Ganymede is rather flat in the wavelength interval common to the *V*-band (490–650 nm) and the bandpass of the Voyager photopolarimeter (570–610 nm) (see Fig. 2). Consequently we may correct for the rotational effect in our data (covering the longitude range 109°–264°) using published light curves, that is, Ganymede's nominal visual magnitude versus the rotational angle[5]. The magnitude of Ganymede as observed by the Voyager 1 photopolarimeter, corrected for rotational effect, and ground-based photometric observations[6] are shown in Fig. 3. These *V*-magnitudes have also been corrected for rotational effect. The data are limited to longitudes between 90° and 270° (the hemisphere facing away from Jupiter). The Voyager data have not been transformed into the *UBV* system but simply normalised to the ground-based observations.

With the extension to 33° phase angle, the shape of Ganymede's phase curve becomes more apparent. Satellite and asteroid phase curves are usually characterised by two parameters: the phase coefficient and the opposition effect. The slope of the straight line of best fit through data points between 6° and the maximum phase angle observable is called the phase coefficient. The magnitude actually observed at zero phase minus that extrapolated from the regression line just defined is called the opposition effect. Ganymede's phase coefficient and opposition effect derived from the data in Fig. 3 are 0.011 ± 0.001 mag per deg and 0.17 ± 0.03 mag, respectively. The error bars represent the statistical scatter in the data, and do not include systematic instrumental errors. There has been a long-term decrease of the instrument's sensitivity during the Voyager 1 mission. The decrease over the 3 days of this observational period is no more than 5%, based on consistency of Jupiter data. Correcting for such a decrease would lower the phase coefficient and increase the opposition effect. These changes are in directions that make the scientific conclusions stronger, as shown below. Other possible sources of systematic error were not checked immediately before Jupiter encounter. We suspect nonlinearity in the instrumental response at high count rates. We expect this effect was less at the count rates observed for Ganymede. However, we are encouraged by the fact that the reduced Voyager data in Fig. 3 are consistent with the phase curve expected from ground-based observations even though the raw data counts span a threefold range.

The derived phase coefficient of Ganymede is remarkably small. Asteroids typically have phase coefficients ranging from 0.020 to 0.055 mag per deg (ref. 7), while the Earth's Moon, Mercury and the martian satellites have values between 0.03 and 0.04 mag per deg (ref. 8). Only Europa[5,6] seems to have a phase coefficient smaller than that for Ganymede. The value for Ganymede approaches that of a Lambert sphere (a diffusely reflecting sphere, with a phase coefficient of 0.006 mag per deg at 20° phase angle).

Theoretical techniques for interpreting an observed phase curve to derive information on the surface of an atmosphereless object have been discussed by Veverka[9], Johnson and Matson[10], and others. The discussion below is modelled on that given by

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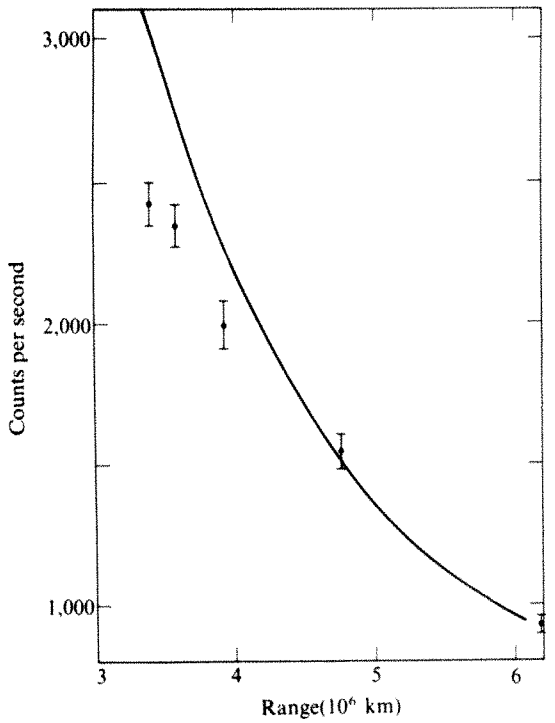


Fig. 1 Whole-disk brightness of Ganymede at 590 nm as a function of the distance between the satellite and the Voyager 1 photopolarimeter. Points are averages of many individual measurements (see text). Error bars are standard deviations. The smooth curve represents brightness variation according to the inverse square law.

Veverka. In essence there are three scales of surface roughness that determine the shape of an object's phase curve: the macroscopic or large-scale topography, the particulate or crystalline nature (microstructure), and small-scale texture intermediate to these two extremes. Shadowing by topographical features is of major importance at all but the smallest phase angles. The greater the large-scale roughness, the greater will be the phase coefficient. The albedo and scattering phase function of the individual particles or crystals are determined by their size, shape, and complex refractive index. Multiple scattering among the particles is generally important, except for the very darkest materials. The greater the particle albedo, the greater the multiple scattering, and the less will be the phase coefficient. For transparent or translucent particles, diffuse transmission and reflection are also involved. The intermediate scale of roughness, the 'texture' of the surface, is determined mostly by the packing characteristics of the particles. The porosity of a surface strongly influences the phase curve at small phase angles.

Modelling of scattering by surfaces is primitive; it is not yet possible to derive surface parameters from observed phase curves only. However, if information on two of the three scales of roughness is available one may set limits on the third. The Hapke–Irvine model for approximating the opposition effect of a dark surface is a case of such an analysis. This model applies to dark particles, for which multiple scattering may be neglected, and to very small phase angles, so that shadowing by large-scale topographic features is not important. With these simplifying assumptions the opposition effect of such a layer is determined largely by the packing characteristics, described by the compaction parameter.

$$D = \frac{3}{4\pi} \times \frac{\rho}{\rho_0}$$

where ρ is the density of a macroscopic volume element and ρ_0 is the density of a single particle. The Hapke–Irvine model seems to fit available observations of dark surfaces and seems useful in establishing relative values of D. Veverka[9] used this model to compute opposition surges for a variety of compaction parameters. A surface with $D \sim 0.13$ exhibits no opposition effect. As D decreases the surface becomes more porous, and the opposition effect develops. For the leading (trailing) side of Callisto, which has an opposition surge of 0.25 mag (0.13 mag), D would be approximately 0.025 (0.04). The surface material on Callisto thus seems to be rough and porous. All other factors being equal a larger opposition effect means a more porous surface.

Although it is tempting to use the Hapke–Irvine model in analysing the phase curve of Ganymede, we must bear in mind that this model is valid only for dark surfaces like that of the moon (visual albedo 0.12) and Callisto (0.17). The visual albedo of Ganymede, 0.43, is too high for the simplifying assumptions to be valid. However, the topographies of Callisto and Ganymede seem to be similarly rough (Callisto is more heavily cratered but Ganymede is extensively grooved), and the degrees of roughness of the two satellites at 13 cm scale are similar. (In

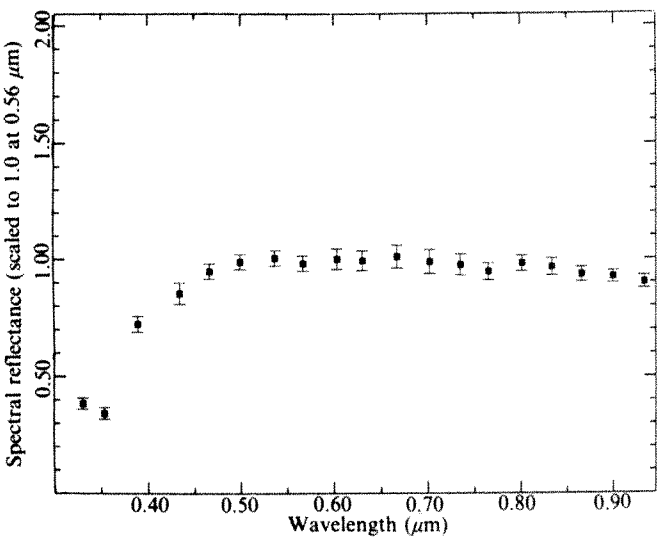


Fig. 2 The relative spectral reflectance of Ganymede as observed from the 224-cm telescope on Mauna Kea Observatory on 28 December 1978. Error bars represent standard deviations of different measurements taken during the same night.

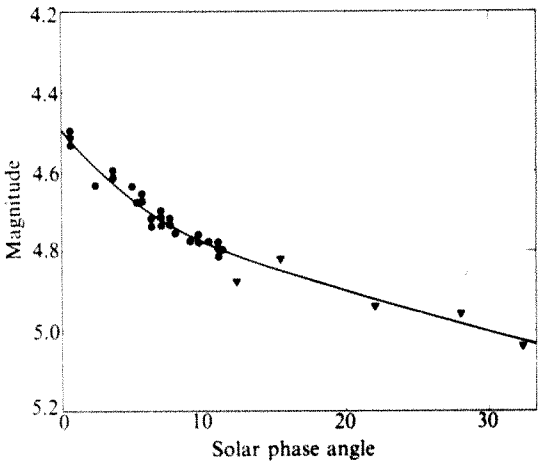


Fig. 3 The phase curve of Ganymede for the hemisphere facing away from Jupiter (longitudes between 90° and 270°). Rotational brightness variations have been corrected for. The Voyager data (▼) are normalised to the ground-based observations (●) of Millis and Thompson[6].

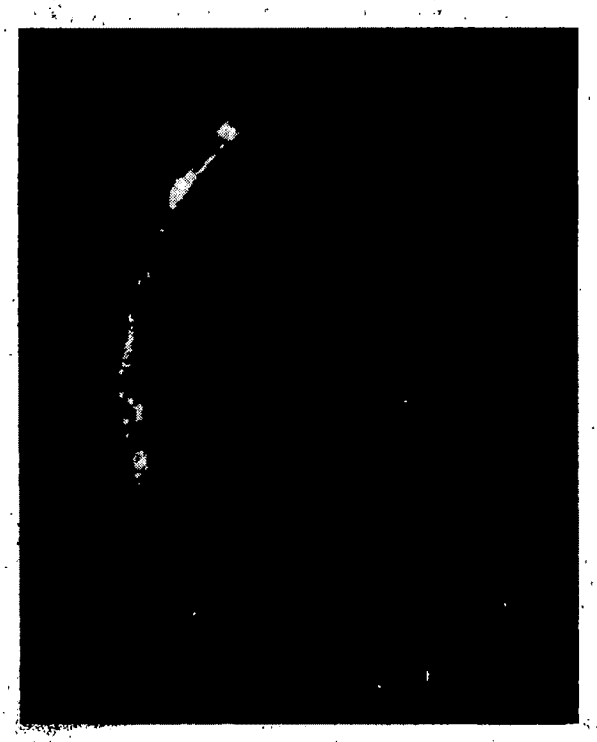
contrast to Ganymede and Callisto the small-scale texture of Io appears to be smooth. 13-cm roughness estimates are from radar observations by Pettengill.[3]) The opposition effect of Ganymede (0.17 mag) is slightly larger than that of the trailing side of Callisto (0.13 mag). Now if the topographical and 13-cm scale degrees of roughness of the two satellites are similar and their opposition surges are nearly the same, but Ganymede is 2.5 times brighter than Callisto, then it seems probable that the surface of Ganymede is the more porous of the two. If all three scales of roughness were the same, Ganymede would have a smaller opposition effect because, having brighter surface particles, multiple scattering of light would tend to wash out the effects of shadowing. Increased shadowing effects from a more porous surface on Ganymede compensate for the multiple scattering reduction of the opposition effect.

We arrive at a similar conclusion by comparing the phase coefficients of Ganymede and Callisto. Callisto's phase coefficient (0.025 mag per deg) is more than twice as large as that of Ganymede (0.011 mag per deg). Assuming that the large and intermediate scale roughnesses of the satellites are similar their phase coefficients should be the same if their albedos were the same. The greater albedo of Ganymede results from greater single particle albedo, which implies more multiple scattering, therefore a lower phase coefficient. A porous surface layer is necessary to effect the multiple scattering. Ground-based polarisation studies of Ganymede led to a similar conclusion[11]. Thus, within the limitations of our simplifying assumptions, we conclude that the surface layer of Ganymede (the hemisphere facing away from Jupiter) is porous, and more so than for Gallisto (the trailing side). In contrast to Ganymede, the transient south polar cap of Mars has a quasi-specular surface[12]. The difference could be due to annual resurfacing on Mars, while micrometeoroids continuously roughen the surface of Ganymede.

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VOYAGER I

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articles

Voltage-dependent caesium blockade of a cation channel from fragmented sarcoplasmic reticulum

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Cs^+ ions block the K^+ conductance of a voltage-gated ionic channel derived from fragmented sarcoplasmic reticulum (SR) and incorporated into an artificial phospholipid bilayer. The block follows a single-site titration curve with a voltage-dependent dissociation constant of 18 mM at +50 mV. Blockade observed with the macroscopic conductance can be fully accounted for by the reduction of the apparent single-channel conductance in the presence of Cs^+. The voltage dependence is explained by assuming that Cs^+ binds to the open state of a single channel on a site located about 40% of the way through the membrane from the cis *side (the side to which the SR vesicles are added). The blocking site is accessible only from the* cis *side.*

ELECTROPHYSIOLOGICAL measurements in a variety of excitable membranes have demonstrated that Cs^+ ions block K^+-conducting ionic channels[1-7]. In the case of the well characterised squid axon K^+ channel, Cs^+ blockade seems to be due to the size of the ion and the geometry of the channel's inner 'mouth' (refs 4, 8). The blocking mechanism, as outlined by Armstrong[8], involves the formation of a Cs^+-site complex inside the channel which prevents the passage of K^+ ions.

We are attempting the characterisation of a voltage-gated, cation-selective channel present in the sarcoplasmic reticulum (SR) membrane of mammalian skeletal muscle[9-12]. The channel is studied by incorporating SR membrane vesicles into an artificial phospholipid bilayer by a process similar to membrane fusion[9,10]. Thus, we may conveniently investigate the conductance properties conferred on the planar bilayer the the SR vesicles. Channels are incorporated irreversibly and with at least 98% orientation[10,11], allowing the definition of *cis* and *trans* sides of the membrane (the former being defined as the side on which the SR vesicles are added). By using agents that modify the system's properties from only one side, we found that the channel contains a Ca^{2+}-blocking site on the *cis* side[10], a transition metal cation binding site on the *trans* side[11], sulphydryl groups accessible from either side[11] and a *trans*-facing arginine or lysine residue accessible to attack by pronase-derived alkaline proteinase B (ref. 12).

A membrane containing a large number of SR channels displays a voltage-dependent K^+ conductance[10,11]. The steady-state conductance increases e-fold per 23 mV (ref. 12) (*trans* side defined as zero voltage). Each individual channel, on the other hand, operates by a mechanism involving a single conducting state in which the probabilities of being in the conducting (open) or non-conducting (closed) states are voltage dependent; the open-state conductance is independent of voltage and has a value of 130 pS in 0.1 M K^+ (refs 10, 11). The channels are ideally selective for K^+ over Cl^- and the single-channel K^+ conductance is 3.5 times the Na^+ conductance and 26 times the Li^+ conductance (unpublished). Cs^+ is unique among the group IA cations in exerting a profound inhibitory effect on the channel's K^+ conductance. In this article, we discuss this latter observation and propose a mechanism for the inhibition.

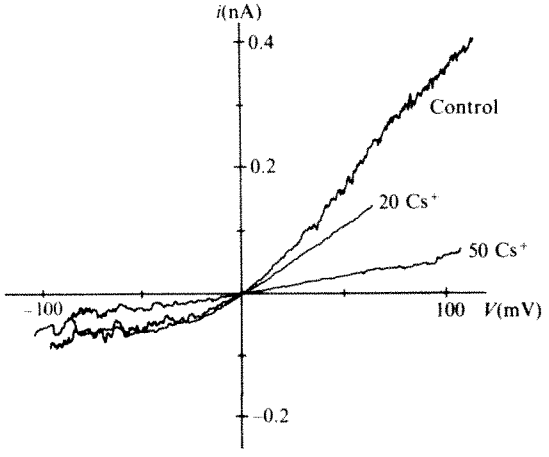


Fig. 1 Steady-state current–voltage relationship and Cs^+ blockade of the K^+ current. Planar bilayer membranes were painted on a 1-mm hole, and electrical measurements were made in voltage-clamp conditions[10,11]; the unmodified membrane conductance was $1-5\times10^{-8}$ S cm^{-2}. The phospholipid mixture was composed of a 50 mg ml^{-1} decane solution of soybean asolectin (Sigma) and egg phosphatidylethanolamine, in a mol P ratio 1 : 1. Incorporation of rabbit back muscle SR was accomplished by adding SR protein (50 μg ml^{-1}) to the *cis* chamber in 'fusion conditions' (ref. 10). All experiments were done at room temperature (21–23 °C). The aqueous phase was composed of symmetrical solutions of 100 mM $K^+ + x$ mM $Cs^+ + (50-x)$ mM Li^+ as the sulphate salts, buffered with 5 mM HEPES–Tris–10 μM EDTA, *p*H 7.5. The Cs^+ concentration is indicated in each trace, while the control trace contains no Cs^+. The current–voltage curves were made in different membranes, and the recordings presented were chosen to have similar conductance at −80 mV (background conductance). They were obtained by applying a slow voltage ramp (20 mV min^{-1}) and recording the current response. Li^+ was chosen as an 'inert' ion on the basis of its low conductance through the open state of the SR channel and its lack of blocking properties (unpublished). Similar results were obtained replacing Li^+ by $Tris^+$. The Cs^+ channel conductance is undetectably low (<3 pS in 0.1 M Cs^+).

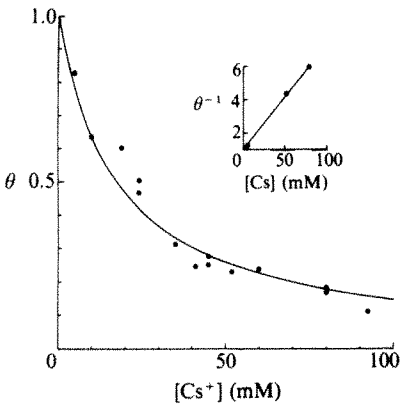


Fig. 2 Titration of Cs^+ block at constant ionic strength. Conditions were as in Fig. 1, except that the aqueous phase was 100 mM K^+-glucuronate–100 mM Li^+-glucuronate, 0.1 mM EDTA–10 mM 2[*N*-morpholino]ethane sulfonic acid (MES)-Tris *p*H 6.7; the lipid mixture was composed of asolectin, 100 mg ml^{-1} in *n*-decane. Cs^+ was added by perfusing the *cis* chamber[11] with known volumes of 100 mM K^+-glucuronate–100 mM Cs^+-glucuronate, 0.1 mM EDTA–10 mM MES-Tris, *p*H 6.7. The steady-state conductance at +50 mV and the background conductance were measured before Cs^+ addition. Cs^+ solution was then perfused into the *cis* chamber to the desired Cs^+ concentration. The new conductance values were established not later than 10 s after Cs^+ addition; the background conductance remained unchanged in all experiments, but the +50 mV conductance was reduced after Cs^+ addition. The channel-mediated fraction of conductance remaining after Cs^+ addition, θ, was calculated as

$$\theta(c, V) = [g(c, V) - g_b]/[g(0, V) - g_b]$$

where $g(c, V)$ is the steady-state conductance at a given Cs^+ concentation, c, and applied voltage, V (+50 mV in this case); $g(0, V)$ is the +50 mV conductance before the addition of Cs^+ and g_b is the background conductance and corresponds to the membrane conductance at −50 mV. The solid line corresponds to a single-site binding curve (equation (1)) with $K(V) = 18$ mM. Data are taken from 12 different membranes. Inset: reciprocal plot of equation (1), with data taken from a single membrane. All data were taken from multi-channel membranes with background conductances >0.8 μS cm^{-2} and +50 mV conductances of the order of 10 μS cm^{-2}.

Cs^+ inhibition of macroscopic conductance

Although Cs^+ itself does not permeate the channel to any appreciable extent (see Fig. 1 legend), increasing concentrations of Cs^+ eliminate the rectification of the K^+ current (Fig. 1). In the presence of 50 mM Cs^+, the K^+ conductance at +50 mV is at most 50% higher than the conductance at −80 mV. This latter value serves as a convenient measure of the poorly understood background conductance of the system[10]. Control experiments (not shown) demonstrate the complete reversibility of Cs^+ inhibition.

To quantify this inhibition, we studied the effect of various Cs^+ concentrations on the K^+ conductance at a fixed voltage. By maintaining both K^+ concentration and ionic strength constant, we eliminated changes in surface potentials[13,14].

Figure 2 shows results of such titrations at +50 mV applied voltage, with Cs^+ added to the *cis* side in place of Li^+. The inhibition follows a single-site titration curve of the form

$$\theta = \left[1 + \frac{[Cs^+]}{K_d(V)}\right]^{-1} \quad (1)$$

where θ is the fraction of the channel-mediated conductance not blocked by Cs^+, and $K_d(V)$ is the macroscopic apparent dissociation constant at that voltage. The pooled data taken from 12 membranes are somewhat scattered around the theoretical curve but the single-site inhibition is clearly seen when data from the same membrane are plotted (Fig. 2, inset). Although *cis* Cs^+ is able to block the conductance at positive and negative voltages, our results indicate that *trans* Cs^+ up to at least 80 mM does not affect the K^+ conductance (data not shown). We will show later that a quantiative estimate for the *cis* and *trans* accessibilities of Cs^+ can be obtained from single-channel fluctuation measurements.

Titration of the single-channel conductance

A probable mechanism for the Cs^+ inhibition of the K^+ conductance of membranes containing many channels is an elimination of the K^+ current through a single channel in the open state, whenever that channel binds Cs^+. As we could observe single channel fluctuations directly, we tested the above proposal for Cs^+ action by allowing the fusion of only a single SR vesicle with the bilayer[10–12]. As shown in Fig. 3 inset, we found that Cs^+ produced a gradual reduction in the open-state conductance. The main part of Fig. 3 demonstrates that the open-state K^+ conductance followed a single-site inhibition curve. The apparent dissociation constant measured here is 18 mM at +50 mV.

The inhibitory effect of Cs^+ can be explained on the basis of Woodhull's theory of ionic blockade[15]. Following this theory, we shall assume that when Cs^+ binds to a site in the open channel, a non-conducting 'blocked' state is formed. If an equilibrium exists between free and bound Cs^+, we expect a given channel to fluctuate between three states: closed, open and blocked. We further assume that the lifetime of the blocked state is much shorter than the time resolution of our recording system (~5 ms). Thus, in the presence of Cs^+, the apparent open-channel conductance corresponds to a time-averaged value determined by the rapid blocking–deblocking rates. It follows that increasing concentrations of Cs^+ should reduce this time-averaged conductance in a single-site binding scheme, as is observed.

The appearance of a short-lived blocked state has been experimentally observed by Neher and Steinbach[16] in the acetylcholine receptor channel of frog muscle when blocked by lidocaine derivatives. An important consequence of the appearance of a blocked state is that the apparent mean open time of the channel (the 'mean group time' of Neher and

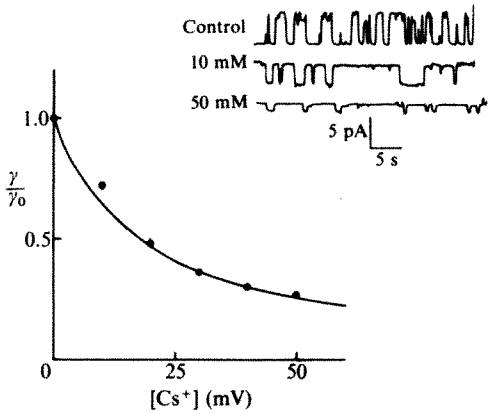


Fig. 3 Cs^+ titration of single-channel conductance. Recordings of single-channel fluctuations were made in conditions described in Fig. 1 at an applied voltage of +50 mV. All the recordings and data points in the graph were made in different membranes. The channel conductance, at a given Cs^+ concentration, was computed from 20–35 conductance fluctuations in 2–4 different membranes. Conductance histograms demonstrate the unitary character of the measured open-state conductance[10–12]. The solid curve was drawn according to

$$\frac{\gamma}{\gamma_0} = \left[1 + \frac{[Cs^+]}{K_b(V)}\right]^{-1}$$

where γ is the channel conductance at a given Cs^+ concentration, γ_0 is the channel conductance in the absence of Cs^+, and $K_b(V)$ is the blocking constant, defined in the text. The curve was fitted with $K_b(V) = 17.8$ mM.

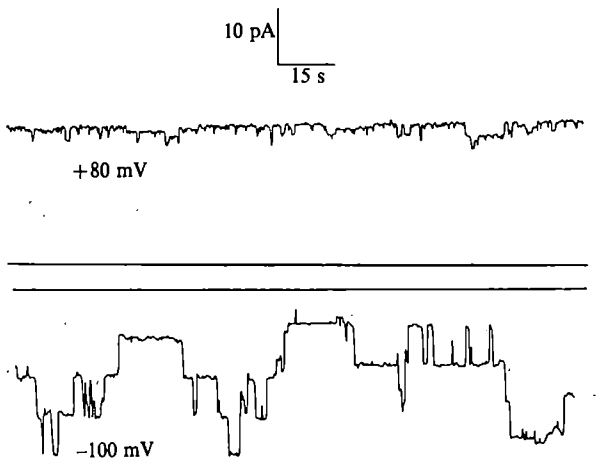


Fig. 4 Single-channel recordings in symmetrical solutions of Cs^+. Single-channel fluctuations at the indicated voltages were obtained from the same membrane, with 100 mM K^+ buffer and 50 mM Cs^+ in the symmetrical aqueous solutions. The current increases upwards in the +80 mV trace and downwards in the −100 mV trace. The solid lines correspond to the conductance of the unmodified bilayer.

Steinbach[16]) should increase with increasing blocker concentrations. Figure 3 (inset) shows that this prediction is fulfilled at least qualitatively; there is a large increase in the mean open times as Cs^+ concentration increases. Although these recordings are from different membranes, they were all taken while only one channel was fluctuating in the membrane.

Cs^+ confers voltage dependence on the single-channel K^+ conductance

In symmetrical solutions containing a fixed Cs^+ concentration, the single-channel K^+ conductance becomes voltage dependent, as indicated in Fig. 4. At 50 mM Cs^+ the channel conductance is 70 pS at −100 mV and only 23 pS at +80 mV. In the absence of Cs^+, the channel conductance is independent of the applied potential (Fig. 5). Voltage-dependent blockade of ionic channels has been a common finding in electrophysiology[1–3,15,16]. Although there is no single interpretation for this behaviour[17], the simplest one assumes that the blocking ion moves part of the way across the membrane to form the blocking complex[15]. In such conditions, the electrical field acting on the charged ligand would contribute to the standard free energy of the binding reaction[15]. Thus, $K_b(V)$, the dissociation constant for the Cs^+ binding reaction, should vary exponentially with the voltage according to

$$K_b(V) = K_b(0) \exp(-\delta FV/RT) \quad (2)$$

where $K_b(0)$ is the zero-voltage dissociation constant, δ is the fraction of the total electrical potential drop across the membrane found at the Cs^+ binding site, and F, R, T have their usual meanings. Combining the single-channel titration curve (Fig. 3) with equation (2), we obtain

$$\frac{\gamma}{\gamma_0} = \left[1 + \frac{[Cs^+]}{K_b(0)} \exp(\delta FV/RT)\right]^{-1} \quad (3)$$

This expression is similar to that of Adelman and French[3] for the Cs^+ blockade of the instantaneous K^+ current in the squid axon K^+ channel, and may be linearised as follows

$$\ln\left(\frac{\gamma_0}{\gamma} - 1\right) = \ln\frac{[Cs^+]}{K_b(0)} + \delta FV/RT \quad (4)$$

By measuring γ as a function of voltage, at a constant and symmetrical Cs^+ concentration, we can test whether this model is sufficient to describe the voltage dependence of Cs^+ blockade; in addition, a linearised plot of equation (4) will immediately determine δ and $K_b(0)$. Figure 5 shows the channel conductance measured over a wide range of voltages in the presence of 50 mM Cs^+ (filled circles). Starting from the largest open-state conductance that we were able to measure, about 85 pS at −150 mV, the conductance gradually falls, reaching 15 pS at +150 mV. In the absence of Cs^+ the channel conductance is independent of voltage (open circles), with a value of 92 pS in these conditions. A plot of $\ln[(\gamma_0/\gamma)-1]$ against voltage is linear with only a slight deviation at positive voltages (see Fig. 5 inset). The parameters obtained from this log plot are: $\delta = 0.35$ and $K_b(0) = 43$ mM. Similar values ($\delta = 0.38$, $K_b(0) = 40$ mM) were obtained from titration experiments as described in Fig. 6. Thus, assuming a constant intramembrane electrical field, we suggest that the site of Cs^+ action is at about 40% of the way across the membrane from the *cis* side.

The observation that in symmetrical solutions of 50 mM Cs^+, the channel conductance at negative voltages approaches the value for the open channel demonstrates that the blocking site is not readily accessible to *trans* Cs^+. We have extended the above treatment to the case in which the site is accessible from both

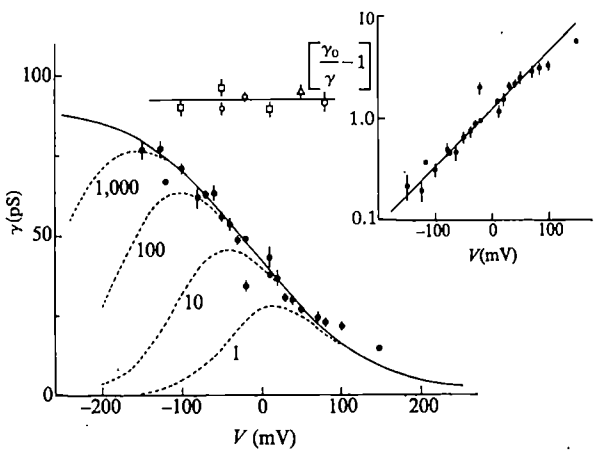


Fig. 5 Voltage-dependent single-channel conductance and *cis-trans* accessibilities of Cs^+. The figure presents pooled single-channel conductance measurements in symmetrical solutions containing 100 mM K^+ buffer and either 50 mM Cs^+ (filled circles) or other non-blocking cations (open symbols). Over 250 determinations were made, from 15 different membranes. Bars indicate the s.e.m. of the measurement; circles without error bars correspond to the means of less than four determinations. Values for the unblocked channel conductance were determined in 100 mM K^+ buffer plus 50 mM Li^+ (○), or 25 mM Li^+ + 25 mM $Tris^+$ (△), or 50 mM Na^+ after subtraction of the contribution of the Na^+ conductance (23.6 ± 2 pS (±s.e.m.)) (□). Data obtained in 100 mM K^+ buffer plus 50 mM $Tris^+$ or 50 mM glucosamine$^+$ also gave open-channel conductance in the range of 90–95 pS, and are not included. The control channel conductance, from all our Li^+ data, was 92 ± 2 pS (±s.e.m.). The solid line in the main graph corresponds to equation (3) in the text, with $K_b(0) = 42.7$ mM and $\delta = 0.35$, values that were obtained from the linearised plot (inset). The family of segmented lines was calculated from a single-binding site model in which *trans* and *cis* Cs^+ reach the site,

$$\frac{\gamma}{\gamma_0} = \left[1 + \frac{[Cs^+]_{cis}}{K_b(0)_{cis}} \exp\frac{\delta FV}{RT} + \frac{[Cs^+]_{trans}}{K_b(0)_{trans}} \exp\frac{(\delta-1)FV}{RT}\right]^{-1}$$

where $[Cs^+]_{cis}$, $[Cs^+]_{trans}$ denote the *cis* and *trans* Cs^+ concentrations and $K_b(0)_{cis}$, $K_b(0)_{trans}$ are the zero-voltage apparent dissociation constants for *cis* and *trans* Cs^+ binding respectively[15]. The curves were calculated with $\delta = 0.35$. $[Cs^+]_{cis} = [Cs^+]_{trans} = 50$ mM and ratios $K_b(0)_{trans}/K_b(0)_{cis}$ are indicated in each curve.

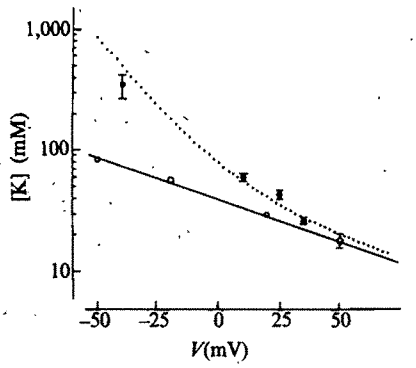


Fig. 6 Comparison of the macroscopic and single-channel blocking constants. All experiments were done in the conditions of Fig. 1. The macroscopic titration (●) was done by Li^+–Cs^+ exchange as described in Fig. 2; the bars indicate the s.e.m. of the slope from which the macroscopic apparent blocking constant, K_d, is determined. The value of K_d at −42 mV was determined by computing the time-averaged conductance in membranes in which 5–10 channels were fluctuating. The total conductance in this case was obtained by integrating the current trace above the 'zero-level' conductance[11], using a digital planimeter. The blocking constant for the macroscopic conductance was obtained, as in Fig. 2, from compiled data from four or more different membranes for each voltage. The single channel dissociation constants were obtained as in Fig. 3. The s.e.m. of the slope from which the channel dissociation constant is determined is contained within the open dots. The value of the macroscopic dissociation constant at +50 mV was 18.4±2.7 mM (±s.e.m.). The value of the single-channel dissociation constant at that same voltage was 18.2±0.06 mM (±s.e.m.). The solid line is the experimentally fitted curve and is drawn according to equation (2), with $K_b(0) = 40$ mM and $\delta = 0.38$. The dotted line corresponds to equation (6) of the text, with $K_b(V)$ as above and $K_0(V) = K_0(0)[\exp(-zFV/RT)$. The parameters $K_0(0)$ and z, the effective gating charge, were measured as described elsewhere[12]; for these conditions, $K_0(0) = 1.0$, and $z = -1.1$.

sides of the membrane but with different accessibilities, expressed as differences in the apparent dissociation constants for *cis* and *trans* Cs^+ (see Fig. 5 legend). The family of bell-shaped curves shown in Fig. 5 (segmented line) was calculated for different $K_b(0)_{trans}/K_b(0)_{cis}$ ratios; thus, it is clear that the Cs^+ binding site is at least 100 times more accessible from the *cis* side than from the *trans* side.

Do the macroscopic and single-channel blockades agree?

When the macroscopic and single-channel dissociation constants are compared in identical experimental conditions (Fig. 6), we observe that they have similar values at positive potentials but are always different at negative potentials. At −42 mV, the macroscopic dissociation constant is about four times larger than the value obtained from single-channel measurements.

The reason for the discrepancy between the two measured 'blocking constants' is apparent from a consideration of the equilibria involved

$$\text{Closed} \overset{K_0(V)}{\rightleftharpoons} \text{Open} \overset{K_b(V)}{\underset{[Cs^+]}{\rightleftharpoons}} \text{Blocked} \qquad (5)$$

where $K_b(V)$ is the voltage-dependent dissociation for Cs^+ binding, and $K_0(V)$, defined as the ratio of the number of open to closed channels, is the voltage-dependent equilibrium constant for channel opening[12]. The measured single-channel blocking constant reflects only the binding of Cs^+ to the channel, and is equal to $K_b(V)$. In contrast, $K_d(V)$, the macroscopic blocking constant, is determined by both the above equilibria, and it can be shown that

$$K_d(V) = K_b(V)\left(1 + \frac{1}{K_0(V)}\right) \qquad (6)$$

Thus, at highly positive voltages, where $K_0(V) \gg 1$, $K_d(V)$ approaches the value of $K_b(V)$; at increasingly negative voltages, where $K_0(V) \ll 1$, the values of the two dissociation constants diverge. The dotted line in Fig. 6 is a theoretical curve for the macroscopic dissociation constant, $K_d(V)$, calculated on the basis of the equilibria described above, in which Cs^+ binds to the site in the open state only. This curve is fully determined by parameters measured independently (see Fig. 6 legend); the agreement between theory and experiment here is thus particularly noteworthy. Based on this departure of the macroscopic dissociation constant from the 'true' single-channel dissociation constant we conclude that the channel must be open before it can be blocked by Cs^+. In addition, Cs^+ must be released from the site before the channel can close.

Conclusions

We consider that the data presented here are consistent with a blocking model in which *cis* Cs^+ enters the channel, binds to a site and prevents the movement of K^+ through the channel as long as it occupies the site. We have not needed to invoke more complicated phenomena such as current-dependent blocking[18,19], multisite blocking[17,20], or a Cs^+ binding step before the opening of the channel. Although we have not yet carried out experiments to investigate single-site competition between K^+ and Cs^+, the chemical similarities between them suggest that Cs^+ may bind to a site normally engaged in the K^+ conduction process.

An important conclusion of this work is that when a blocking reaction is determined by macroscopic steady-state measurements, the observation of a voltage-dependent blocking constant does not imply that the blocking reaction is itself voltage dependent. Only when other voltage-driven equilibria are eliminated, as we have done here by single-channel measurements, can voltage-dependent blocking be demonstrated. In macroscopic electrophysiological measurements, this problem is eliminated by the use of instantaneous, rather than steady-state currents[3,19].

Finally, we should emphasise that the SR cation channel is being studied not in its native membrane, but in an artificial phospholipid bilayer. We do not know the extent to which the channel's properties are modified by this reconstitution procedure. Nevertheless, it is gratifying to find in a bilayer system Cs^+ blocking properties so far described only in electrophysiological preparations.

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In vitro formation of a complex between cytoskeletal proteins of the human erythrocyte

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The formation of a high-molecular weight complex between spectrin and F-actin depends on the presence of a third cytoskeletal constituent, protein 4.1. Electron microscopy shows that in this ternary complex the actin filaments are linked by bridges, which have the appearance of spectrin. The spectrin must be in the tetrameric state for such bridges to form: the dimer is evidently univalent, for it binds but forms no cross-links. G-actin also fails to form extended complexes. It is inferred that in the native cytoskeleton the spectrin is tetrameric and associated with 4.1 and probably oligomers of actin.

THE unique physical and functional properties which characterise the behaviour of the mammalian erythrocyte are known to be controlled by a cytoskeletal apparatus[1]. This is a complex of proteins associated with the cytoplasmic surface of the cell membrane, and its predominant constituent is the high-molecular weight protein, spectrin. However, almost nothing is known of the stoichiometry, patterns of interactions and structural nature of the cytoskeleton. An irregular fibrous network can be discerned in the scanning electron microscope[2], and a complex of proteins associated with a considerable quantity of lipid, obtained by extraction of erythrocyte ghosts with non-ionic detergent[3], probably contains all the elements of the cytoskeleton and evidently retains some of its properties[4]. Another preparation which can be regarded as a fragmented form of the cytoskeleton is the oligomeric complex, containing predominantly spectrin, which is extracted from the membrane into media of low ionic strength[5]. We have observed that both these forms of cytoskeletal complex contain, in addition to spectrin and actin, another membrane protein, termed 4.1 in the indexing system of Fairbanks *et al.*[6] (referring to mobility in a standard gel-electrophoretic system, containing SDS). This is a protein of apparent molecular weight 78,000, as judged by SDS electrophoresis, and it is present in roughly equivalent proportions to spectrin, taken as a tetramer of molecular weight 960,000. We describe here the requirements for the formation of a complex between the above three constituents of the cytoskeleton, its structural characteristics as seen in the electron microscope and the implications for the structure of the cytoskeleton in the cell.

Formation of the high-molecular weight complex

Earlier work[7] revealed that preparations of spectrin, chromatographed on Sephadex G-200, and therefore containing dimers and tetramers as well as the oligomeric complex in which the spectrin is associated with actin and 4.1, was able to stimulate the polymerisation of muscle G-actin, and subsequent studies linked this effect to phosphorylation of spectrin[4]. These results could not be reproduced with the dimer or tetramer fraction recovered from Sepharose 4B chromatography, however, and it became clear that they were a property of the oligomeric fraction (containing endogenous actin and protein 4.1). These results (Pinder *et al.*, unpublished) will be reported in detail elsewhere. They are relevant here only to the extent that they exclude any inference of a binary interaction of high affinity between spectrin and actin. Indeed, our attempts to demonstrate strong binding of spectrin to muscle G- and F-actin by analytical and preparative sedimentation techniques and by column chromatography in a variety of conditions were uniformly unsuccessful, except when the oligomeric complex or a trace of 4.1 was present.

In the present study, the formation of stable high-molecular weight complexes was detected by zone sedimentation in sucrose gradients. Spectrin and protein 4.1 were prepared from fresh human erythrocytes. The spectrin was recovered as the tetramer by extraction in the cold, followed by column chromatography on Sepharose 4B. Protein 4.1 was obtained in pure form from spectrin-depleted ghost vesicles, followed by ion-exchange chromatography on DE-52 cellulose (Fig. 1). Endogenous actin was prepared by extraction of an acetone-treated ghost residue into a medium of low ionic strength[8] (details are given in Fig. 2 legend). Muscle actin was obtained from rabbit skeletal muscle[9]. These proteins were electrophoretically pure (Fig. 2). When spectrin and F-actin are centrifuged through a sucrose gradient, the protein distribution is indistinguishable

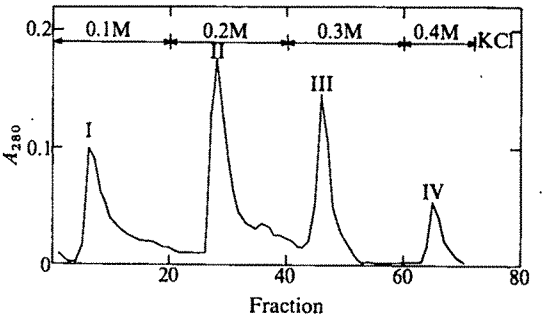


Fig. 1 Preparation of purified protein 4.1 from human erythrocyte membranes. In a typical preparation, 5 ml of packed vesicles remaining after extraction of spectrin at low ionic strength, are further extracted with 0.8 M potassium chloride, 10 mM sodium phosphate, 0.1 mM dithiothreitol and 3 mM phenylmethylsulphonyl fluoride, *p*H 8.0, in a total volume of 20 ml at 0 °C with stirring for 5 h. The vesicles are then removed by centrifugation at 80,000*g* for 1 h. The supernatant is dialysed against 7.5 mM sodium phosphate and 0.1 mM dithiothreitol, *p*H 8.0, and applied to a DE-52 ion-exchange column (10×0.9 cm) equilibrated with the same buffer. The column is eluted, first with 40 ml of the initial buffer, followed by two steps with added potassium chloride. A typical elution curve is shown. We have subsequently found that 0.16 M potassium chloride at the second step gives optimal resolution. The haemoglobin and most of band 6 are eluted before addition of salt. The peaks shown in the elution profile comprise: residual band 6 and minor protein constituents (I), protein 4.1 (II), residual spectrin, further traces of 4.1, as well as 2.1 and some minor protein bands (III) and remaining residual spectrin and some 2.1 (IV). The yield of protein 4.1 is 50–60% of the total present in the ghosts.

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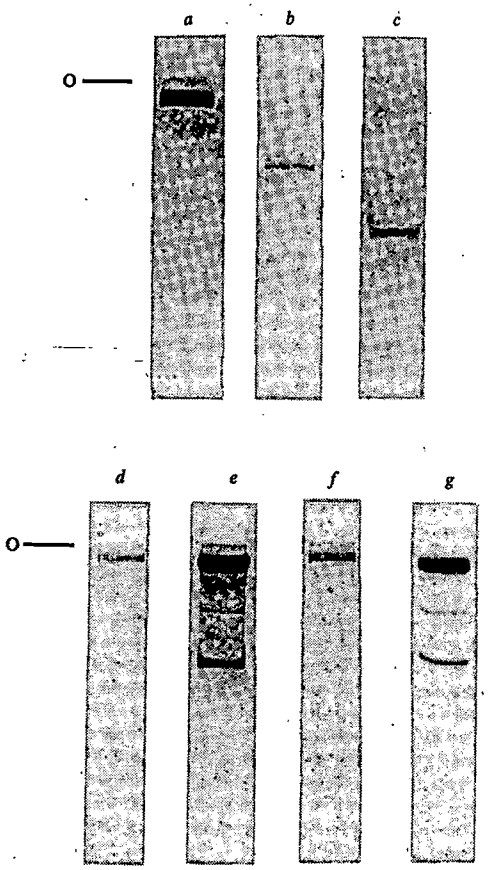


Fig. 2 Demonstration of ternary complex formation: analysis of sucrose gradient sedimentation fractions by gel electrophoresis in the presence of SDS[6]. *a–c* Show the starting materials, which are, respectively, spectrin tetramer, prepared by extraction of ghosts at 4 °C, followed by chromatography on a column of Sepharose 4B as described earlier[11], protein 4.1, prepared as described above, and erythrocyte G-actin. The latter is prepared by a method differing in detail from that described by Sheetz *et al.*[8]. In particular, after washing the ghosts from 60–70 ml packed human erythrocytes with 0.5 M potassium chloride and 10 mM Tris, *p*H 7.4, they were twice more washed with 1 mM Tris, 0.1 mM ATP and 0.1 mM dithiothreitol, *p*H 7.4, rather than distilled water. The acetone powder[8] was freed of excess acetone by suction on a medium sinter, rather than centrifugation, and was extracted according to the method of Spudich and Watt[9]. The actin was purified by three successive polymerisation–depolymerisation cycles (1–2 mg yield). Rabbit muscle G-actin was prepared by the method of Spudich and Watt[9]. To form the complex, the proteins were mixed and left for 60 min on ice in 0.1 M sodium chloride, 30 mM Tris, 0.1 mM magnesium chloride and 0.5 mM dithiothreitol, *p*H 8.0. The protein concentrations in the final mixture were: spectrin 1 mg ml^{-1}, F-actin 0.1 mg ml^{-1}, and protein 4.1 0–0.1 mg ml^{-1}. Aliquots of 0.2 ml of the mixture were applied to 4.5 ml linear sucrose gradients (10–30% w/v) in the above buffer, with a 0.5-ml cushion of 45% sucrose. After centrifugation at 4 °C and 100,000*g* for 14.5 h in a Beckman SW50 rotor, fractions were collected; pelleted proteins were dissolved in 0.2 ml of 0.1% SDS. Aliquots of 20–50 μl were analysed on the gels, which show the pellet fraction collected from a protein mixture of: *d*, 1 mg ml^{-1} spectrin and 0.1 mg ml^{-1} erythrocyte F-actin, with no 4.1; *e*, 1 mg ml^{-1} spectrin, 0.1 mg ml^{-1} F-actin and 0.1 mg ml^{-1} protein 4.1; *f*, the same, with erythrocyte G-actin in place of F-actin; *g*, the same mixture as in *e*, but with muscle in place of erythrocyte F-actin. The origin of migration is indicated.

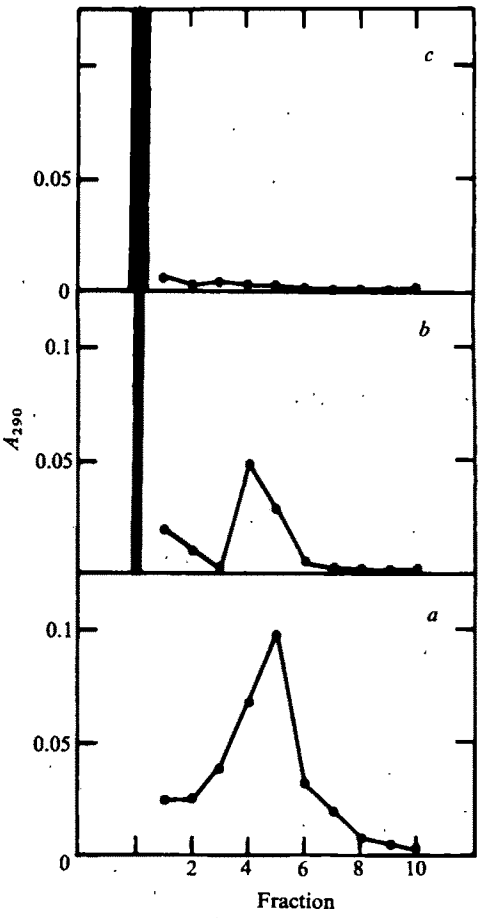


Fig. 3 Sucrose gradient velocity sedimentation of mixtures containing 0.5 mg ml^{-1} spectrin, 0.1 mg ml^{-1} erythrocyte F-actin and no added protein 4.1 (*a*), 30 μg ml^{-1} 4.1 (*b*) or 75 μg ml^{-1} 4.1 (*c*). The shaded region is the pellet. The experimental details are as described in Fig. 2 legend. Sedimentation is from right to left. In these conditions the presence of 75 μg ml^{-1} protein 4.1 is sufficient to cause the incorporation of all the spectrin (0.5 mg ml^{-1}) into a high-molecular weight complex.

from the superimposed patterns of the two proteins sedimenting separately. Similarly, there is no evidence from these experiments of the formation of a stable complex between spectrin and protein 4.1 alone. However, when all three proteins are mixed, a rapidly sedimenting component is obtained which, with an increasing proportion of 4.1, ultimately incorporates all the spectrin (Fig. 3). The composition of this stable ternary complex is reflected by the gel electrophoresis patterns in Fig. 2*e*. Complexes containing actin, spectrin tetramer and protein 4.1 in the molar ratio of about 3 : 1 : 1 have been obtained, but studies of the possible range of stoichiometries are still in progress. At all events, the results at this ionic strength show the assembly of a complex only if all three cytoskeletal constituents are present. Furthermore, when the actin is kinetically trapped in the depolymerised state (G-actin), at a concentration sufficiently low that no significant polymerisation occurs within the time scale of the experiment, only very small traces of a rapidly sedimenting complex appear (Fig. 2). This does not demonstrate that G-actin is incapable of forming a ternary complex with the other components, but does show that in these (physiological) solvent conditions and in the accessible concentration range, it is not able to participate in the formation of an extended network.

The results shown in Figs 2*e* and 3 were obtained with purified erythrocyte actin. Muscle actin behaves in a similar manner (Fig. 2*g*). Muscle actin was also used in the experiment shown in Fig. 4, which demonstrates the progressive incorporation of spectrin and F-actin into the complex with the addition of increasing proportions of 4.1. In Fig. 4*a* the small amount of complex formed reflects the concentration of residual 4.1, present in the spectrin preparation.

The formation of ternary complexes between cytoskeletal proteins reveals itself in other types of experiment, which also show that the formation of a network requires that spectrin be in the tetrameric state. In a mixture containing F-actin (1 mg ml^{-1}), protein 4.1 (0.05 mg ml^{-1}) and spectrin tetramer (0.4 mg ml^{-1})

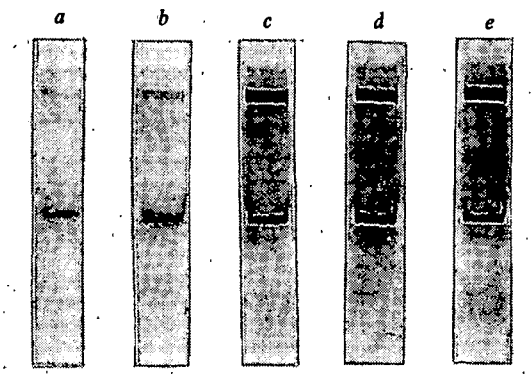


Fig. 4 Gel electrophoresis of the ternary complex, recovered as a pellet after sucrose gradient centrifugation (conditions as in Fig. 2 legend), as a function of the proportion of protein 4.1. In this case muscle F-actin was used. The total spectrin tetramer and F-actin concentrations were kept constant at 1 and 0.1 mg ml^{-1}, respectively. In *a* the pellet contains the complex formed as a result of the residual trace of 4.1 present in the spectrin. In *b–e* purified protein 4.1 was added at a concentration of 16, 40, 80 and 120 μg ml^{-1}, respectively.

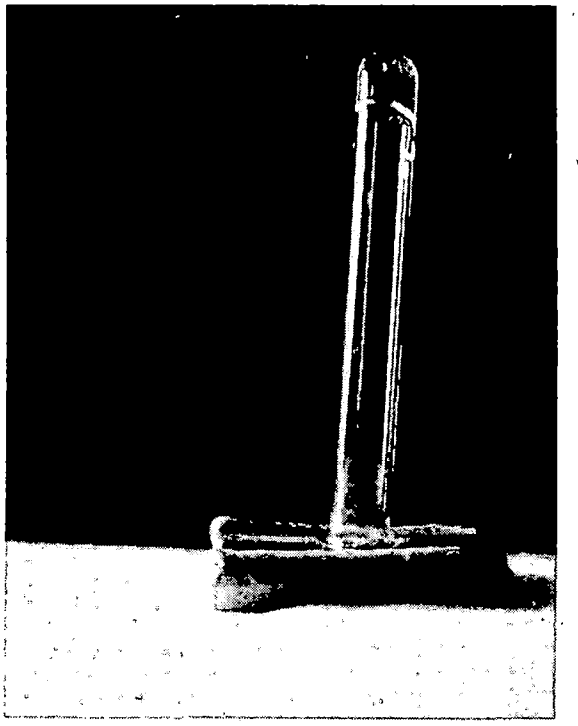


Fig. 5 Gel formed from a mixture containing 0.9 mg ml^{-1} muscle F-actin, 0.4 mg ml^{-1} spectrin tetramer and 0.05 mg ml^{-1} protein 4.1 (all purified as described in Fig. 1 legend). The medium contained 50 mM sodium chloride, 10 mM imidazole, 1 mM ATP, 1 mM dithiothreitol, 1 mM EGTA and 1 mM magnesium chloride, *p*H 7.2. No gel is formed in the absence of protein 4.1, in the absence of spectrin, or with the dimer form of spectrin in place of the tetramer.

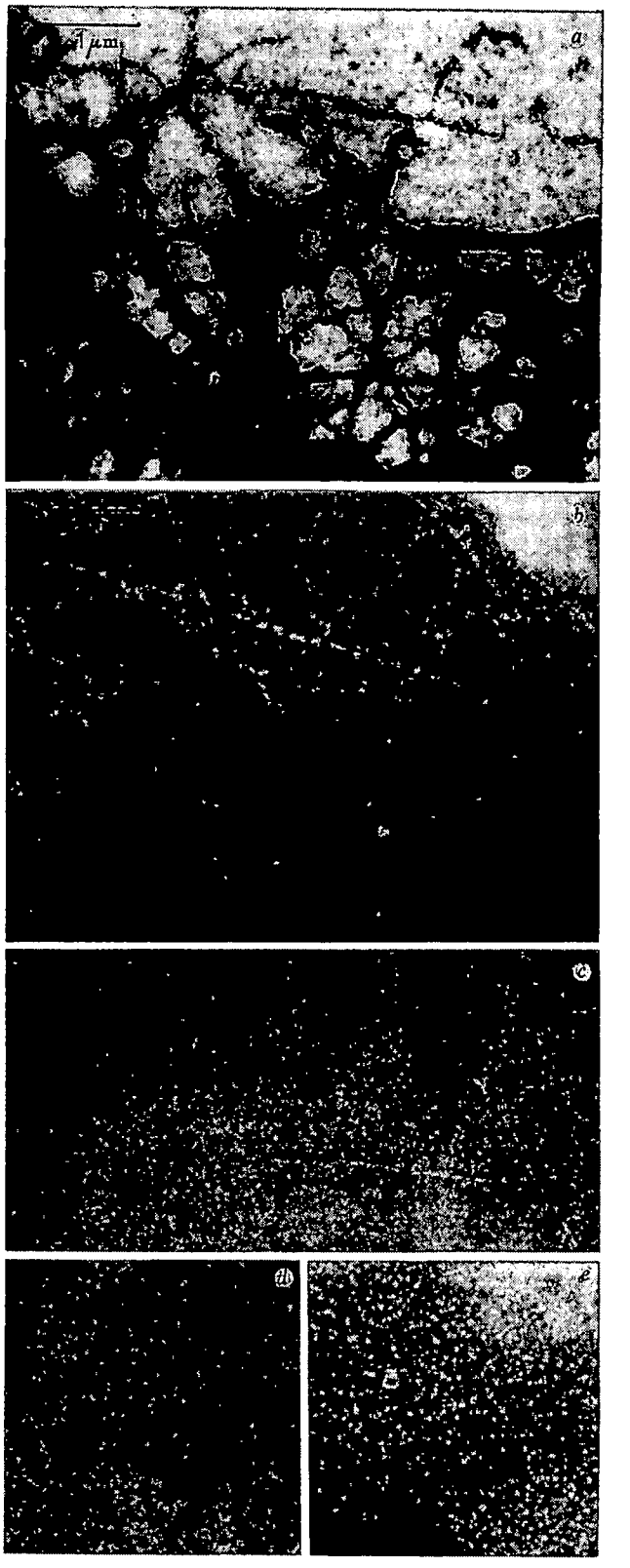


Fig. 6 Appearance in the electron microscope of complexes of F-actin with spectrin tetramer and protein 4.1. Complexes were prepared at a total protein concentration of about 1 mg ml^{-1}. After 10-fold dilution they were applied to carbon-coated grids, negatively stained with 1% uranyl acetate and examined in a Philips EM 200 instrument at 80 kV. *a*, Part of a field of complexed material showing a network of connected fibres. *b*, Complex shown at higher magnification, prepared as in *a* and freed of uncomplexed components by passage through a column (10 × 1 cm) of Sepharose 4B. The actin filaments are associated with thin flexible fibres, which form curved bridges where neighbouring filaments lie close together. Some of these are marked by arrows. *c*, Complex formed with spectrin dimers in place of tetramers in the ternary system. Appendages can be seen on the actin filaments, but no bridges between filaments. *d*, Spectrin tetramers alone, observed in the same conditions. *e*, Preparation of purified protein 4.1.

in 10 mM phosphate, 0.1 M sodium chloride, 2 mM magnesium chloride and 0.1 mM dithiothreitol, a gel is formed (Fig. 5). When two such mixtures, containing respectively spectrin dimer and tetramer, were centrifuged for 15 min at 20,000*g* to bring down high-molecular weight complex, 15 and 60% of the total protein was recovered as a pellet in the two cases. No gelation occurs in any binary combination of the proteins, nor when the actin is unpolymerised or the spectrin dimeric. The presence of the ternary complex in the cytoskeleton is presumably responsible for the failure of more than a part of the erythrocyte actin to extract into the low ionic-strength buffer in the G-form from the acetone powder. The remainder stays, together with spectrin and 4.1, in the pellet.

Electron microscopic evidence for ternary complex formation

Figure 6 shows the appearance of the complex in the electron microscope. Adjoining F-actin filaments are linked at intervals by curved bridges, leading to a separation in these regions of usually about 50 nm. In some cases the bridges seem to be more extended, corresponding to an inter-filament separation of up to 100 nm. The length and aspect of these bridges are consistent with their identification as spectrin tetramers as they appear when negatively stained (Fig. 6*d*). The tetramers linking the actin filaments are evidently not as extended as they appear after deposition from solutions containing 70% glycerol and shadowing. Shotton *et al.*[10] have shown that in these conditions the tetramers have a shadowed length of nearly 200 nm. By contrast, protein 4.1 when negatively stained is seen as a compact and presumably globular species (Fig. 6*e*). The simplest model for the complex, consistent with its appearance in the electron microscope, would involve the attachment of spectrin tetramers, through sites at their extremities, to 4.1 and actin. That the spectrin tetramers should in fact be bifunctional species follows from the electron microscopic observations of Shotton *et al.*[10], for the tetramer is evidently derived from the dimer by end-to-end association, and studies on self-association of the dimer[11] have shown that no (isodesmic) associated states higher than the tetramer are formed; in other words that, with the individual polypeptide chains of each dimer apparently lying parallel[10], the self-association sites are internally satisfied (D_2 symmetry).

The postulated structure of the complex seen in Fig. 6 implies that the spectrin must be divalent if a network is to be formed, and that the dimer will not suffice. Parallel experiments with the purified dimer, which is indefinitely stable in the cold[11], lead to no such networks as those shown in Fig. 6*b*, although binding evidently occurs. In the electron microscope the spectrin can be recognised as threads attached to the F-actin filaments (Fig. 6*c*), but as expected from the present results, no bridges can be discerned.

The interactions leading, as we have described, to the formation of stable ternary complexes of the cytoskeletal proteins can be readily reconciled with several earlier observations, including our own (already referred to) on the efficacy of the oligomeric complex, as opposed to pure spectrin, in inducing polymerisation of G-actin. The formation of a complex between muscle F-actin and (crude) spectrin was, in fact, first inferred by Tilney and Detmers[12] from an increase in viscosity accompanying mixing. Moreover, the attachment of actin filaments to erythrocyte membrane vesicles, only when these contain the oligomeric complex, has recently been reported by Cohen and Branton[13]. Puszkin *et al.*[14] have described the association of G-actin, evidently by way of polymerisation, to unfractionated spectrin (eluted from Sephadex G-100 and therefore containing the oligomeric complex) adsorbed to latex beads.

Clearly, we cannot rule out the possibility (indeed it must be postulated) that binary interactions between spectrin, actin and 4.1 occur, and these might be expected to reveal themselves in solvents of lower ionic strength. It should be recognised that the removal of all traces of 4.1 from spectrin is extremely difficult, and that even material purified on a Sepharose 4B column contains detectable amounts. (This can probably be ascribed to a self-associated form, the elution profile of which overlaps that of spectrin.) Because few cross-links are required to generate a fraction of high molecular weight, which will manifest itself in hydrodynamically based assays, such spectrin preparations are expected to exhibit artefactual binding to F-actin. This would be most obtrusive when sensitive methods such as low-shear viscometry are used. We consider it likely that a recent brief report of such interactions[15] can be explained in these terms.

Discussion

Our results agree with our earlier conclusion[11] that the spectrin is present in the cytoskeletal network *in situ* as a tetramer, rather than a dimer. They also imply that actin in the erythrocyte cytoskeleton is unlikely to be in the monomeric G-form, and that models based on such a view[16] are over-simplifications. However, neither is it likely to be present as long filaments, as in the cytoplasm of many cells, for the quantity of actin present in the erythrocyte is so small that such filaments, even if arranged in a uniform two-dimensional net, would leave such large areas between the intersections that it would be hard to envisage the functional utility of such an arrangement. The inference seems inescapable that the actin is present as very short oligomeric segments. This would also be compatible with the character of the oligomeric spectrin–actin–4.1 complexes extracted from the membrane, which contain no F-actin filaments, and would also explain the failure of electron microscopic examination to detect such filaments in ghosts or in the cytoskeletal complexes prepared by extraction with non-ionic detergent. The only evidence known to us which suggests the presence of actin in the self-associated state, comes from cross-linking experiments[17], in which oligomeric actin species are indeed found. If we assume a 50-nm end-to-end distance for the spectrin tetramers, the amounts of the proteins present in the cell would be sufficient to cover the cytoplasmic surface with (for example) a square lattice, constructed of spectrin tetramers, with connecting elements at the junctions, containing 5–10 actin monomers.

Many other aspects of the protein interactions in the cytoskeleton remain unclear. Thus, whereas there is evidence to link phosphorylation of spectrin to changes in its interaction with its neighbouring proteins[4,18] and with the control of cell shape[19], we have no evidence that it modifies the formation of the ternary complexes which we have described. Further investigations in this regard are in progress. We note finally that another protein constituent, which migrates in SDS gels in a position very close to that of actin, and therefore probably has a subunit molecular weight of about 45,000, is found associated with the cytoskeleton complex, regardless of how it is prepared. We still know nothing about the nature and function of this species.

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Nucleotide sequence of the gene coding for the major protein of hepatitis B virus surface antigen

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DNA extracted from hepatitis B virus Dane particles has been cloned in bacteria using a plasmid vector. A full-length clone has been examined by restriction endonuclease analysis, and the nucleotide sequence of an 892-base pair fragment from cloned hepatitis B viral DNA encoding the surface antigen gene is reported. The amino acid sequence deduced from the DNA indicates that the surface antigen is a protein consisting of 226 amino acids and with a molecular weight of 25,398. The portion of the gene coding for this protein apparently contains no intervening sequences.

VIRAL HEPATITIS is a major worldwide public health problem. Its aetiology is associated with at least three distinct viral families: hepatitis A, hepatitis B and non-hepatitis A/hepatitis B (ref. 1). The characterisation of the viruses, the pathology associated with the infection and the development of effective means of control have been severely hampered by the very narrow host range specificity of the viruses, and the inability to replicate the virus and obtain cytopathology in tissue culture systems. Nevertheless, progress has been made in identification and preliminary characterisation of the viruses from the sera of infected individuals and from livers at autopsy. The hepatitis A virus is apparently a picornavirus detected as a 27-nm particle[2,3]. In addition to being infective in humans and chimpanzees, it has recently been transferred to marmosets and has been replicated in tissue culture[4-6].

The plasma of individuals infected with heptatitis B show three major particulate structures containing the antigenic determinants apparently specified by the hepatitis viral genome. These include the predominant 22-nm particles, 22-nm filaments of various lengths, and the 42-nm spherical form known as the Dane particle[7,8]. The Dane particle is probably the hepatitis B virion. Recently, a human hepatoma cell grown in tissue culture was shown to produce small quantities of hepatitis B antigens[9].

In instances where productive infections and high titres of viruses cannot be obtained in alternative hosts or tissue culture cells, recombinant DNA methods can be used to obtain large quantities of the virus genome for characterisation and biological activity studies. Structural analysis of the various antigens may allow identification and sequence determination of the various genes of the virus. This information, plus the availability of the viral genome, will greatly aid the study of the pathology and eventually the development of antiviral therapy. In particular, it may be possible, using segments of the viral genome, to produce appropriate viral antigens in alternative hosts such as bacteria or yeast for the development of a vaccine.

Hepatitis B is a particularly attractive paradigm for the development of this approach. The hepatitis B viral genome is relatively simple, perhaps consisting of only 3,200 bases[10,11]. The surface coat, which can be removed by treatment with detergents, contains two main polypeptides, P1 (22–24,000 MW) and P2 (25–29,500 MW)[12]. These molecules are antigenically indistinguishable and protein components are probably identical. Their amino acid compositions are the same within the limits of the analyses used, and the first 19 residues from the N-terminus and the three amino acid residues at the carboxy-terminus seem to be identical[13]. The higher molecular weight molecule (P2) is a glycoprotein, and it has been suggested that the major virus-specific surface antigens are contained within a single gene. Five to seven other polypeptides ranging upward in size to 97,000 MW have been reported to be present in surface antigen preparations[14]. It is not known whether any or all of these molecules are coded for by the viral genome. Viral core preparations contain a major polypeptide of MW 17,000 to

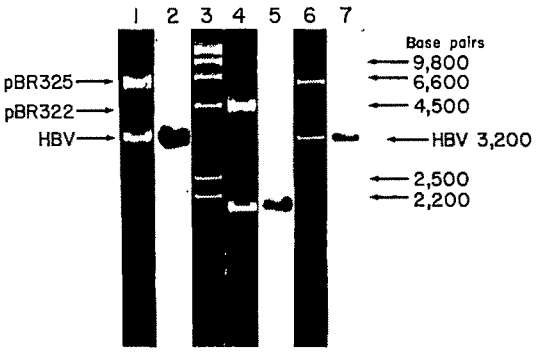


Fig. 1 Cloning and hybridisation of hepatitis B viral DNA. Double-stranded DNA was synthesised in Dane particles (isolated from human sera by Merck Laboratories) by the endogenous DNA polymerase reaction[10]. Incubation was for 3 h at 37 °C in a reaction mixture containing Dane particles (250 ng of DNA), 50 mM Tris-HCl (*p*H 7.5), 0.1 M NaCl, 20 mM $MgCl_2$, 40 mM NH_4Cl, 0.4% NP40, 10 mM 2-mercaptoethanol and 0.2 mM each dATP, dGTP, dCTP and dTTP. DNA was isolated by phenol extraction and ethanol precipitation after digesting the Dane particles for 60 min at 56 °C with proteinase K (0.8 mg ml^{-1}). Dane DNA was digested to completion with endonuclease *Eco*RI (producing a single 3,200-base pair fragment) and with *Bam*HI (yielding two fragments of 2,100 and 1,100 base pairs). Digested Dane DNA (250 ng) was ligated to the appropriate plasmid (50 ng of *Eco*RI digested pBR325 (ref. 18), or 680 ng of phosphatase-treated, *Bam*HI-digested pBR322 (ref. 30)) for 15 h at 14 °C in a reaction mixture containing 50 mM Tris-HCl (*p*H 8), 1 mM ATP, 10 mM $MgCl_2$, 20 mM dithiothreitol and 1 unit of T4 DNA ligase (New England Biolabs). The reaction mixture was used to transform *E. coli* HB101 (in P3/HV1 containment conditions) or *E. coli* χ1776 (in P2/HV2 containment conditions). Recombinant colonies derived from the DNA ligated to the *Bam*HI site of pBR322 were selected by their ampicillin resistance and tetracycline sensitivity[17] and screened for plasmid size[19]. A clone (pHBV-2100) of approximately 6,500 base pairs was identified by a modified toothpick assay[19]. Recombinant colonies derived from the DNA ligated to the *Eco*RI site of pBR325 (ref. 18) were selected by their sensitivity to chloramphenicol. A clone (pHBV-3200) containing plasmid DNA of approximately 8,600 base pairs was identified by the toothpick assay. Supercoiled plasmid DNA was isolated from either clone by a cleared lysate procedure[31], purified by CsCl–ethidium bromide gradient centrifugation[31], digested with *Eco*RI or *Bam*HI and analysed in 1% agarose gels. DNA fragments were hybridised according to Southern[32] to Dane particle DNA labelled by nick translation[33]. Lane 1, ethidium bromide staining of fragments derived from digestion of pHBV-3200 with *Eco*RI. Lane 2, hybridisation of the same fragments to ^{32}P-Dane particle DNA and autoradiography. Lane 3, *Hin*dIII-digested λ DNA as MW standards. Lane 4, ethidium bromide staining of fragments derived from digestion of pHBV-2100 with *Bam*HI. Lane 5, hybridisation of the same fragments to ^{32}P-Dane particle DNA and autoradiography. Lane 6, ethidium bromide staining of fragments derived from digestion of pHBV-64 (a clone of pHBV-3200 viral DNA inserted in the *Pst*I site of pBR322). Lane 7, hybridisation of the same fragments to ^{32}P-Dane particle DNA and autoradiography.

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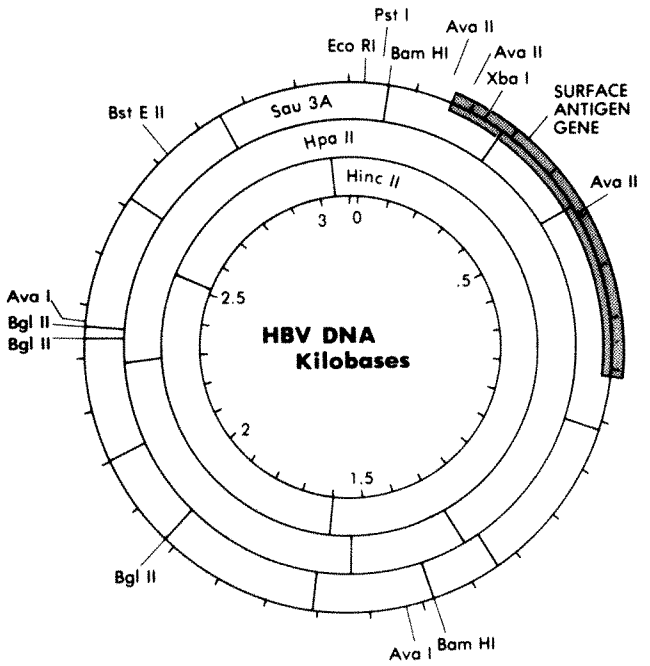


Fig. 2 Restriction endonuclease cleavage map of cloned HBV DNA in plasmid pHBV-3200. Cleavage with restriction endonucleases was carried out in the conditions described by the suppliers with excess amounts of each enzyme. Analysis of the resulting fragments was by electrophoresis in 7% or 10% acrylamide slab gels in 50 mM Tris-borate (*p*H 8.3) and 1 mM EDTA or in 1–2% agarose slab gels in 50 mM Tris-acetate (*p*H 8.3) and 1 mM EDTA.

19,000 and a variety of other larger polypeptides of MWs 25,000 to 200,000 (ref. 12). Whether these polypeptides are virus-specific determinants is unknown. A third class of antigens associated with hepatitis infection are the e antigens[15], which recent studies suggest are a dissociated form of the core antigen[16].

Here we report the cloning in bacteria and structural analysis of DNA from hepatitis B virus (HBV). We have defined the location of the viral surface antigen gene in the restriction endonuclease cleavage map of the cloned HBV genome. Nucleotide sequence of an 892-base pair region encoding the surface antigen gene allows us to deduce the complete amino acid sequence of this protein.

Cloning of DNA from hepatitis B virus Dane particles

DNA from Dane particles (prepared from pooled sera received from Merck, Sharp and Dohme) was labelled with ^{32}P-dATP and ^{32}P-dCTP using the endogenous DNA polymerase reaction[10], and purified as described by Landers *et al.*[11]. Several different DNA preparations were cleaved by the restriction endonuclease *Eco*RI into a single fragment of approximately 3,200 base pairs and by *Bam*HI into two fragments of approximately 2,100 and 1,100 base pairs (results not shown). For cloning, the Dane particle DNA was digested with *Bam*HI endonuclease and separately with *Eco*RI, ligated to the respective sites of pBR322 (ref. 17) and pBR325 (ref. 18) and used to transform *Escherichia coli* χ1776. Ampicillin-resistant colonies derived from the DNA ligated into the *Bam*HI site were further screened for their sensitivity to tetracycline[17] and the size of the plasmids they contained[19]. Similarly, recombinant colonies derived from the DNA ligated into the *Eco*RI site of pBR325 were screened for their sensitivity to chloramphenicol[18] and by analysis of their plasmids[19].

Putative recombinant plasmids were isolated and examined by agarose gel electrophoresis after digestion by *Eco*RI and *Bam*HI. One clone from the *Eco*RI experiment (named pHBV-3200) was found to contain a hybrid plasmid with an inserted DNA fragment of approximately 3,200 base pairs, the reported size of linearised viral DNA[20]. A clone from the *Bam*HI experiment (named pHBV-2100) was found to contain a plasmid with a 2,100-base pair insert. The identities of the inserts within pHBV-3200 and pHBV-2100 were verified by cleavage of the plasmids with *Eco*RI and *Bam*HI and hybridisation of the fragments with ^{32}P-labelled Dane particle DNA (Fig. 1). Physical mapping of both inserted fragments with restriction endonucleases showed that pHBV-2100 contains a *Bam*Hi-derived insert also present in pHBV-3200.

Location and sequence analysis of the fragment containing the HBV surface antigen gene

A physical map containing the cleavage sites for several restriction endonucleases was prepared from pHBV-3200. This map has been obtained by a combination of enzyme digestions and DNA sequence analysis (Fig. 2). No restriction sites were found for *Xma*I, *Sst*I, *Kpn*I, *Hpa*I, *Xho*I and *Sac*I endonucleases. The map is similar, but not identical, to those recently described by others for cloned hepatitis viral DNA[21–23]. For example, the map reported by Charnay *et al.*[23] shows an extra *Bam*HI site in the region containing the surface antigen gene. Examination of the nucleotide sequence in this region (Figs 3, 4) shows that the sequence GGATCA coding for the amino acids Gly-Ser may be converted to a *Bam*HI site GGATCC by a change of one base in the third position of the codon, which would therefore not result in a change in the amino acid sequence.

The HBV surface antigen gene was located by extensive nucleotide sequence analysis of the entire cloned viral genome to identify the sequence coding for the 19 amino acids present at the amino-terminus of the protein[13]. The location of the gene within the physical map of the cloned viral DNA is shown in Fig. 2.

The nucleotide sequence of an 892-base pair region encoding this gene was determined as summarised in Fig. 5 legend. The sequence, including the restriction enzyme cleavage sites, is presented in Fig. 3. The translation of this sequence in one of the phases (Fig. 4) predicts precisely the sequence of the 19 N-terminal amino acids of the proteins P1 and P2, except for residue 15, in which the DNA sequence predicts a leucine instead of the serine. Re-examination of the original data of Peterson and Vyas has shown that residue 15 is indeed leucine, and that the original report was incorrect. Furthermore, the amino acid sequencing data up to residue 31 is completely compatible with the amino acid sequence predicted by the DNA, with the exception of residue 24 (arginine), in which insufficient amounts of the phenylthiohydantoin amino acid were formed for analysis (D. Peterson, personal communication). The reported C-terminal sequence, Val-Tyr-Ile[13], is in phase from 204 amino acid residues towards the 3′ RNA terminus just before the ochre termination codon, UAA. The polypeptide encoded by these sequences is 226 amino acids long and has a MW of 25,398, in satisfactory agreement with the mass for P1 (22–24,000) determined by SDS gel electrophoresis[12]. The amino acid composition also agrees very closely with that reported for this protein[13]. Note also the relatively high content of proline (10.2%), tryptophan (5.8%), aromatic amino acids (Trp, Tyr, Phe) (15.0%) and hydrophobic amino acids (Val, Ile, Leu) (26.6%).

Because of the prevalence of intervening sequences in eukaryotic genes (see ref. 24 and refs therein), it is not possible to presume the colinearity of a gene with the amino acid sequence of the protein product. However, there is no evidence for an intervening sequence in the surface antigen gene, as the molecule predicted by the DNA sequence closely approximates the characteristics of the isolated surface antigen P1. Any intervening sequence(s) would have to be small (<150 bases); most intervening sequences in structural genes are longer. The N-terminal and C-terminal ends of the molecule are in phase, thus any intervening sequence must also maintain the phase. Furthermore, preliminary studies suggest that the number of major peptides resolved from trypsin-treated aminoethylated

P1 agrees quite well with those predicted from the amino acid sequence derived from the gene (D. Peterson, personal communication). Further studies on P1 or its mRNA should resolve this issue decisively. In the meantime, it seems justified to assume colinearity of the gene with the mRNA for purposes of examination of the primary structure of P1. The high concentration of proline residues and their dispersion throughout the molecule preclude the possibility of a high content of α-helix. Indeed, according to the Fasman rules[25], there is only approximately 4% α-helix. However, there is a significant amount of β form (~30%) and also more than 10% β-turns. Thus, the molecule probably has a globular configuration. Perhaps the most striking primary structural feature of the molecule is a 19-amino acid long hydrophobic region. The sequences flanking this region are particularly rich in proline and cysteine residues. This hydrophobic region may be the site for intermolecular interaction of P1 monomers, and the abundant cysteine residues could make intra- and intermolecular linkages to form the surface coat of the virus. Because of the likelihood that P1 is glycosylated, we searched for possible sites of glycosylation at asparagine residues (Asn-X-Ser/Thr)[26]. There are three such sites at amino acid positions 3, 59 and 146.

Directions for future study

Although P1 and P2 are the major peptides, five or six other proteins of larger size (up to ~100,000 MW) have been observed in surface antigen preparations[12]. One of these, P6 (~72,000 MW), is sometimes present as a major component[12]. This family of molecules cross-reacts antigenically and hence must be related structurally. Whether they represent aggregates of P1/P2 or are distinct molecules is not known. We have not detected nucleotide sequences encoding similar amino acid sequences in the other regions of the viral genome. The possibility that they are formed by processing from a single peptide precursor should also not be overlooked. However, the DNA sequence suggests that it is unlikely that a precursor could extend from the C-terminus because several other termination codons exist in the 3′ RNA direction. Furthermore, the nucleotide sequence which could code for a protein of 190 amino acids begins with the methionine codon AUG, 41 bases after the termination codon of the surface antigen gene (Fig. 5). Screening of the sequence of the entire virus suggested that this may be the gene coding for the major core protein. Further structural analysis of the core protein must be carried out before a

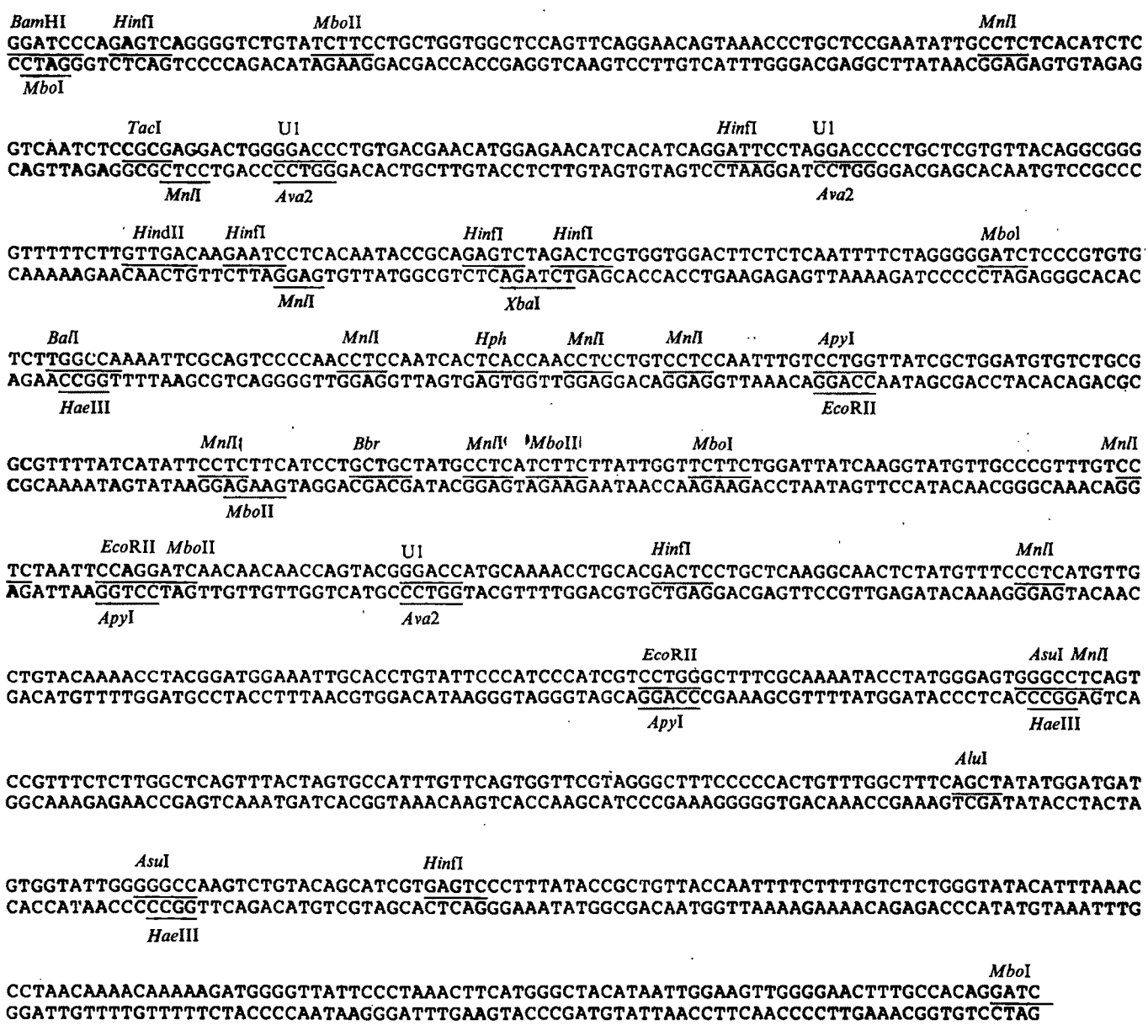


Fig. 3 Nucleotide sequence of the HBV surface antigen gene and adjacent regions. Sequence analysis was carried out by the method of Maxam and Gilbert[34] as outlined in Fig. 4. The enzyme *Mbo*I is an isoschizomer of *Sau*3A. U1 indicates Sau_{961} sites.

```
G GAU CCC AGA GUC AGG GGU CUG UAU CUU CCU GCU GGU GGC UCC AGU UCA GGA ACA GUA AAC CCU GCU CCG AAU AUU GCC UCU CAC AUC

                                                  1                                                  10
                                                 met glu asn ile thr ser gly phe leu gly pro leu leu val leu gln ala
UCG UCA AUC UCC GCG AGG ACU GGG GAC CCU GUG ACG AAC AUG GAG AAC AUC ACA UCA GGA UUC CUA GGA CCC CUG CUC GUG UUA CAG GCG

          20                                        30                                                  40
gly phe phe leu leu thr arg ile leu thr ile pro gln ser leu asp ser trp trp thr ser leu asn phe leu gly gly ser pro val
GGG UUU UUC UUG UUG ACA AGA AUC CUC ACA AUA CCG CAG AGU CUA GAC UCG UGG UGG ACU UCU CUC AAU UUU CUA GGG GGA UCU CCC GUG

          50                                        60                                                  70
cys leu gly gln asn ser gln ser pro thr ser asn his ser pro thr ser cys pro pro ile cys pro gly tyr arg trp met cys leu
UGU CUU GGC CAA AAU UCG CAG UCC CCA ACC UCC AAU CAC UCA CCA ACC UCC UGU CCU CCA AUU UGU CCU GGU UAU CGC UGG AUG UGU CUG

          80                                        90                                                  100
arg arg phe ile ile phe leu phe ile leu leu leu cys leu ile phe leu leu val leu leu asp tyr gln gly met leu pro val cys
CGG CGU UUU AUC AUA UUC CUC UUC AUC CUG CUG CUA UGC CUC AUC UUC UUA UUG GUU CUU CUG GAU UAU CAA GGU AUG UUG CCC GUU UGU

          110                                       120                                                 130
pro leu ile pro gly ser thr thr thr ser thr gly pro cys lys thr cys thr thr pro ala gln gly asn ser met phe pro ser cys
CCU CUA AUU CCA GGA UCA ACA ACA ACC AGU ACG GGA CCA UGC AAA ACC UGC ACG ACU CCU GCU CAA GGC AAC UCU AUG UUU CCC UCA UGU

          140                                       150                                                 160
cys cys thr lys pro thr asp gly asn cys thr cys ile pro ile pro ser ser trp ala phe ala lys tyr leu trp glu trp ala ser
UGC UGU ACA AAA CCU ACG GAU GGA AAU UGC ACC UGU AUU CCC AUC CCA UCG UCC UGG GCU UUC GCA AAA UAC CUA UGG GAG UGG GCC UCA

          170                                       180                                                 190
val arg phe ser trp leu ser leu leu val pro phe val gln trp phe val gly leu ser pro thr val trp leu ser ala ile trp met
GUC CGU UUC UCU UGG CUC AGU UUA CUA GUG CCA UUU GUU CAG UGG UUC GUA GGG CUU UCC CCC ACU GUU UGG CUU UCA GCU AUA UGG AUG

          200                                       210                                                 220                 226
met trp tyr trp gly pro ser leu tyr ser ile val ser pro phe ile pro leu leu pro ile phe phe cys leu trp val tyr ile OC
AUG UGG UAU UGG GGG CCA AGU CUG UAC AGC AUC GUG AGU CCC UUU AUA CCG CUG UUA CCA AUU UUC UUU UGU CUC UGG GUA UAC AUU UAA


ACC CUA ACA AAA CAA AAA GAU GGG GUU AUU CCC UAA ACU UCA UGG GCU ACA UAA UUG GAA GUU GGG GAA CUU UGC CAC AGG AUC
```

Fig. 4 The amino acid sequence of the HBV surface antigen gene. The nucleotide sequence presented in Fig. 3 was translated in one reading frame so as to correspond to the known first 19 amino acids of the N-terminus of the HBV surface antigen[13].

definitive assignment can be made.

It is possible that a putative precursor could extend from the N-terminus. In certain instances, promoter sites in eukaryotic genes may be identified from the DNA sequence. Hogness and Goldberg (personal communication) have postulated that the canonical sequence TATAAATA may interact with RNA polymerase, as *E. coli* polymerase interacts with the Pribnow box[27]. Initiation of transcription usually occurs 23 ± 1 nucleotides from this site. We have been unable to find this sequence in the 200 nucleotides immediately preceding the N-terminal methionine. However, more distant variations on the Hogness–Goldberg sequence occur (D. S. Hogness, personal communication; E. Ziff, personal communication and ref. 28). For example, the sequence TATATT is found 184 bases in the 5′ RNA direction from the initiator methionine codon of the surface antigen gene and also occurs in the IgG2 light chain[29]. However, there are no specific data and the site of initiation of transcription and the structure of the initial gene product of the HBV surface antigen gene therefore remain in doubt.

Tiollais and coworkers have defined the single-stranded regions in the DNA of the virus[20]. From their map, it is clear that single-stranded regions of DNA extend into the surface antigen gene, but probably not as far as the initiation of transcription. These observations localise the coding strand of the gene on the full-length DNA strand.

The availability of abundant quantities of hepatitis B viral DNA through molecular cloning procedures will allow a comprehensive study of its gene products and the pathology of the virus. The present work enables specific studies on the expression of the gene coding for the major surface antigen to be carried out. The availability of this gene may also provide an alternative means for production of the antigen which in turn can be used as a vaccine against hepatitis B infection.

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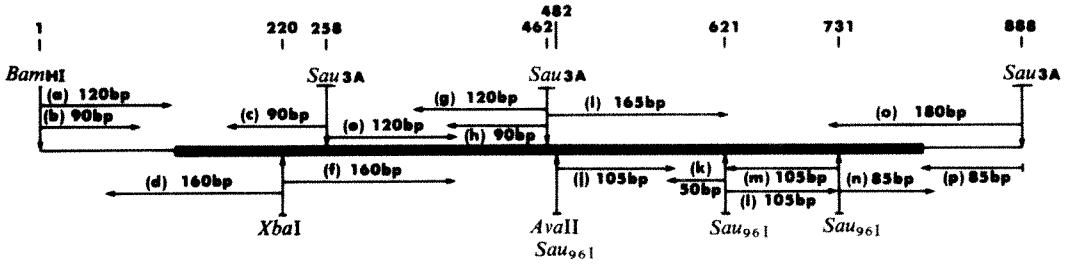


Fig. 5 Sequence strategy for the 889-base pair fragment containing the HBV surface antigen gene. Nucleotide residues are numbered in the direction from 5′ to 3′ in the message strand, beginning at the *Bam*HI site. The solid bar represents the 678-base pair region coding for the protein. Only the restriction sites used as starting points for sequencing are shown. For labelling, DNA was treated with 5 μg of bacterial alkaline phosphatase (Worthington) at 37 °C for 60 min in 20 mM Tris-HCl (*p*H 8.0), phenol extracted and treated with 10 units of T4 polynucleotide kinase (Boehringer) at 37 °C for 30 min in a reaction mixture containing 50 mM glycine (*p*H 9.5), 10 mM $MgCl_2$, 10 mM dithiothreitol, 0.1 mM spermidine and 0.001 mM [γ-^{32}P]ATP (~4,000 Ci mmol^{-1}). Fragments labelled only at one end were isolated by gel electrophoresis after either digestion with a restriction enzyme or strand separation. DNA sequence was carried out by the method of Maxam and Gilbert[34]. Arrows indicate the direction and number of base pairs sequenced in each experiment. Sequences were determined in both strands or in duplicate for all but 50 residues. In this 50-base pair region the data were very reliable. (Sequence data are available from the authors on request.)

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letters

Discovery of IR bursts from Liller I/MXB1730−333

THE first detection of IR bursts from the object[1] known as Liller I which has been identified[2] with the rapid X-ray burster MXB1730−333 is reported here. The rapid burster is the only one of its kind that has been extensively studied in the X-ray region[3–5] and some of its known characteristics can be summarised as follows. When it is active it gives several thousand X-ray bursts per day. It operates in two modes: mode I (usually in March and September every year), mode II (usually in April and October every year). In mode I it gives large X-ray bursts with energy in the range 10^{39}–10^{40} erg and also small bursts with energy in the range of 10^{38}–10^{39} erg; while in mode II it primarily emits bursts with energy typically close to 10^{39} erg. These bursts are called type II on the basis of the constant character of their spectrum during the decay phase. The rapid burster occasionally also gives bursts known as type I which are characterised by the softening of the X-ray spectrum during the decay phase. The energy in these bursts is usually in excess of 10^{39} erg and their frequency is about one every few hours.

Liller[1] searched for an optical counterpart of the rapid burster and found a highly reddened compact cluster with a red magnitude $m_r = 21$ in the error box of the rapid burster[6]. This has been identified as a globular cluster by Kleinmann *et al.*[7], with the help of IR observations. The identification of this cluster with the rapid burster was supported by Doxsey *et al.*[2] who obtained a more precise position for the X-ray burster. Kleinmann *et al.*[7] observed the globular cluster continuously for about an hour on 29 May 1976 and did not detect any variation greater than 10% of the total IR flux. However, the rapid burster may have ceased activity in April 1976.

Two of us (K.M.V.A. and S.M.C.)[8] have proposed a model for the X-ray emission from the rapid burster based on accretion of matter onto the poles of a rotating magnetic neutron star. More recently (unpublished data), the cyclotron emission of electrons in the accreting column above the poles of the magnetised neutron star has been considered and emission in the IR and optical regions suggested; these calculations have prompted us to undertake the present experiment. The optical emission from the rapid burster may, however, be difficult to detect because of the large interstellar extinction[1,7] of $A_v = 11 \pm 1$ mag. On the other hand, the interstellar extinction in the IR is very small which makes it possible to observe the radiation.

The observations were made on the 1-m telescope of the Indian Institute of Astrophysics at Kavalur (lat. 12° N), India on the night of 4–5 April 1979 between 21.29 and 00.10 UT. At the Cassegrain focus a liquid-nitrogen cooled photometer was used with a set of IR filters, three apertures and a Fabry mirror imaging the primary of the telescope on a 0.5 mm × 0.5 mm InSb detector. The incoming beam was $f/20$ giving the scale of 10 arc s mm^{-1} at the focal plane. For the present measurement we used a 2 mm aperture corresponding to 20 arc s field of view. The incoming beam was chopped alternately on the source and a neighbouring part of the sky with a throw of 20 arc s by a tertiary mirror inclined at 45° to the incident beam with a frequency of 16 Hz. The servo-controlled square wave gave a duty cycle of 75%. The Liller I signal was detected with the phase sensitive detector followed by the low pass filter with a time constant of 0.6 s. In the case of standard stars, the phase sensitive detector

Table 1 IR bursts from Liller I/MXB1730−333

Burst No.	Time of occurrence (UT)	Rise time (s)	Duration (s)	FWHM (s)	Peak luminosity (10^{37} erg s^{-1} in 0.3 μm interval at 1.6 μm)	Burst energy (10^{38} erg in 0.3 μm interval at 1.6 μm)
1	21 h 33 min 00 s	2	37	12	2.2	3.0
2	22 41 24	2	36	12	1.9	2.0
3	23 45 06	3	36	12	2.0	2.6
4	23 47 30	3	24	9	1.7	1.5
5	00 03 00	2	36	10	2.2	2.5
6a	00 04 54	2	20	6	2.3	1.7
6b	00 05 12	2	19	6	1.6	0.9

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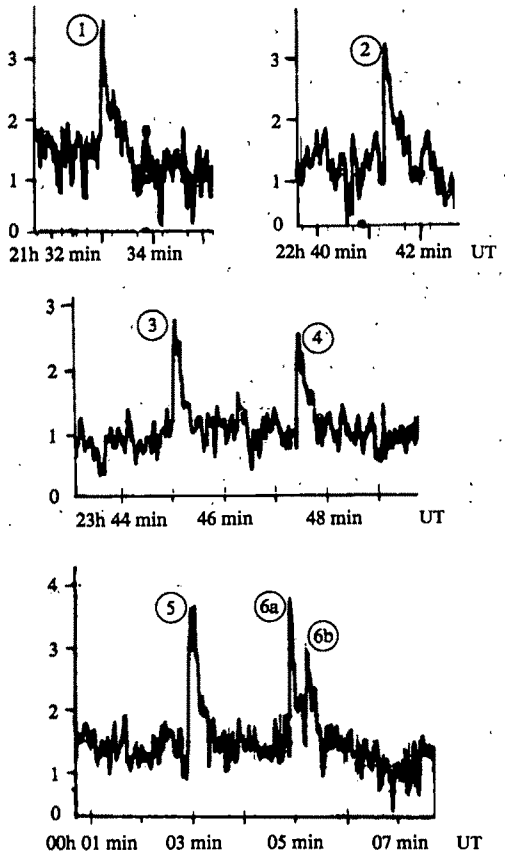


Fig. 1 Six IR bursts from the source Liller I/MXB1730−333, the rapid burster. Note the composite structure of burst number 6; the two components are designated a and b. The ordinate numbers are in units of 10^{-16} W cm^{-2} μm^{-1}.

output was linearly integrated for 6 s. The measurements reported here were made with an H-filter (λ peak 1.6 μm, half width 0.3 μm, maximum transmission ~75%). Several standard IR sources were observed before the observation of Liller I.

The steady IR flux from Liller I was observed and its magnitude calculated as $m_H = 7.5 \pm 0.4$ using a nearby star[9] IRC-30317 for calibration. Kleinmann *et al.*[7] have reported $m_H = 6.4$ for Liller I using 24 arc s beam size and 90 arc s chopper throw. The present measurement has (1) a smaller beam and (2) a smaller chopper throw. Taking the flux distribution of Liller I as given by Kleinmann *et al.*[7] we estimate that our measurement will be higher by $m_H = 0.54$. After accounting for (1) and (2) above and subtracting 0.54 from our m_H value, it is still higher by 0.56 ± 0.4 than the flux value given by Kleinmann *et al.*[7].

Six IR bursts detected during the 2.5 h observing time are shown in Fig. 1. The details of the time of occurrence, rise time, duration, FWHM, peak intensity and energy in the bursts are summarised in Table 1. Kleinmann *et al.*[7] have given interstellar extinction in the K-band $A_k = 1.1$ mag for Liller I. Assuming an inverse wavelength dependence for extinction, we estimate $A_H = 1.4$ mag. While calculating peak luminosity and energy we have subtracted $A_H = 1.4$ mag from observed m_H values. The energy in the bursts is estimated by integrating the area under the burst profiles and assuming a distance of 10 kpc to the object[3]. Note the close similarity of the various characteristics of the successive IR bursts. They seem to rise rapidly in a time period of nearly 2 s and decay gradually over time of about 30 s. Note that burst number 6 is composed of two bursts, the characteristics of which are separately given in Table 1. The occurrence of burst numbers 3, 4 and 5, 6 in rapid succession (~150 s) may be significant.

The remarkably close similarity of the rise time, duration and gradual decay of intensity of the IR bursts with the corresponding characteristics of type-I X-ray bursts[3,4] strongly indicate the association between the two. The low rate of occurrence of the IR bursts accords with this association.

The observed IR emission from the bursts is unlikely to be of black body type. If a region of 2 light seconds in size is assumed then the peak burst luminosity implied a brightness temperature of 7×10^7 K at 1.6 μm if the radiation were thermal. This is greater than the X-ray burst temperature and would imply a much greater total luminosity.

The observations of the rapid burster at the X-ray and IR wavelengths suggest that there could be emission of burst radiation at all intermediate wavelengths. It is likely that in spite of large interstellar extinction the optical bursts from the rapid burster would be detected. It will also be interesting to observe other X-ray burst sources at different IR wavelengths.

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Possible chemical impact of planetary lightning in the atmospheres of Venus and Mars

THE importance of lightning as a source of trace gases in the Earth's atmosphere has long been recognised. In 1827, von Liebig proposed lightning as a source of atmospheric fixed nitrogen[1] and recent investigations have supported this hypothesis[2–7]. In the Earth's pre-biological atmosphere lightning may have produced organic molecules, such as HCN, which led to the evolution of life[8]. After life evolved, but before photosynthesis and biological nitrogen fixation developed, lightning may have provided an abiotic source of organic C and fixed N to the growing biota[9,10]. The presence of cloud layers on Venus[11] and synoptic scale dust storms on Mars[12] suggest that lightning may also occur on these planets, and indeed there have been reports of lightning being detected on Venus by American and Soviet spacecraft. The implications for atmospheric chemistry of lightning on Venus and Mars are discussed here.

The production of gases by an electrical discharge involves high-temperature chemical reactions in and around the discharge channel[13]; the air around the channel is heated by the radially propagated shock wave. As the temperature, T, of the heated air decreases at some rate characterised by a cooling time, $\tau_T(T)$, a species X may attain a final concentration in

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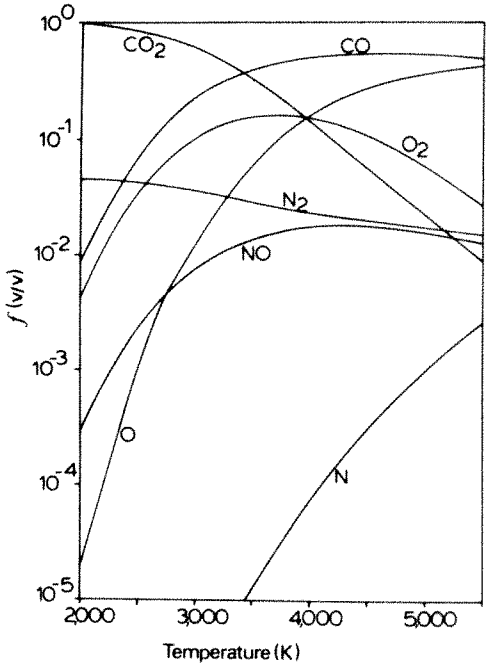


Fig. 1 Equilibrium mixing ratios of CO_2, N_2, O_2, O, NO and N as a function of temperature in heated Cytherean air. To obtain these results, the equilibrium abundances of the 24 species previously listed[7] plus S, S_2, S_2O, SO, SO_2, SO_3, COS, CS_2 and H_2SO_4 were determined as a function of temperature using the thermochemical data from the JANAF tables[14]. Ambient conditions were chosen for an altitude of 55 km, the lower cloud region; ambient $T = 310$ K (ref. 15), ambient $CO_2 = 0.66$ atm, $N_2 = 0.032$ atm, $SO_2 = 1.4 \times 10^{-4}$ atm, $O_2 = 4 \times 10^{-5}$ atm, $H_2O = 4 \times 10^{-4}$ atm, and $\rho = 1.2$ kg m^{-3} (ref. 16).

excess of its ambient value, thus leading to a net production of X. The final concentration of X is approximated by the equilibrium concentration $f_x^\circ(T_F(X))$ (v/v) at $T_F(X)$, the 'freeze-out' temperature for X. T_F is given by the temperature at which

$$\tau_x(T_F(X)) = \tau_T(T_F(X)) \quad (1)$$

where τ_x is the chemical lifetime of species X. When $T > T_F$, then $\tau_x < \tau_T$ and the chemical reactions are sufficiently rapid to keep X in thermochemical equilibrium. However, for $T < T_F$, $\tau_x > \tau_T$ and chemical reactions are too slow to adjust to the rapidly decreasing T; X, therefore, 'freezes-out' with mixing ratio $f_x^\circ(T_F(X))$. While a lower abundance in X is favoured thermodynamically at low T, the kinetics are too slow for readjustment. P, the net yield of X due to this process, is approximated by[7]

$$P(X) \simeq \frac{f_x^\circ(T_F) M(E_0, T_F(X))}{E_0} \quad \text{(molecules J}^{-1}\text{)} \quad (2)$$

where M is the number of molecules per metre heated to or above T_F in the region where X is being produced, and E_0 is the discharge energy (in J m^{-1}). Generally, T_F is between 1,000 and 5,000 K.

Figure 1 illustrates the thermochemical equilibrium concentrations of several species of interest for air in the cloud layer of Venus heated to temperatures of 2,000–5,500 K, for $A = \rho/\rho_0 = 1$ where ρ is the mass density within the heated air and ρ_0 is the ambient mass density. The variation of martian equilibrium composition with temperature is quite similar to that of Venus. For both planets we find significant enhancements of CO, O_2, NO and O for $T > 2{,}000$ K, indicating that these species may be produced by lightning on Venus and Mars. Production of these gases by lightning could have significant implications for the chemistry and evolution of the atmospheres of Venus and Mars and needs, therefore, to be investigated.

Whether lightning does, in fact, produce trace species depends on the cooling characteristics of the discharge; an estimate of the source rates requires knowledge of the energy properties and the frequency of planetary lightning. Thus, a reliable prediction of the chemical impact of lightning on Venus and Mars will have to await appropriate observations. However, we estimate the source rates by assuming that lightning of Venus is similar to that on Earth, as an illustration.

The source rates of CO, O_2, NO, and O on Venus with Earth-like lightning are estimated using an earlier reported method[7]; $\tau_T(T)$ and $M(T, E_0)$ are calculated from Lin's[17] differential equations describing a cylindrical shock wave. Yields predicted by this method for NO, N_2O and CO in discharges in present atmospheric conditions have compared favourably with laboratory experiments[4,7,18]. The shock wave differential equations are solved with a specific heat ratio, $\gamma = 1.16$, appropriate to a CO_2 atmosphere at temperatures between 1,500 K and 5,000 K[19]. As these equations indicate that, for the temperature region of interest, $1 \leq A = \rho/\rho_0 \leq 10$, f_x° and τ_x are determined for $A = 1$ and 10 as limiting values. Finally, as CO_2, which comprises about 95% of the ambient atmosphere, is significantly dissociated at temperatures above 2,000 K, stoichiometrically it follows that virtually all O atoms in our system must originate from CO_2 dissociation and we therefore require that $P(CO) = P(NO) + 2P(O_2) + P(O)$.

Using the earlier method

$$\tau_T(T) \sim \left(\frac{1}{T(R)} \frac{dT(R)}{dt}\right)^{-1} = \frac{5.7 \times 10^{-5} E_0^{1/2}}{\rho_0^{1/2} T} \quad \text{(s)} \quad (3)$$

for $\gamma = 1.16$, where R is the shock front radius, and t is time. For $E_0 \sim 10^5$ J m^{-1}, as on Earth[20], and $\rho_0 = 1.2$ kg m^{-3} at 55 km, the lower portion on Venus' cloud layer, $\tau_T \sim (5\text{–}7) \times 10^{-6}$ s for $2{,}500 \leq T \leq 5{,}000$ K, as depicted in Fig. 2. Figure 2 also shows τ_{CO}, τ_{O_2} and τ_{NO}. Using equation (1) to determine T_F, Fig. 2 indicates that CO freezes-out first; for $A = 1$, $T_F(CO) = 5{,}500$ K with $f_{CO}^\circ(5{,}500\ \text{K}) = 0.5$ and for $A = 10$, $T_F(CO) = 3{,}500$ K with

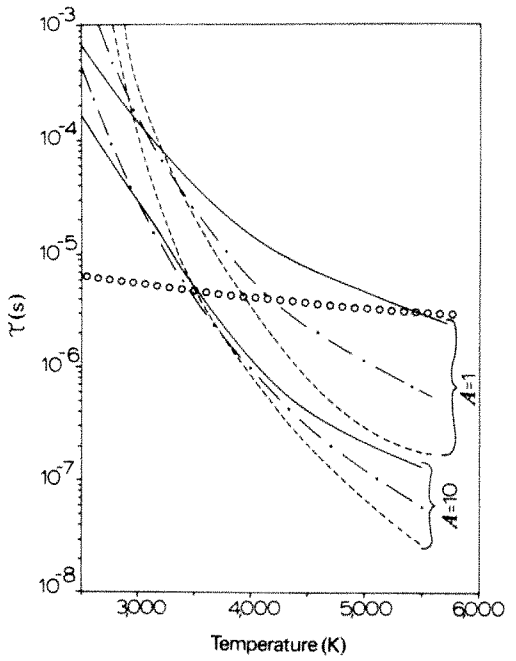


Fig. 2 Temperature dependence of the cooling time, τ_T (OO), and chemical lifetimes; τ_{CO} (———), τ_{O_2} (— · —); and τ_{NO} (– – –) for $A = 1$ and 10 where $A = \rho/\rho_0$. The chemical lifetimes were determined from densities taken from the thermochemical equilibrium calculations and the appropriate kinetic data[21,22].

Table 1 Estimated production rates for Earth-like lightning on Venus

Species	$f^{\circ}_{x}(T_F(X))$* (v/v)		$P(X)$ (molecules J^{-1})		$S(X)$† (molecules cm^{-2} s^{-1})	
	$A=1$	$A=10$	$A=1$	$A=10$	$A=1$	$A=10$
CO	0.50	0.26	1.4×10^{17}	1.1×10^{17}	1.4×10^{10}	1.1×10^{10}
O_2	0.16	0.12	4.5×10^{16}	5×10^{16}	4.5×10^{9}	5×10^{9}
NO	0.018	0.013	5×10^{15}	6×10^{15}	5×10^{8}	6×10^{8}
O	0.16	0.018	4.5×10^{16}	0.8×10^{16}	4.5×10^{9}	0.8×10^{9}

* For cooling time characterised by $E_0 = 10^5$ J m^{-1}, $\rho = 1.2$ kg m^{-3}.
† For 10^{-7} W cm^{-2} dissipated by lightning.

$f^{\circ}_{CO}(3{,}500\ K) = 0.26$. For $\gamma = 1.16$, Lin's[17] equations yield

$$M(T_F, E_0) = \frac{1.55\times10^{21}}{T_F} E_0 \quad \text{(molecules m}^{-1}\text{)} \qquad (4)$$

and, substituting equation (4) into equation (2), $P(CO) \sim (1.1\text{–}1.4)\times10^{17}$ molecules J^{-1}.

The yields of O_2, NO and O are controlled by the CO kinetics. As CO_2 dissociation is the only source of O atoms, O_2, NO and O cannot be produced outside the CO-producing region. In the CO-producing region, the equilibrium abundances and chemical lifetimes of O_2, NO and O below $T_F(CO)$ may be determined from the thermochemical calculations with the CO abundance set at $f^{\circ}_{CO}(T_F(CO))$. The final number of O_2, NO and O molecules or atoms produced is then given by their respective freeze-out abundances multiplied by $M(T_F(CO), E_0)$, the numbers of molecules heated to or above $T_F(CO)$. We find that O_2 and NO freeze-out at approximately the same temperature; for $A=1$, $T_F(O_2) \sim T_F(NO) \sim 4{,}000$ K, and for $A=10$, $T_F(O_2) \sim T_F(NO) \sim 3{,}300$ K. The final concentration of O, which freezes-out at a lower T, is determined by conservation of oxygen. Table 1 summarises the estimated final abundances of the species and their yields, and also indicates the resulting global source rates, S, obtained by assuming that lightning on Venus, as on Earth, dissipates about 10^{-7} W cm^{-2}.

Before this study it was believed that O, O_2 and CO were produced exclusively above the clouds by photochemistry[23,24]. Downward transport is thought to supply the lower atmosphere with O_2 and CO and thereby fuel the conversion of S and SO_2 to H_2SO_4–H_2O particles and SO_2 to liquid S, components of the clouds of Venus[25–29]. Photochemical models[23] estimate downward fluxes at 62 km of 1.4×10^{12} CO molecules cm^{-2} s^{-1} and 5.5×10^{11} O_2 molecules cm^{-2} s^{-1}. Comparison with the numbers in Table 1 indicates that, if lightning on Venus were similar to that on Earth, it would contribute a small, additional, local source of CO and O_2 to augment the upper atmospheric source. However, if lightning on Venus were 100 times more energetic or frequent, and this is certainly a possibility, the CO and O_2 discharge sources would be comparable with the downward transport source and thus could have a key role in maintaining the clouds. If in the 55 km region SO_2 is about 100 p.p.m. (ref. 16), the major sink for lightning-produced O is probably $O + SO_2 + M \rightarrow SO_3 + M$, further augmenting SO_4^{2-} production.

While we predict 2–3 times more CO production than O_2 production by lightning, Oyami *et al.*[16] found at least 60 times more O_2 than CO in the lower atmosphere of Venus. However, note that the abundance of an atmospheric constituent is a function of both the loss and source terms. In the terrestrial atmosphere, for example, CO is considerably more reactive than O_2 and thus, while the O_2 source is only 100 greater than that of CO[30,31], the O_2 abundance is 10^6 greater than the CO concentration. While a similar effect may cause the apparent enhancement of O_2 relative to CO on Venus, complete photochemical model calculations which include all the relevant sources and sinks are needed to explain the observed composition of the atmosphere of Venus.

The production of NO by lightning may result in the formation of HNO_2 and HNO_3 and thus imply the trace presence of NO_2^- and NO_3^- in the clouds. The NO_2^- and NO_3^- are probably removed in precipitation and may either be recycled back to N_2 or remain on the surface. For the NO yield obtained, the time to consume atmospheric N_2 is about 5 billion years and thus, in the absence of recycling, roughly equal amounts of nitrogen would reside in the atmosphere and lithosphere. A similar effect on Mars could help to explain the apparent deficiency of nitrogen on this planet relative to Earth. However, on Mars we find rather low yields for lightning with energies of 10^5 J m^{-1} and cooling rates of about 10^{-6} s; the thin martian atmosphere leads to slow chemical reaction rates, high freeze-out temperatures, and low chemical yields. For lightning on Mars to lead to significant chemical production cooling times of 0.01–1.0 s are required which, according to equation (3), would imply extremely energetic discharges.

We find that lightning, if present, may produce CO, O_2, NO and O in the atmospheres of Venus and Mars. Calculations for lightning on Venus indicate that this process could conceivably influence the atmospheric nitrogen budget and the sulphur chemistry that maintains the Venus cloud layer. A final determination of the chemical role of planetary lightning, however, will have to await systematic observations of the presence and properties of electrical discharges on Venus and Mars.

The National Astronomy and Ionosphere Center is operated by Cornell University under contract with the NSF. This work was supported partly by the NSF under Grant ATM77-20179 to W.L.C.

Note added in proof: Since completion of this work, documentation of the evidence for lightning on Venus gathered by American and Soviet spacecraft has appeared[32,33], further emphasising the potential importance of planetary lightning.

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Photoinduced electron transfer across a water–oil boundary as a model for redox reaction separation

THE creation of artificial solar energy conversion and storage systems that mimic the photosynthetic pathway has evoked great interest in recent years[1]. One approach to the photolysis of water involves the mediation of two photosystems in the generation of reduced and oxidised species that are the active components in the decomposition of water[2,3]. However, homogeneous (aqueous) solutions of these components suffer from the basic limitation that the reducing and oxidising agents can react with each other and thus no net reaction can be observed. Several kinds of synthetic 'photosynthetic membranes' have been suggested as a means of separating the two redox units and overcoming these fundamental difficulties[3]. Recently, photosensitised electron transfer across vesicle walls has been demonstrated and suggested as a means for generating oxidising and reducing agents in separate water compartments[4]. We report here a photochemical electron transfer across the interface of a water-in-toluene microemulsion, and propose this system as a model for the separation of oxidised and reduced

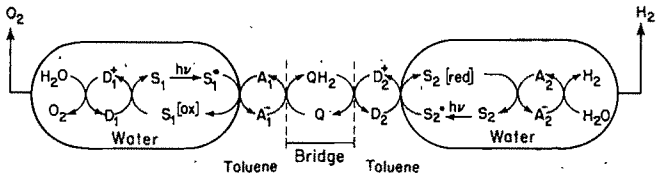


Fig. 1 General scheme for water photodecomposition using two half cells of water-in-toluene microemulsions.

species. It is well known that surfactant molecules aggregate to reversed micelles in organic solvents[5]. Reversed micelles entrap water to form 'water pools' in a continuous oil phase. The proposed general model for the compartmentalisation of two water phases and its utilisation in the photolysis of water is shown in Fig. 1.

The model system involves the generation of an oxidised donor (D_1^+) and a reduced acceptor (A_2^-) in the water droplets of two separate half cells using the two sensitisers S_1 and S_2 respectively. The complementary redox agents of this process (A_1^- and D_2^+) are confined to the organic phase, so that back reactions are inhibited. The two half cells are bridged by electron and proton carriers to regenerate A_1 and D_2. Regeneration of D_1 and A_2 is coupled to oxidation and reduction of water, so these must be charge storage catalysts[2,3]. Thus, all the components, except water, are recycled.

In principle, the electron transfer process of one half cell can be divided into two distinct parts: (1) from the water to the interphase, (2) from the interphase into the continuous organic solution. Electron transfer from the water phase to the interphase was investigated in a microemulsion in which the 'water pools' included EDTA as the donor, and tris(2,2'-bipyridine)ruthenium(II) ($Ru(bipy)_3^{2+}$) as the photosensitiser. The acceptor, 1,1'-dihexadecyl-4,4'-bipyridinium chloride (HV^{2+}), is expected to be located at the interphase boundary as a result of its amphiphilic structure. The microemulsion was prepared by adding 0.15 ml of 0.3 M ammonium EDTA solution (pH = 8.5) and 21 μl of 10^{-2} M $[Ru(bipy)_3]Cl_2$ solution to 2.9 ml of toluene containing 220 mg of dodecylammonium propionate as surfactant (0.3 M with respect to the surfactant). The mixture was vortex stirred to clarity. Into the resulting solution 2 mg of HV^{2+} were dissolved by vortex stirring. The microemulsion solution was transferred into a cuvette of 1-cm pathlength, and an inert atmosphere in the cell was generated by evacuation followed by nitrogen bubbling through the solution for 15 min. The contents of the sealed cuvette were stirred while being

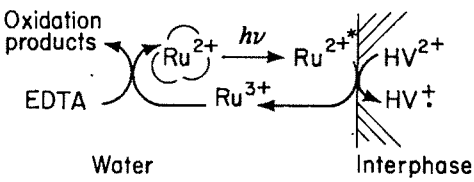


Fig. 2 Electron transfer from the aqueous phase to an acceptor in the interphase.

illuminated with blue light (incident photon flux ~10–16×10^{-7} einstein s^{-1}). The formation of HV^+ was followed spectroscopically[6]. After 4 min of illumination 75% of the parent HV^{2+} had been reduced ($\phi_{max} = 1.3 \pm 0.4\%$). Introducing air into the cuvette reoxidised HV^+ to HV^{+2} and revealed that no change in the original $Ru(bipy)_3^{2+}$ concentration occurred. Furthermore, the fact that the mole ratio HV^{2+}:$Ru(bipy)_3^{2+}$ was 13:1 emphasises that the photosensitiser acted as a catalyst and was recycled during the HV^+ formation.

The size of the water droplets[5,7] ensures numerous collisions of the sensitiser with the interface during its excited state lifetime (~0.6 μs)[8]. Hence, the results are rationalised by the electron transfer from photoexcited $Ru(bipy)_3^{2+}$ to the interphase located acceptor HV^{2+}. The resulting $Ru(bipy)_3^{3+}$ is reduced by EDTA and thus the photosensitiser is recycled. Therefore, electron transfer from a donor in the water phase to an acceptor in the interphase has been demonstrated (see Fig. 2).

However, in order to simulate the general scheme proposed in Fig. 1, electron transfer into the continuous organic phase is required. For this, an acceptor that is originally located in the interphase, while its reduced form is extracted from this region, into the bulk organic phase, seems to be desirable. Furthermore, coupling of this acceptor with a second acceptor present in the continuous organic phase should regenerate the acceptor at the interface. Benzylnicotinamide (BNA^+) seems to fulfill these requirements, as it is expected to concentrate at the interface, while its reduced form should migrate into the bulk organic solution[9]. Hence, we have investigated the photosensitised reduction of the dye, 4-dimethylaminoazobenzene (dye) present in the continuous organic phase, mediated by BNA^+ at the interface and $Ru(bipy)_3^{2+}$ in the water droplets. As in the previous experiment, EDTA was used as donor. The microemulsion system was prepared as follows. 230 mg of dodecylammonium propionate and 3.7 mg of BNA^+ (4.8×10^{-3} M) were dissolved in 2.9 ml of a 5×10^{-5} M solution of 4-dimethylaminoazobenzene in toluene. 0.1 ml of ammonium EDTA solution (0.3 M, pH = 8.5) and 3 μl of Ru $(bipy)_3^{+2}$ solution (10^{-2} M) were added to the toluene solution. The resulting microemulsion solution was illuminated under an inert atmosphere with blue light. The progress of the electron transfer was followed by the disappearance of the dye absorption at λ = 402 nm (ε = 22,000 mol^{-1} cm^{-1}). The change in the dye concentration as a function of illumination time is shown in Fig. 3. After 4 min of illumination, 80% of the dye had been reduced

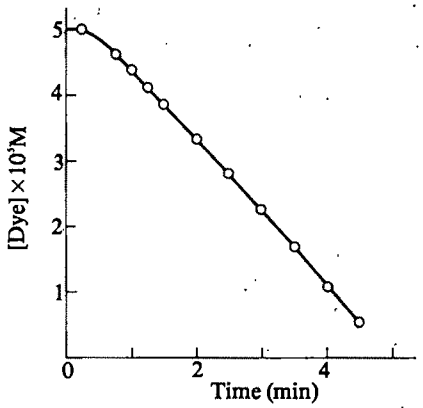


Fig. 3 The reduction of 4-dimethylaminoazobenzene as a function of illumination time.

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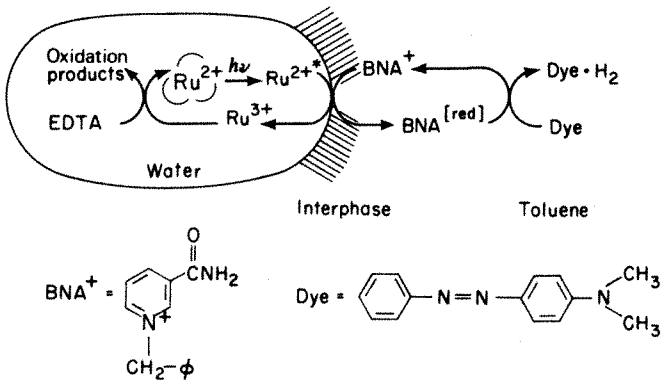


Fig. 4 Cyclic mechanism for electron transfer across the interface.

($\phi_{max} = 0.13 \pm 0.04\%$). The reduced dye product (Dye H_2) was identified as the corresponding hydrazo derivative. On the introduction of I_2 or H_2O_2, the reduction product Dye H_2 could be reoxidised to the diazo-dye[10]. At the end of the dye photoreduction, the $Ru(bipy)_3^{2+}$ concentration seemed to be unchanged. The experimental mole ratio of the dye: $Ru(bipy)_3^{2+}$ (5:1) indicates the photocatalytic activity of the ruthenium complex. Furthermore, in control experiments excluding any of $Ru(bipy)_3^{2+}$, EDTA, or BNA^+, no reduction of the dye could be observed on illumination. Hence, all the components of the system are crucial in the reduction process. The fact that BNA^+ is required in the process emphasises that it acts as a mediating agent in photoreduction of the dye.

These results are rationalised by a cyclic redox mechanism as shown in Fig. 4. The electron transfer from photoexcited $Ru(bipy)_3^{2+}$ to BNA^+ is followed by the reduction of the dye dissolved in the continuous organic phase. The photosensitiser is in turn regenerated by the oxidation of EDTA. The thermodynamic balance of the net reaction, reduction of the dye by EDTA to the corresponding hydrazo derivative, is uphill in free energy ($\Delta G^\circ \sim 37$ kcal per mol of EDTA consumed)[11]. Thus, the process represents a net storage of energy.

Our results demonstrate that an endoergic redox reaction across a water–oil interface has been carried out. The compartmentalisation of the redox reaction may serve as a key solution in the photolysis of water. Further steps, involving reversible acceptor–donor couples separated by this interface, as well as the introduction of adequate agents for the oxidation and reduction of water, are being investigated.

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Strong electric field effects on proton transfer between membrane-bound amines and water

DURING water demineralisation by electrodialysis much of the current is carried by hydrogen and hydroxyl ions[1,2]. This is attributed to an effect known as water splitting, which occurs near those surfaces of the ion exchange membranes which are depleted of ions during current flow (see Fig. 1). I have found[3] that water splitting only occurs in sulphonic acid membranes if certain impurities are present. But in contrast, it is an intrinsic property of anion exchange membranes in which the functional group is a tertiary alkyl amine. It is also intrinsic to membranes with quaternary ammonium ions if the surface groups have been converted to the tertiary form. An investigation of water splitting for the latter type of membrane (Negev Institute A)[4] is described here. It will be shown that the effect could be due to a 1,000-fold increase in the forward rate constant for reaction (2), caused by the strong external electric field (10^7–10^8 V m^{-1}) at the interface.

The measurements were made in a cell with eight chambers (Fig. 2.). The central two were separated by the anion exchange membrane under study. The others isolated the central chambers from the *p*H changes occurring at the electrodes. Water splitting originated in the surface layer of the anion exchange membrane, on the cathode side (see Fig. 1). The current carried by the hydrogen and hydroxyl ions (J_W), was calculated from the rate of p_H change of the central solutions.

Water splitting was only observed when the current exceeded a threshold. It then generally accounted for about one-third of the total current. The maximum value of J_W at room temperature exceeded 50 A m^{-2}. At higher temperatures J_W increased, saturating at a value of about 400 A m^{-2} at 100 °C, in 10 mM and 0.1 M NaCl solutions. The measurements were reproducible to within 10%; however, the temperature at the surface of the membrane was uncertain over a wide range of currents because of local heating.

To appreciate the magnitude of the effect consider the production of H^+ and OH^- ions in a membrane from ordinary water dissociation. For a membrane 30 μm thick with an average water concentration (C_W) of 10 M the rate is 6×10^{-6} mol m^{-2} s^{-1}. These ions recombine; however, even if they did reach the membrane boundaries the contribution to J_W would be only 0.6 A m^{-2}. Considering that water splitting only originates near the surface, the experimental value of 50 A m^{-2} at room temperature is several orders of magnitude greater than what could possibly be attributed to ordinary water dissociation.

It is now shown that the reaction layer is in the space charge region at the surface of the membrane (see Fig. 1). We define boundaries $x = 0$, δ of the reaction layer according to $J_H = 0.99\, J_W$ at $x = 0$ and $J_{OH} = 0.99\, J_W$ at $x = \delta$. Outside this region reaction flow is negligible and $\mu_W + \mu_H + \mu_{OH} = 0$, where μ denotes electrochemical potential. The outward flow (ϕ) of water ions is balanced by an inward flow of water molecules; $C_W \gg C_H, C_{OH}$. Thus

$$\left|\frac{d\mu_W}{dx}\right| \ll \left|\frac{d\mu_H}{dx}\right| \quad \text{at } x = 0 \text{ and } \left|\frac{d\mu_W}{dx}\right| \ll \left|\frac{d\mu_{OH}}{dx}\right| \text{at } x = \delta$$

using $\phi = -Cu(d\mu/d_x)$, where u denotes mobility. It follows that $d\mu_H/dx \approx -d\mu_{OH}/dx$ at $x = 0$, δ. Therefore $C_H u_H / C_{OH} u_{OH} \approx 99$ at $x = 0$ and $C_{OH} u_{OH} / C_H u_H \approx 99$ at $x = \delta$. We assume that u_H/u_{OH} and $C_H \cdot C_{OH}$ have the same values in the aqueous pathways in the membrane as in free solution, that is 1.8 and 10^{-14} mol^2 l^{-2} respectively. The average water concentration is 55 M in the external solution and about 10 M in the membrane interior, where it forms aqueous pathways. The calculated concentrations just outside the reaction layer, are therefore $C_H = 7.4 \times 10^{-7}$ M, near $x = 0$, and 1.4×10^{-9} M, near

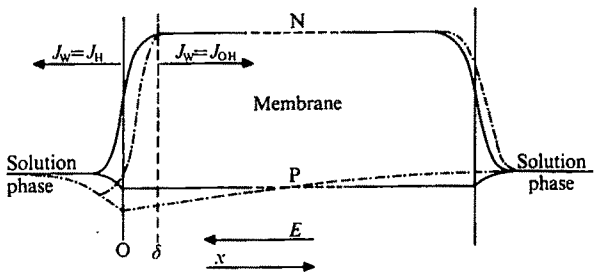


Fig. 1 Concentration profiles for positive (P) and negative (N) mobile ions, in an anion exchange membrane, at equilibrium (———) and during current flow (– · – · – · –).

$x = \delta$. These, and the values for C_{OH}, are negligible compared with the average concentration, that is ≈0.7 M, of ionisable groups in the membrane. Thus since such a large fraction of the total current is carried by the ions of water, it is inferred that the total concentration of mobile ions in the reaction layer is negligible compared with that of the fixed charge and that the reaction layer is therefore in the region of uncompensated fixed charge (Fig. 1).

A plausible mechanism which takes account of the tertiary amino groups (B) in the surface region is that the H_3O^+ and OH^- ions originate in protonation and deprotonation reactions that is

$$B + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} BH^+ + OH^- \quad (1)$$

$$BH^+ + H_2O \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} B + H_3O^+ \quad (2)$$

The times required for the H_3O^+ and OH^- ions to migrate out of the reaction layer under their gradients in electrochemical potential would need to be comparable or less than their recombination times with the amino groups. This condition seems to be fulfilled (unpublished work).

We shall estimate a lower limit for the pseudo-first-order rate constant k_2'. Since the diffusion flow equals the integrated reaction flow

$$J_W < Fk_2[BH^+][H_2O]\delta \quad (3)$$

where $[BH^+]$ has been treated as being constant with x, and F is the Faraday constant. The change in electrical field intensity (E) across the reaction layer satisfies

$$E(0) - E(\delta) = \frac{F[BH^+]\delta}{\varepsilon_0 \varepsilon_r} \quad (4)$$

using Poisson's equation, where ε_0 is the permittivity of free space and ε_r is the average dielectric constant. Using equations (3) and (4)

$$k_2' > J_W/\varepsilon_0\varepsilon_r[E(0) - E(\delta)] \quad (5)$$

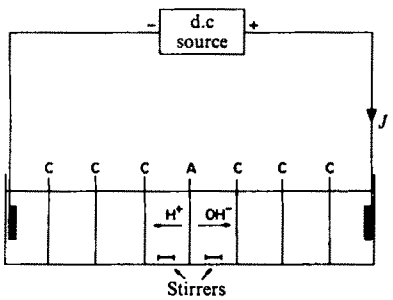


Fig. 2 The experimental system. The central chambers contained NaCl solutions of various concentrations. The other chambers were separated by cation exchange membranes and contained 0.1 M NaCl.

The upper limits for $[E(0) - E(\delta)]$ and ε_r are about 10^8 V m^{-1} and 20 respectively. Thus to account for $J_W = 50$ A m^{-2}, k_2' must exceed 2,800 s^{-1}.

This estimate may be compared with that for the same amino group, but in the membrane interior. The functional group in the reaction layer seems to be the same as that in the Negev Institute tertiary alkyl amino ion exchange membranes[3] which have an apparent pK_a of about 9.9 (unpublished work). Using $k_{-2} < 2.1 \times 10^{10}$ l mol^{-1} s^{-1} (ref. 5) we obtain $k_2' > 2.5$ s^{-1}, for groups inside the membranes. This is less than a 1,000th of the value in the reaction layer.

Note that the major assumption underlying this result is that the great enhancement in the rate of water dissociation may be accounted for by reactions (1) and (2).

In considering how k_2' might increase, the reactions are presented as occurring in two steps

$$\underset{a}{B + H_2O} \rightleftharpoons \underset{b}{BH^+ \ldots OH^-} \rightleftharpoons \underset{c}{BH^+ + OH^-} \quad (6)$$

$$BH^+ + H_2O \rightleftharpoons B \ldots H_3O^+ \rightleftharpoons B + H_3O^+ \quad (7)$$

where $BH^+ \ldots OH^-$ and $B \ldots H_3O^+$ are encounter pairs in the same solvent cage, k_{bc} and k_{cb} are the rate constants for the separation and encounter processes respectively and k_{ab} and k_{ba} are those for the chemical transformations. Applying the steady-state approximation to the encounter pair, the expressions for the overall rate constants are[5]

$$\vec{k} = \frac{k_{ab}k_{bc}}{k_{ba} + k_{bc}}; \qquad \overleftarrow{k} = \frac{k_{cb}k_{ba}}{k_{ba} + k_{bc}} \quad (8)$$

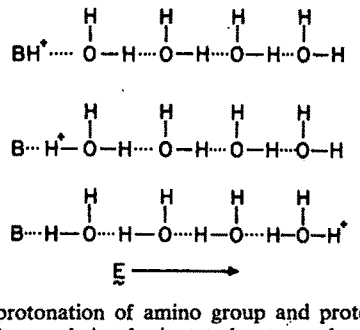


Fig. 3 Deprotonation of amino group and proton conduction along a chain of orientated water molecules.

Most of the difference in $\vec{k}$ between the membrane interior and the reaction layer would probably be due to a difference in $k_{bc}/(k_{ba} + k_{bc})$ rather than to k_{ab}, since the height of the potential barrier between states a and b is unlikely to alter by more than kT in the applied field. Therefore since the maximum value of $k_{bc}/(k_{ba} + k_{bc})$ is less than unity, the model requires that the ratio should be less than about 10^{-3} in the membrane interior.

From data for ammonia, the methylamines and triethylamine in water it seems likely that the reverse reactions are diffusion controlled[6]. For diffusion-controlled protolytic reactions in water k_{bc} is between 10^{10} and 10^{11} s^{-1} and $k_{ba} > 10^{12}$ s^{-1} (ref. 5). The constant k_{bc} is probably at least an order of magnitude smaller in the membranes because of the smaller ion diffusion coefficients[7]. However, the constant k_{ba} could again exceed 10^{12} s^{-1}, since the molecules may be correctly orientated for the reaction when the solvent cage is formed[8]. Thus the ratio $k_{bc}/(k_{ba} + k_{bc})$ could be less than 10^{-3} in the membrane interior, as required. We shall consider two ways by which it might be increased by the strong electric field in the reaction layer.

The first way resembles the second Wien effect. Onsager[9] has shown for reaction (1), that k_{bc}, but not k_{cb}, depends on the strength of an external field. In reaction (2) the site in B, for its recombination with H_3O^+, is a lone pair so that the charge product is effectively negative when the proton is sufficiently close to the reaction site. The dissociation of the protonated

amino group is, in this respect, analogous to that of a neutral group. It follows, from a consideration of Onsager's treatment, that k_{bc} would increase with the electric field intensity for reaction (2) also, although the magnitude of the increase is uncertain and depends on the variation of the interaction energy with the distance of separation and the role of the solvent in proton transfer[10].

The second possibility involves the degree of alignment of the water molecules in the reaction layer. In free solution actual proton transfer is very fast and probably consists of quantum mechanical tunnelling. The rate-determining step seems to be the necessary reorientation of water molecules between successive transfers (structural diffusion)[5,11]. It is conceivable that there is a bridge of water molecules in the reaction layer, already favourably orientated for proton transfer by the strong electric field, when reaction (2) occurs (Fig. 3). If this were the case then the rate-determining step in proton transfer would no longer be structural diffusion but quantum mechanical tunnelling through adjacent hydrogen bonds. The constant k_{bc} would then be much greater than in the membrane interior. It might even exceed k_{ba}, particularly if there were concerted proton transfer. Information relevent to this proposition should be obtainable from dielectric constant measurements for d.c. currents both below and above the threshold for water splitting.

Finally, note that since the electric field strength in the reaction layer is of the same order of magnitude as in biological membranes it is possible that proton transport in biological membranes involves water splitting and that the degree of protonation of amino groups there, depends on field strength. Such effects could be significant in several aspects of membrane function.

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Natural atmospheric ^{14}C variation and the Suess effect

THE dilution of the atmospheric $^{14}CO_2$ concentration by large amounts of fossil-fuel derived CO_2 which do not contain any ^{14}C is commonly called the Suess effect. Its magnitude can be calculated with the same geochemical models as the global carbon cycle that also predict the future rise of atmospheric CO_2 to be caused by the combustion of fossil fuels. Validation of a CO_2 predictive model with the Suess effect ^{14}C data is important because these two phenomena have a common cause, and therefore register model responses at roughly the same frequencies. Measurements of the Suess effect yield values between −15‰ and −25‰ in $\Delta^{14}C$ (in 1950)[1], while different model predictions also cover about this range[2,3]. The requirement that a model correctly reproduces the Suess effect becomes a strong constraint when the accuracy of the measurement is

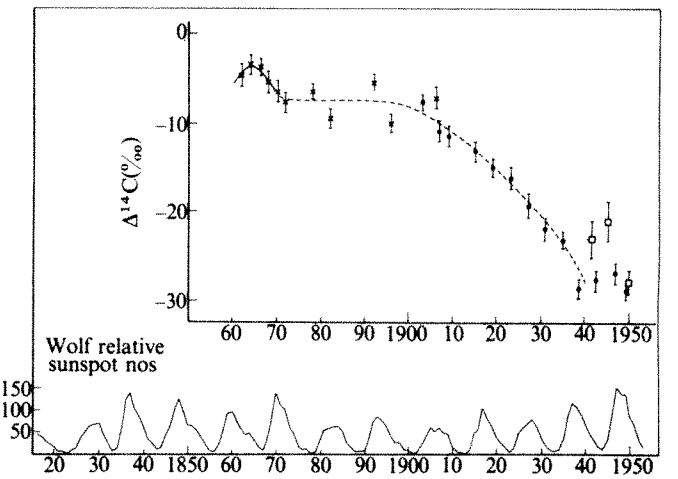


Fig. 1 $\Delta^{14}C$ in tree rings from Drenthe, The Netherlands: ●, *Quercus rubra* L. (Sleen, 52° 45′ N, 6°45′ E); ×, *Quercus robur* L. (Sleen); □, *Quercus robur* L. (Norg, 53°4′ N, 6°27′ E). The line has been fitted by eye. The sunspot record has also been included for comparison.

improved to better than 2‰. ^{14}C measurements in tree rings to an accuracy of 1.2‰ are reported here. The results indicate that the natural fluctuations of atmospheric ^{14}C as yet preclude determination of the Suess effect to the accuracy required by the models.

The design and the operating characteristics of the new set of proportional counters that were used for these measurements have been described elsewhere[4]. The samples were taken from three large mature oak trees from the province of Drenthe, The Netherlands, that had been felled by a severe windstorm in November 1972. The first 20 years of growth of each tree have been discarded. Only latewood was used from one of the trees, *Quercus rubra* L. which had very wide yearly rings. ^{14}C measurements were made on cellulose since this is the component of wood unlikely to contain carbon derived from other years of growth[5]. The amount of wood needed for every sample, before the cellulose extraction, was very large, ~200 g.

The results are listed in Table 1 and plotted in Fig. 1 as $\Delta^{14}C$ values defined by

$$\Delta^{14}C = \frac{A - A_{ox}}{A_{ox}} \times 1{,}000 \quad (‰)$$

where A_{ox} is equal to 0.95 × activity of NBS oxalic acid, corrected for age and fractionation to 1950 and −19.0‰ respec-

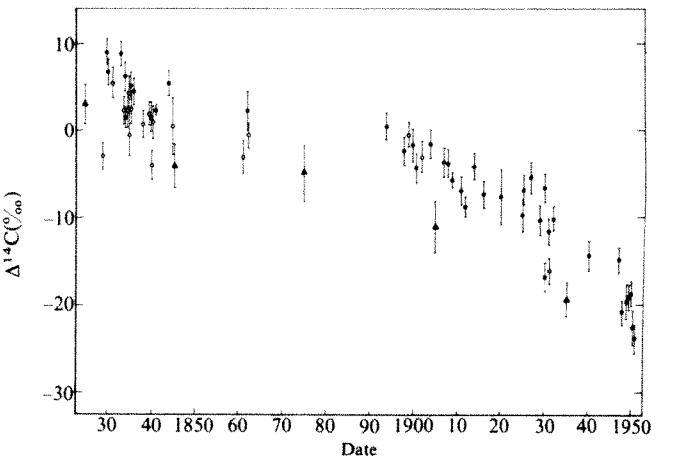


Fig. 2 Suess effect measurements by Lerman *et al.*[1]: ●, Northern Hemisphere; ○, Southern Hemisphere. A few older results of de Vries, corrected for fractionation by Lerman *et al.*[1], are also included (▲).

Table 1 Radiocarbon content in tree rings calculated with a half life of 5,730 yr

Year of growth	Laboratory no.	$\delta^{13}C$(‰)	$\Delta^{14}C$(‰)
a Quercus rubra L. (only latewood)			
Post bomb period			
1972	GrN-8016	−23.51	474.6±2.0
1971	8017	−23.88	498.5±2.0
1967	8066	−23.68	637.3±1.6
1961	8067	−24.86	229.4±1.6
1959	8070	−22.55	277.9±1.5
Pre bomb period			
1950	8069	−24.17	−29.0±1.1
1947	8136	−23.74	−27.0±1.3
1943	8137	−23.68	−27.9±1.3
1939	8138	−24.09	−28.9±1.1
1935	8139	−23.29	−23.3±0.9
1931	8161	−24.05	−21.8±1.3
1927	8162	−24.94	−19.3±1.4
1923	8185	−25.62	−16.2±1.3
1919	8192	−25.46	−15.0±1.0
1915	8193	−25.53	−13.2±1.2
1909	8198	−25.26	−11.5±1.3
1907	8195	−25.24	−10.9±0.9
1903	8196	−25.12	−7.7±1.1
b Quercus robur L. (Sleen)			
1906	8364	−24.65	−7.1±1.3
1895–1896	9003	−24.33	−9.7±1.1
1892	8363	−24.26	−5.3±0.9
1882	8362	−24.08	−9.5±1.2
1878	8770	−24.18	−6.3±1.0
1872	8199	−24.25	−7.7±1.1
1870	8200	−24.37	−6.5±1.2
1868	8201	−23.37	−5.3±1.2
1866	8202	−24.77	−3.7±0.9
1864	8203	−23.88	−3.4±1.3
1862	8204	−24.11	−4.7±1.2
c Quercus robur L. (Norg)			
1950	8295	−24.13	−28.1±1.3
1945	8296	−24.39	−21.1±2.2
1941–1942	8297	−24.37	−23.1±2.1

tively, and A is the activity of the sample, also age and fractionation corrected from 1977 to the year of growth ($T_{1/2}$ = 5,730 yr) and to −25.0‰ respectively.

One 11-yr period, 1862–72, could be closely followed because of an extended period of wide annual rings. The results agree with the dependence of atmospheric ^{14}C on the 11-yr solar cycle[6], also depicted in Fig. 1. A precise magnitude for this dependence cannot be obtained until more solar cycles have been investigated and a longer term trend has been established for the 11-yr period to be fully explained.

Another striking feature is that the atmospheric ^{14}C level seems to remain nearly constant between 1939 and 1950. This result is in accordance with earlier measurements for this period by Damon, Long and Wallick[6], who find an almost constant level around $\Delta^{14}C = -32$‰. During this 11-yr period a total of 16.6 G ton of carbon from fossil fuels has been released into the atmosphere[7], which should have resulted in a 4–6‰ decrease of the atmospheric $^{14}C/C$ ratio during this decade. The observed constancy even occurs during a period of high solar activity when the natural ^{14}C production by cosmic rays is expected to be below average. One is tempted to speculate that the increased ^{14}C production which apparently cancels the Suess effect in these years is due to solar flares. An exceptionally large flare could conceivably raise the atmospheric ^{14}C level by several ‰ (ref. 8). Increases in the cosmic ray intensity associated with flares was first reported in 1946 and 1949[9,10].

Furthermore it is not clear from the measurements which ^{14}C concentration should be chosen as the preindustrial level. There

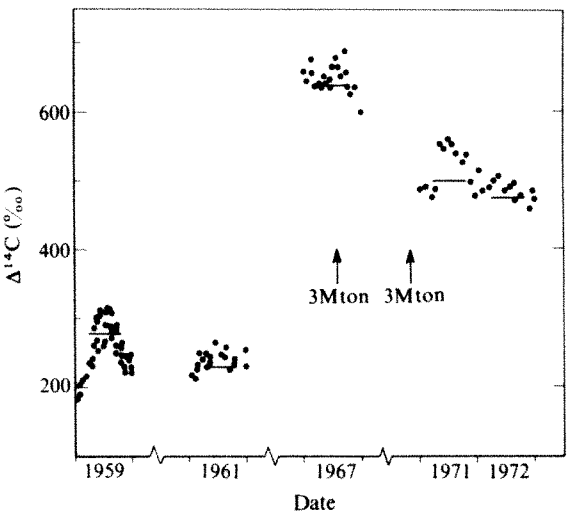


Fig. 3 Comparison of direct atmospheric ^{14}C measurements and tree rings. The 1959 and 1961 data are taken from Münnich[11] and Östlund *et al.*[12]. The other data are from Nydal *et al.*[13]. The dates and estimated magnitudes in megatons TNT of two Chinese thermonuclear explosions are shown by arrows. The tree ring $\Delta^{14}C$ values are represented by horizontal bars through the summer half year.

does not seem to be such a level. Therefore, the Suess effect should be calculated as the difference between the measured time series and an extrapolation of the natural ^{14}C level from 1850 to 1950. This extrapolation would have to be based on an established relationship between atmospheric ^{14}C and the solar record.

When our results are compared with previous Suess effect measurements (Fig. 2) by Lerman, Mook and Vogel[1], our results are seen to be lower than the latter by a constant amount of 5–7‰.

As our trees were located in a rural environment, at a typical distance of 150–200 km from and almost surrounded by the industrialised regions of North Western Europe, a regional fossil fuel contamination exceeding the average global effect cannot be fully excluded. This contamination cannot be estimated using the comparison with the results of Lerman *et al.* because of the large scatter of the older data. Comparing a number of recent growth rings with direct atmospheric ^{14}C measurements (Fig. 3) also does not lead to a definite quantitative conclusion. These data are also very scattered and some of them may be influenced by leakage from the stratosphere of radioactive debris from two recent Chinese thermonuclear explosions. Nevertheless, the fact that the tree $\Delta^{14}C$ values seem to be consistently on the low side of the possible range would suggest that the 1950 ring should be at most 4‰ lower in $\Delta^{14}C$ than the 1950 atmosphere.

A regional contamination is expected to increase almost in proportion with the global Suess effect. A comparison between the results of Lerman *et al.* and our data do not reveal conclusive evidence with respect to a contamination effect in our case.

Our observations of the short term variability of the natural ^{14}C level, however, remain equally valid regardless of the existence of a regional contamination effect.

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Life in the calcium chloride environment of Don Juan Pond, Antarctica

DON JUAN POND, which contains saturated calcium chloride brine, is in the south fork of the dry Wright Valley of Antarctica at latitude 77°33′ S and longitude 167°10′ E, and has been controversial almost since its discovery in 1961. Meyer *et al.*[1,2] reported a sparse microflora of four species of heterotrophic bacteria and a yeast. Cameron, Horowitz and colleagues[3,4], using the Antarctic dry valleys as the best available natural simulation of Mars, reported that many areas were virtually sterile and, at most, limited to sparse bacterial populations. Field work[5–10] has revealed a more abundant and varied microflora of yeasts, blue-green algae, fungi and bacteria, especially in the bottoms of frozen freshwater lakes. However, reports point to an extreme dry valley–exposed rock South-Polar biome consisting predominantly of heterotrophic forms, mainly prokaryotic with occasional fungal associates. If this is correct Don Juan Pond must, like the dry valleys generally, consist only of converter–consumer populations lacking extensive capability for continuous carbon reduction. Cameron has emphasised the ecological restrictions on the activity and distribution of algae in the dry valleys and mentions no algal or other autotrophic forms in his discussion of Don Juan Pond, even though thin organic layers were found 2 m below the 15–20 cm of standing water[11]. However, in the course of a mercury sampling programme during the austral summer of 1978–79 we observed an extensive, irregular pellicle or mat-like structure 2–5 mm thick but extending 500-600 m^2 over much of the western part of the Don Juan Pond salt flats.

The extensive algal mat contained much adhering rock grains and fines and varied from yellow- to green- to black-brown. It could be lifted from the underlying sediment (Fig. 1, top). This mat was calculated to weigh about 1 tonne (0.167 g cm^{-1}) and contained about 130 kg of organic matter. Near the mat were moist areas of active gas bubble formation (Fig. 1, middle). Freshly moistened samples of dried mat contained ATP[12], proteins, polysaccharides and lipids, generally enclosing mineral particles. These samples also gave positive catalase and peroxidase reactions within seconds of moistening with appropriate reagents, and positive dehydrogenase within 45–60 min. In addition, dry mat placed on 1.5% agar containing 1% starch showed zones of hydrolysis when developed with I_2.KI after 4 h at 4°C. These zones increased with longer incubation and are indicative of β-amylase. Enzyme reactions[13–16] of approximately equal intensity appeared whether mat samples were moistened with tap water, Don Juan Pond water or pure solutions of $CaCl_2$ and NaCl.

Microscopic examination of small flakes from the mat moistened with water or saline revealed densely entangled filamentous blue-green algae, principally an *Oscillatoria*-like organism (Fig. 1, bottom left), with non-mottle small blue-green filaments, non-motile green *Chlorella*-like cells, motile *Dunaliella*-like flagellates, non-motile golden-yellow 'tetrads' (Fig. 1, bottom right) and occasional phycomycetous hyphae and diatoms. Bacteria, both motile and non-motile, colourless and red-pigmented, were present but not abundant. At 24° C within 24 h after moistening flakes with distilled water, occasional Ciliata and Tardigrada were seen. Their absence in mat samples moistened with salt solutions may reflect delayed rather than inhibited development from the inactive state. After 5 d at 24°C, irrespective of salinity, most algal filaments and cells appeared faded, fragmented or lysed. Salt-free and low-salinity cultures were also overgrown with phycomycetes. At 4°C growth was slow but evident and cells maintained their osmotic integrity and pigmentation in Don Juan Pond water even after 2 weeks. The clear waters of Don Juan Pond seem naturally devoid of algae and fungi and have few bacteria, but nevertheless they contain significant amounts of ATP (unpublished results of P.L. and B.Z.S.)

The obvious explanation for the nearly barren condition of Don Juan Pond brine—that it is a consequence of osmotic stress combined with electrolyte imbalance— is not compelling. Many enzymes exhibit substantial activity in high salt media and

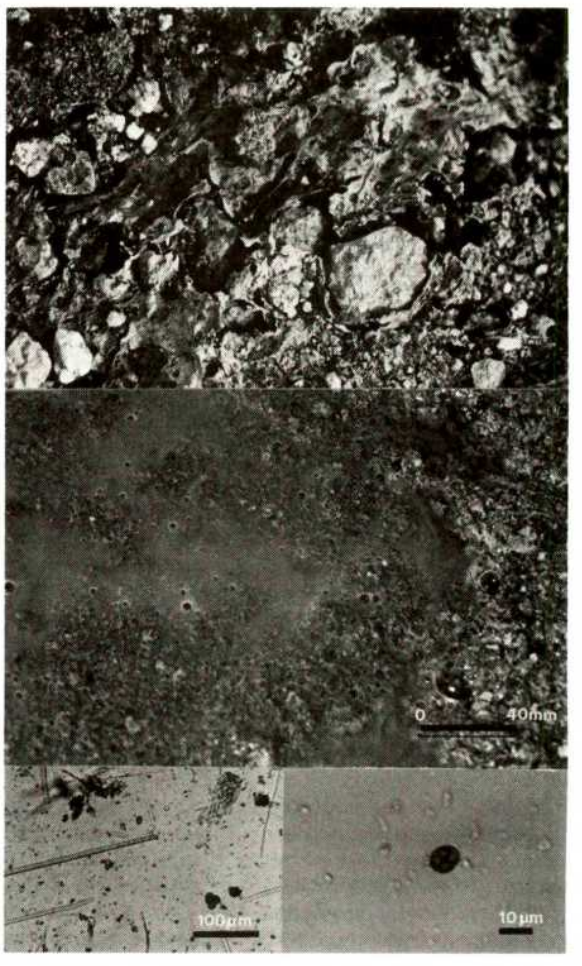


Fig. 1 Don Juan Pond and its microflora: top, surface view of the dry area showing pieces of mat around pebbles and rock fragments; middle, surface view of wet area showing bubble formation under free-standing brine; bottom left, *Oscillatoria*-like filaments and other algal forms in the mat; bottom right, golden-brown 'tetrads'.

Table 1 Major chemical constituents of the free liquid phase of Don Juan Pond

Constituent	Previous analysis (Meyer *et al.*[2])	New analyses *A*	*B*
Ca^{2+}	114,000	167,500	159,000
Mg^{2+}	1,200	2,500	2,480
K^{+}	160	170	201
Na^{+}	11,500	7,050	5,580
Fe^{3+}	24	5.6	—
Mn^{2+}	0.05	2.7	—
Cu^{2+}	—	1.5	—
Co^{2+}	—	6.8	—
Ni^{2+}	—	9.2	—
Hg^{2+}	—	(3.5)	—
Cl^{-}	212,000	—	317,600
SO_4^{-}	11	—	—
NO_3^{-}	13	—	—
HCO_3^{-}	49	—	—
SiO_2	—	—	Trace

All recent analyses were made by autoanalyser and atomic absorption spectrophotometry. Results are given in p.p.m. except for Hg which is in p.p.b. No effort was made to duplicate the sample site of Meyer *et al.* Analysis *A* was made at Hawaii Agricultral Experiment Station and analysis *B* at Hawaii Institute of Geophysics.

various microorganisms have been cultured in brines comparable with that from Don Juan Pond[17–19]. The minor constituents of Don Juan Pond—copper, cobalt and nickel—are in concentrations (1.5, 6.8 and 9.2 p.p.m., respectively) readily equal to if not greater than those required for toxic reactions in various plants and animals[20]. The level of mercury is also relatively high (3.5 p.p.b.).

The gross average composition per 100 g of fresh mat was: 17.4 ± 7.4% water; 67.1 ± 7.2% mineral; 5.7 ± 1.8% soluble material, and 8.7 ± 2.5 organic matter ('loss on ignition'). Salts in the mat represent 32.7% dissolved solid material, or 327,000 p.p.m., compared with about 485,000 p.p.m. in the pond (Table 1, analysis *B*). Of the total soluble material, approximately 33% was precipitated as calcium carbonate which is the expected proportion for a saturated $CaCl_2$ solution. Spectrophotometry of samples of mat revealed major chlorophyll *a* bands at 410, 430, 475 and 663 nm, and minor bands at 580 and 615—together with accessory pigments or extraneous colouring matter. Paper chromatography (ether solvent) revealed up to six bands including yellow-brown and red-carotenoid-like fractions with double maxima around 500 nm. Using the putative organic fraction of the mat as the basis[21], we calculated: in a dry sample, 105 and 1.4 μg per g of chlorophyl *a* and *b* respectively, and in a moist cold-preserved specimen, 265 and 4.9 μg per g, respectively.

Compared with typical chlorophyll values attained using *Oscillatoria* as a control—about 3,000 μg per g of cells—these are quite low, Nevertheless, preliminary manometric experiments have shown that the mat, in its natural medium at 4°C, produces oxygen in light (117 $\mu E \cdot cm^{-2} s^{-1}$ incandescent) and releases CO_2 in darkness.

These observations, although limited, provide evidence that the Don Juan Pond salt flats and brines have a relatively rich and trophically balanced ecosystem worthy of more intensive field and laboratory study.

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Texture contours can facilitate stereopsis by initiating vergence eye movements

IN man the two eyes receive slightly differing views of the world. The visual system utilises this disparity for stereoscopic depth perception. This involves a difficult correspondence problem for establishing binocular fusion: for each surface feature extracted from one eye's image, the brain has to decide which is the equivalent feature in the other eye's image. Ambiguities often exist, particularly for densely-textured visual surfaces, but one aspect of binocular visual processing which helps reduce the scale of the problem is that for any given vergence angle, the visual system considers only those potential left/right fusions with disparities under a certain limiting size (defined by Panum's fusional areas[1,2]). This disparity limit, however, requires that when disparities greater than Panum's area are presented, a suitable vergence change must be initiated to bring corresponding features within the allowable range for fusion. A question currently of considerable theoretical interest concerns the nature of the stimulus characteristics used to initiate these essential vergence movements. The interest stems mainly from Marr and Poggio's model of human stereo vision[3,4] in which false correspondences are avoided by seeking left/right fusions within spatial frequency tuned disparity channels whose disparity range is closely tied to their spatial frequency sensitivity[5]. By setting the disparity range of any given channel to about $\pm w$ (where w = the width of the centre of the receptive fields of the channel in question) Marr and Poggio have demonstrated that false matches are improbable and any that do emerge are easily eliminated using quite simple rules. A further feature of the model is that the outputs of low spatial frequency tuned channels (that is units capable of dealing with large disparities because of

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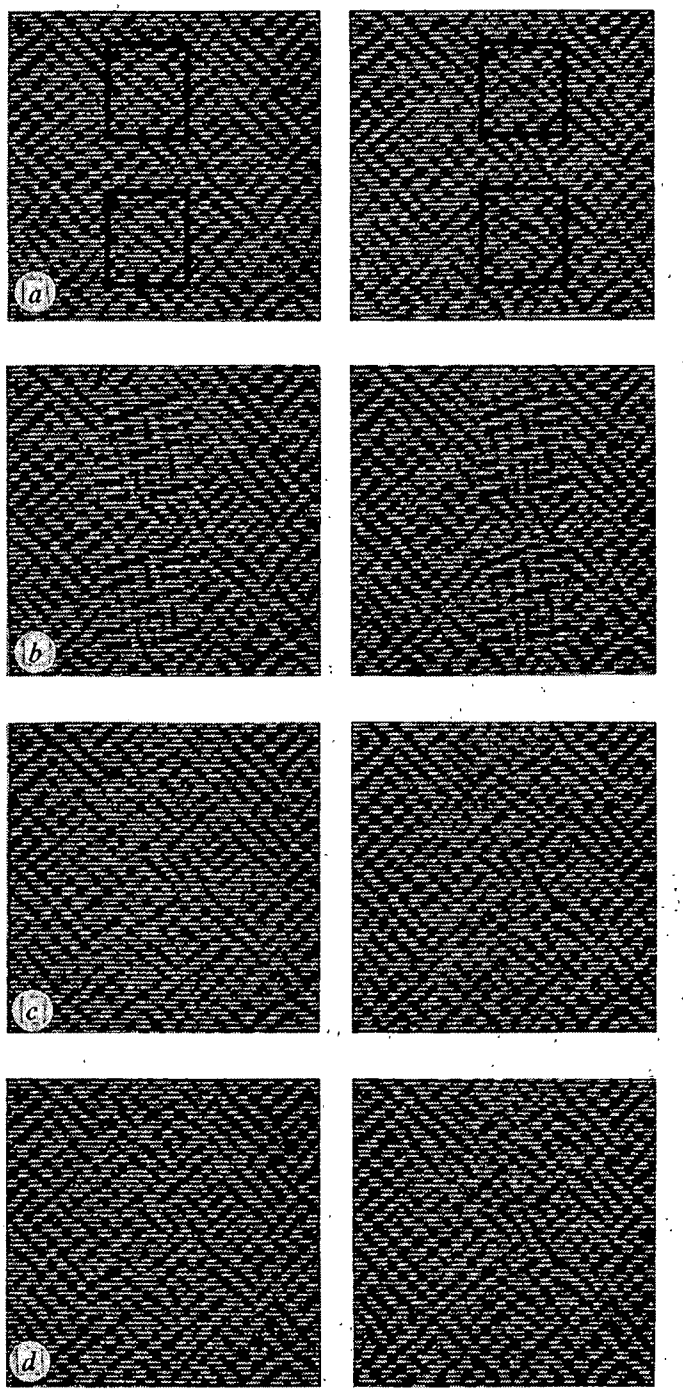


Fig. 1 Photographs of the oscilloscope displays showing examples of stereograms used in the two experiments as follows: *a*, Line contour stereogram. *b*, Texture contour stereogram. *c*, Cyclopean stereogram. *d*, Cyclopean stereogram used for the depth discrimination task in the second experiment. All stimuli were computer-generated in two steps. First, orientated textures were produced by filtering random-dot patterns using gaussian filter functions tuned for orientation and spatial frequency (centre frequency 4.5 cycles deg^{-1}, obtained here by viewing the illustrations from about 11×picture height; bandwidth ±0.9 cycles deg^{-1}; orientation bandwidth ±10°). Textures either had component orientations of 45° and 135° (for example *c* surround) or 75° and 165° (for example, *c* targets). This filtering was done using a 128×128 pixel array for each half. The second step in stimulus production was to reduce each stereo half to a 64×64 pixel array (the resolution appearing in the illustrations), using a sampling procedure which had no important effects on spatial frequency content as judged by examining the resulting power spectra and by obtaining equivalent psychophysical data in pilot trials run using a tachistoscope to present the original 128×128 pixels stimuli as hardcopy outputs produced by a high quality facsimile receiver[6]. The reduction to 64×64 pixels permitted on-line stimulus presentation with a satisfactory refresh rate (20 ms per frame). Stereograms *a*, *b* and *c* contained upper and lower targets, each with a mean disparity of ~35 arc min (the target elements lay in the disparity range 32.7 to 37.4 arc min as a side effect of the sampling procedure used to obtain the 64×64 pixel arrays). Stereogram *d* contained an upper target with a disparity of about 32.7 arc min and a lower target with a disparity of about 37.4 arc min, so presenting the depth discrimination task described in the text. In all stereograms apart from *a*, disparate targets were inset into their surrounds using a cosine contrast ramp over a period of 6 pixels (within the 128×128 array) to preclude discrimination of a monocular contour in the texture contour condition on the basis of trivial edge effects at the boundary of the two textures.

their relatively large receptive fields) can initiate vergence movements bringing into correspondence higher spatial frequency channels mediating smaller disparities. The theory predicts that in the case of a two-planar densely-textured stereogram (for example Fig. 1*a–c*) in which the disparity range exceeds the size appropriate for the lowest spatial frequency channels activated by the stereogram's texture, vergence movements should exhibit only a random-search structure. We report here an experimental test of this prediction which shows that vergence movements are not always random in the stimulus circumstances just specified. Rather, vergence can be guided by texture contours (Fig. 1*b*) depicted by differences of texture orientation between regions of relatively high spatial frequency content for the disparity range incorporated in the stereogram. Moreover, we find that such texture contours can facilitate stereopsis in a way consistent with the idea that they do so by initiating appropriate eye movements.

In our first experiment, we used a nonius line technique for recording vergence changes made in response to tachistoscopic presentations of various stereograms. The stimuli were generated by computer on-line and presented via a pair of oscilloscope displays (one display for each 5×5 cm stereo half) which were viewed at about 57.5 cm and binocularly superimposed using Risely prisms. A trial began with the subject fixating a central fixation spot; above this was a black nonius line (20 mm long, 0.8 mm thick) seen only by the right eye and below was an identical line seen only by the left eye. When ready to proceed (having adopted suitable fixation, as demonstrated by the nonius lines appearing vertically aligned), the subject pressed a hand-held switch. He then heard a 1-s warning tone which was followed after a 2-s pause by an 80-ms presentation of one of the stereograms shown in Fig. 1*a–c*. This presentation was followed by a 40 ms uniform grey field of the same space average luminance as the stereograms (about 4.3 cd m^{-2}). Finally, a second pair of nonius lines with no central fixation spot was displayed for a further 400 ms. The subject's task was to estimate the direction and extent (in mm) of any apparent misalignment of the second pair of nonius lines at their first moment of appearance. (Any subsequent movement of the nonius lines was ignored; further experiments[6] using 60-ms nonius line exposures to remove the problem of nonius shifts during the 400-ms exposure period have confirmed the findings we report here.) A convergent eye movement was indicated if the top line appeared misaligned to the left, vice versa for a divergent movement.

The stereograms contained two disparate targets, one positioned in the upper half field and one in the lower half field (two targets were used for comparison with stimuli used in the second experiment). Both upper and lower targets in each stereogram had the same disparity (either ~35′ convergent or ~35′ divergent), except for control conditions in which both targets had zero disparity. Targets were either cyclopean (not visible until after binocular fusion, as in Fig. 1*c*) or outlined by either a line or texture contour (Fig. 1*a* and 1*b* respectively). The different contour and disparity conditions were randomly presented, with each subject having a total of eight trials on each condition. Data were collected from two experienced observers and Fig. 2*a* shows the mean size and direction of misalignment of the nonius lines following the various stereogram presentations. For both subjects, disparate texture and line contours initiated an appropriate vergence eye movement (that is, one which would lead to bifoveal fixation of the targets). In contrast, the zero disparity texture and line contours produced little if any misalignment of

the nonius lines. Also, all three cyclopean conditions—convergent, divergent and zero disparity—failed to create much misalignment of the nonius lines. Note that nonius alignment/misalignment appeared involuntarily for both subjects. Also, as the task of judging the size of the various shifts required the subjects' full concentration, they were unable to notice any depth effects. However, additional trials omitting the post-stereogram nonius lines have demonstrated that both subjects can reliably discriminate between convergent and divergent disparities from 80-ms exposures of the line and texture contour stereograms but not from similar exposures of the cyclopean stimuli.

The fact that no vergence movements of any importance were initiated by the flashed cyclopean stereograms is in accord with Marr and Poggio's theory. For two-planar disparities of the size we used (approximately $\pm 35'$), the theory predicts vergence movements to be triggered if a disparity channel tuned to spatial frequencies under 1 cycle deg^{-1} was activated. The texture for our stereograms was created using narrow-band filters tuned to 4.5 cycles deg^{-1}, well above the required range, and so the absence of vergence eye movements for the cyclopean conditions is in keeping with the theory's prediction. On the other hand, flashed disparate texture contours did initiate appropriate vergence movements, even though these texture contours did not alter the spatial frequency range of the stimulus texture, implying that human stereopsis does not rely solely on low spatial frequency/large disparity mechanisms to guide vergence eye movements.

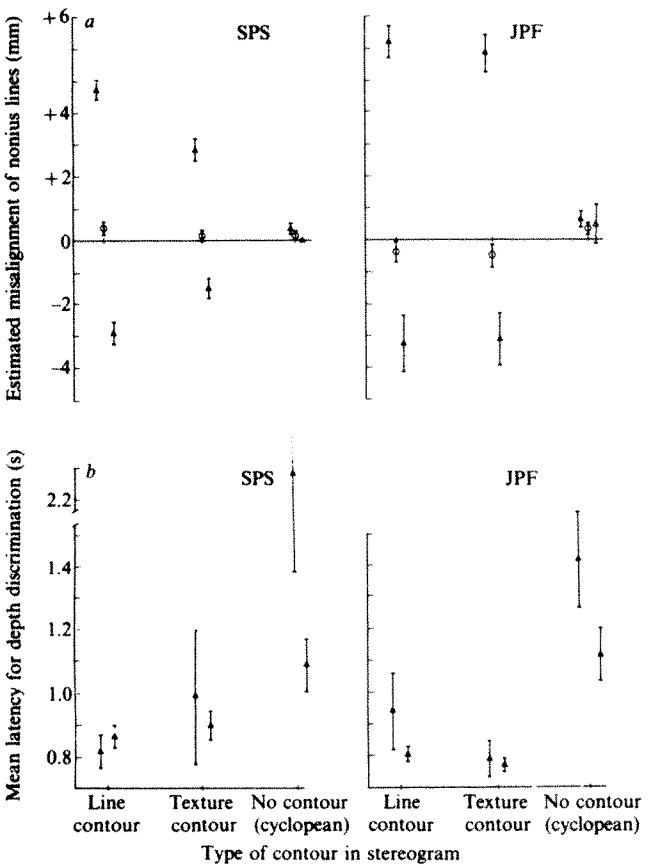


Fig. 2 *a*, Estimated misalignment of the nonius lines following 80-ms presentations of the stereograms shown in Figs 1*a–c*. ▲, convergent disparity; △, divergent disparity; ○, zero disparity. The bars show one standard error to either side of the mean. *b*, Reduction in mean latencies for a cyclopean depth discrimination task (Fig. 1*d*) following 80-ms presentations of stereograms in Fig. 1*a–c* (▲, convergent disparity; △, divergent disparity; bars = ± 1 s.e.m.

Given the above results, it becomes of interest to discover whether a vergence movement triggered by a tachistoscopic presentation of a line or texture stereogram can facilitate a subsequent cyclopean depth discrimination task which requires this vergence movement for its successful execution. Consequently, in our second experiment we measured latencies to make a cyclopean depth discrimination following tachistoscopic presentations of the stereograms used in the first experiment. Experiments began with the subject fixating a central spot. When he was ready to proceed, he pressed a hand-held switch which initiated after a 2-s pause, an 80-ms presentation of one of the stereograms shown in Fig. 1*a–c*. This stimulus was followed by a 40-ms presentation of a uniform grey field of same space average luminance as the stereograms (as before, about 4.3 cd m^{-2}), after which came a cyclopean stereogram presenting a depth discrimination task (Fig. 1*d*). In this latter stereogram, both targets possessed either a convergent or divergent disparity, as in the flashed disparate stereograms that preceded them, but now the targets differed slightly in depth due to a disparity difference of about 4.7'. This difference was created by setting the upper target to a disparity of $\sim 37.4'$ and the lower target to a disparity of $\sim 32.7'$, or vice versa. On the appearance of this cyclopean discrimination stimulus, the subject's forced-choice task was to press a switch up or down to indicate whether the upper or lower target appeared the nearer. This response enabled his stereopsis latency to be measured and it was also used to terminate the cyclopean presentation. Note that the design of the experiment was such that on half the trials a convergent up/down discrimination was required and on the remaining trials a divergent one. In every case, however, the subject's task was to press the switch up or down to indicate which target was the nearer. As in the previous experiment, the different contour and disparity conditions were presented randomly, with each subject having eight trials on each condition.

Data for the same two practised observers are shown in Fig. 2*b*. In conditions where the flashed stereogram contained a line or texture contour outlining the disparate targets, the mean latency for stereopsis was significantly reduced in comparison with the control condition where only cyclopean targets were flashed (for both subjects, $F > 12$, $d.f. = 2, 42$, $P < 0.0001$). Comparison of the data from our two experiments thus strongly suggests that the facilitation of stereopsis evident in Fig. 2*b* is a result of the flashed line and texture contours initiating vergence eye movements as recorded in Fig. 2*a*, movements which would be appropriate for the fusion of the immediately-following cyclopean stereogram. These data thus extend into a tachistoscopic paradigm the already known capability of continuously-present monocular features for facilitating stereopsis from random-dot stereograms[7,8].

For simplicity, we have described our second experiment as though only appropriate disparate flashed cues were used. However, the experiment also included zero disparity and wrong disparity cues (in the latter case, a divergent cue preceded a convergent cyclopean presentation, or vice versa). As would be expected from the vergence hypothesis under consideration, these cues provided either no facilitation or even a hindrance to successful fusion (full data will be reported elsewhere[6]).

A further condition in our second experiment showed that flashed texture cues carried by rivalrous texture targets composed of differently-orientated elements in the two fields[6] provided just as good facilitation as the same-orientation targets shown in Fig. 1*c*. Thus S.P.S. produced mean latencies (seconds) for the different- and same-orientation target conditions of 0.95 (s.e.m. = 0.10) and 0.86 (0.05) respectively (convergent and divergent data pooled). Equivalent means for J.P.F. were 0.82 (0.74) and 0.78 (0.03). This result suggests that some instances of qualitative stereopsis from rivalrous texture stereograms[9–12] may well be mediated by mechanisms whose primary function is the control of vergence shifts.

We conclude that disparate texture cues can trigger vergence movements appropriately and that their ability to facilitate

stereopsis is probably based at least partly on this fact. This conclusion implies that it is insufficient to limit vergence control to spatial frequency channels whose tuning determines their disparity selectivity. Rather, it seems that any reasonably complete model of human stereopsis must allow for the early computation of information about boundaries separating regions of different textures, and it must allow the vergence eye movement control mechanism rapid access to this information.

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The herring ear has a unique receptor pattern

HERRINGS and their relatives in the teleost order Clupeiformes, including sardines and anchovies, have an unusual connection between the gas bladder and the inner ears[1,3] that may indicate an auditory mechanism involving the utricle, a part of the ear generally considered to be a gravistatic organ in all other vertebrates[4,5]. In herring, two slim ducts extend forward from the gas bladder, one to each ear, and connect with gas-filled portions of the otic bullae within the skull. The membranous wall of the utricle is in contact with these pro-otic and pterotic bullae, and thus might be stimulated directly by vibrations of this compressible system. Structural features of the ducts and bullae in clupeids suggest that certain acoustic frequencies may be selectively filtered[6,7], but this requires electrophysiological confirmation[8]. However, although such studies[1,6,7] support the argument of an auditory function for the clupeid utricle, no data have been available regarding ultrastructural features of the herring utricle. We now report that scanning electron microscopic (SEM) investigations of the otolith sensory epithelial surfaces have revealed several additional structural aspects of the herring utricle which may further support a hypothesis for an auditory role for this organ in clupeids. These features seem to be unique to the clupeid utricle and include the presence of large numbers of sensory cells with long ciliary bundles, and a special sensory receptor pattern.

Inner ear sensory surfaces from the Pacific herring, *Clupea harengus pallasi* (a subspecies of the worldwide *C. harengus*), were prepared as in other studies[9,10]. Tissues were fixed in buffered glutaraldehyde solution, post-fixed in buffered osmium tetroxide, and after removal of the statoconia (otoliths) the tissues were dehydrated, then dried by the critical-point method using carbon dioxide. Observation, photography and mapping were done with the scanning electron microscope. Additional data from other clupeiform representative species[11] fully agree with our data for herring.

An unusual feature of the herring utricle is the partition of the sensory epithelium into three laterally directed 'fingers' which are separately innervated[2]. These three regions, named the macula anterior, macula media and macula posterior[2], are shown by SEM observations to be three distinctly separate populations of ciliated mechanosensory hair cells. Even on the medial wall from which the fingers diverge there is a gap without sensory cells between each of these three maculae.

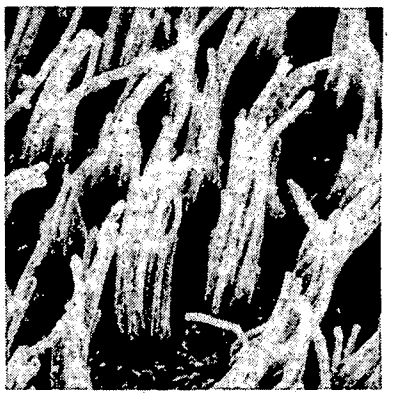


Fig. 1 Tall ciliary bundles typical of those found on herring utricular sensory epithelia (×8,000).

A second unusual feature in the herring utricle is a widespread distribution of a relatively 'tall' form of ciliary bundle. Each bundle, as in other vertebrate hair cells, contains a single kinocilium which is a true cilium, located at one end of an orderly array of sterocilia which are similar to microvilli; the sterociliary array consists of rows of stereocilia which decrease in height the further they are from the kinocilium. In the herring utricle there are large numbers of bundles which have a kinocilium at least 6–7 μm long and some stereocilia which are nearly as long (Fig. 1). There is a relatively dense forest of these tall bundles over much of the macula media, and they are also common on the maculae anterior and posterior. Near the edges of all three maculae, but more broadly inward from the margins of the maculae anterior and posterior, there is another form of bundle with a still longer kinocilium but short (1–2 μm) stereocilia.

The pattern of hair cell orientations in the herring utricle also seems to be a feature unique to this group. The asymmetry of the ciliary bundle allows determination of a morphological polarisation vector for each hair cell[12]. In each of the three maculae in the herring utricle the cells can be divided into two populations of opposing orientations (Fig. 2). For example, hair cells in the anterior half of the macula media are orientated in a generally posterior direction (with respect to the axis of the fish), whereas those in the posterior half are orientated anteriorly. An imaginary, nearly straight line can be drawn to divide the opposing populations, running in a medial to lateral direction, and bisecting the macula media in the transverse axis of the fish. A similar but more curved line can be drawn for the anterior and posterior maculae, also transverse to the fish and following the curve of these maculae. Although the map in Fig. 2 shows a flat projection of the maculae, the utricular pouch is actually quite rounded. As the anterior macula lies well up the slope of the anterior utricular wall, its hair cells should be considered to have considerable upward or downward orientation vectors, rather than simply anterior–posterior vectors.

We now have sufficient comparative data to put the features of the herring utricle in some perspective. In all vertebrates studied to date the utricular macula is a single dished or crescentic patch, sometimes with a postero-lateral process, the lacinia[4]. Generally, there is a distinctive antero-lateral band, the striola, in which the hair cells differ markedly in size or in ciliary bundle form from the hair cells in the rest of the macula[13]. Further, the usual orientation map of the vertebrate utricle, in both bony

fishes[9,12,14,15] and tetrapods[13,16], shows a wide antero-lateral area containing hair cells with their kinocilia on the side inward from the periphery, whereas the large medial-posterior area has hair cells orientated facing outward towards the periphery. The dividing line between these two populations curves along the middle of the striola (and lacinia, when present), usually in a lateral to posterior curve that parallels the macular edge. An exception to this pattern occurs in at least one elasmobranch fish, the thornback ray, which has a mixture of patches of opposing orientations in the utricle[17], although the macula is not structurally split.

The presence of the tall ciliary bundles and the absence of a striola in the herring utricle also occur in the herring saccule and lagena[11]. In several other teleosts these features are found in the saccule and lagena[10], whereas the utricle retains the usual striola and lacks a large proportion of tall bundles[9,14,15]. The functional significance of bundle morphology is unclear, although there is some evidence that tall bundles may be sensitive to a higher frequency range than shorter bundles[18]. However, bundle forms may depend for both their form and function on properties of the overlying otolith membrane.

The orientation patterns of the herring utricular maculae may have phylogenetic origins in the general vertebrate utricular pattern. This is suggested by the fact that all vertebrate utricles have cells orientated towards the line of division between populations, and that the herring macula posterior has a gross location and polarisation pattern similar to that of the lacinia in other fishes. The utricular opposition in herring differs significantly from that in the saccule in clupeids[11] and many other bony fishes, where hair cells are orientated away from lines of population division[9,10,14,15].

Because the directional sensitivity to shear has a morphological basis in the asymmetry of the ciliary bundle, the orientation patterns in the vertebrate ear are thought to have a functional significance[19,20]. The macula anterior of the herring utricle, with its opposed populations on the anterior wall, may be a bidirectional detector for dorso-ventral shearing displacements, and the large macula media could be the major bidirectional detector for rostral-caudal displacements. The sensitivity of this arrangement could be enhanced by the presence of the pro-otic fenestra from the pro-otic bulla that forms a fluid connection to the utricle[7]. This fenestra opens between the macula anterior and macula media and is in a prime location for driving displacements of hair cell bundles on those maculae.

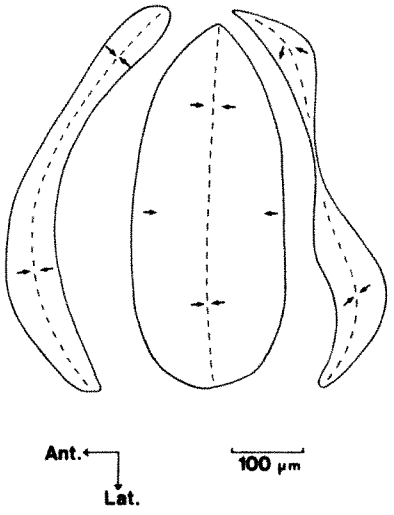


Fig. 2 Map of hair cell orientation patterns in the herring utricle (left ear, 50 mm fish). Arrows correspond to the morphological polarisation of hair cell populations in a sampled region, given by the asymmetric location of the kinocilium on the hair cell ciliary bundles. Dashed lines indicate divisions between populations of different orientations; two opposed populations occur in each of the three maculae. Note that this is a schematic flat projection map of surfaces from the rounded utricular pouch; the macula anterior actually lies on the curve of the anterior wall, giving its cells a substantial dorso-ventral orientation component.

These ultrastructural findings indicate that hair cell ciliary bundles and hair cell orientation patterns in the herring utricle are distinctly different from the utricular structure of any other vertebrate yet examined. Coupled with the known relationship between the gas bladder, bullae and utricle, these features are not incompatible with the argument that the herring utricle may respond to acoustic stimuli. Sensitivity could be enhanced by the gas-filled otic bullae, and input processed by the complex differentiation of the tripartite macula in a manner unique among vertebrates.

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A phosphoglycolate phosphatase-deficient mutant of *Arabidopsis*

CURRENT concepts of photosynthetic and photorespiratory carbon metabolism have been derived almost entirely from the results of biochemical and physiological investigations. To examine these concepts by genetic analysis, we have isolated several mutants of a C_3 species with defects in CO_2 assimilation and photorespiration. We report here the isolation and preliminary characterisation of the first of these, a mutant deficient in phosphoglycolate phosphatase activity.

The mutants were recovered as conditional lethals which are phenotypically indistinguishable from the wild type when grown in air enriched to 1% CO_2, but which are inviable in normal atmospheric conditions of 0.03% CO_2. The rationale for the screening procedure is that conditions of elevated CO_2 limit ribulosebisphosphate (RuBP) oxygenase activity[1], thereby reducing the flow of carbon through the photosynthetic carbon oxidation (PCO) cycle[2]. As chemical inhibition of enzymes in the PCO cycle reduces the photosynthetic rate in conditions which normally permit photorespiration, but does not affect photosynthesis at high CO_2 or low O_2 concentrations[3], we postulated that mutants with defects in the PCO cycle would be

viable only in conditions of high CO_2 or low O_2. These growth conditions should also permit the recovery of mutants with a greatly increased ratio of RuBP oxygenase to RuBP carboxylase activity.

Wild-type *Arabidopsis thaliana* (L.) Heynh. seed was mutagenised with ethyl methane sulphonate[4], brought to maturity in normal cultural conditions, and bulk collected. The M2 generation was grown to the four-leaf stage in air enriched to 1% CO_2. At this time, chlorotic plants and those which lacked vigour were removed. The CO_2 concentration was reduced to 0.03%, and plants which remained normal in appearance after 4 d were discarded. The remaining plants were returned to a 1% CO_2 regime for 4 d. Those plants which failed to recover were discarded. This procedure resulted in a 500-fold reduction in population size. The remaining plants were taken to maturity in 1% CO_2 without further screening. A similar screening procedure, applied in the M3 generation to the individual lines established from the M2, resulted in the retention of 40 probable mutants from an initial population of approximately 40,000 M2 plants. The putative mutants were advanced to the M5 generation by single seed selection without additional screening.

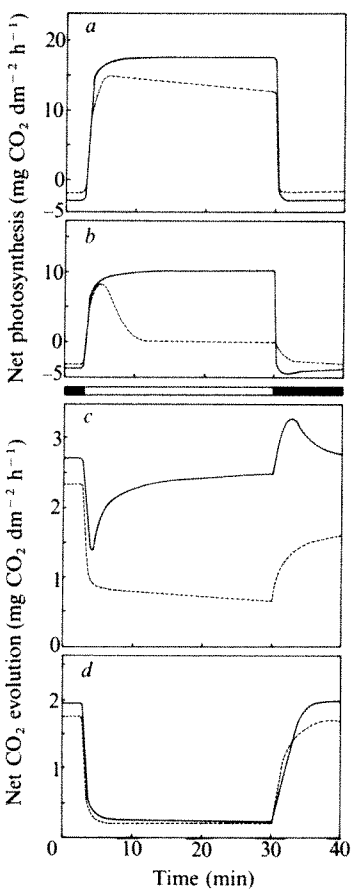


Fig. 1 Net CO_2 exchange of wild-type and mutant *Arabidopsis* in various gas regimes. Plants were grown in 1% CO_2, 21% O_2 and bal. N_2 at 25 °C and 75% RH under continuous fluorescent illumination (170 μ einsteins m^{-2} s^{-1}) on vermiculite irrigated with half-strength Hoagland's solution. Gas exchange was monitored on intact plants (at floral initiation stage) from which the lower leaves had been removed. The plants were placed in a glass chamber of 20 ml volume and continually flushed with a humidified (75% RH) gas stream at a flow rate (100–150 ml min^{-1}) sufficient to maintain a ΔCO_2 of less than 15 μl l^{-1}. The CO_2 concentration of the exiting gas was continuously monitored with an IR gas analyser. The total volume of the system, including measuring cuvette, was 46 ml. Light (300 μeinsteins m^{-2} s^{-1}) was provided by a 300 W tungsten lamp filtered through 15 cm of water. The plant chamber was maintained at 25 °C by immersion in circulating water. The composition of gas entering the chamber was: *a*, 355 μl l^{-1} CO_2, 2.0% O_2, bal. N_2; *b*, 352 μl l^{-1} CO_2, 21% O_2, bal. N_2; *c*, 50% O_2, bal. N_2; *d*, 2% O_2, bal. N_2. The response of the wild-type plants is indicated by the solid line, and that of the mutant by the broken line. The open or closed bar inserted in the figure represents light or dark conditions, respectively.

Table 1 Products of $^{14}CO_2$ assimilation by wild-type and mutant *Arabidopsis*

	Wild-type	CS119	Wild-type HBA-treated*	CS119 HBA-treated*
Basic fraction	21.4†	11.0	3.2	7.6
Glycine	10.5	0.5	0.3	0.4
Serine	8.4	0.9	0.3	0.5
Alanine	2.0	9.0	1.4	5.9
Neutral fraction	6.1	14.8	3.6	9.3
Acid-1 fraction	36.7	23.3	61.7	23.9
Glycolate	2.7	0.9	28.8	1.8
Acid-2 fraction	8.8	29.1	6.2	28.1
Phosphoglycerate	6.3	5.6	3.8	7.8
Phosphoglycolate	0.7	19.6	0.7	19.6
Acid-3 fraction	24.4	17.6	23.7	27.4
Recovery	97.4	95.8	98.4	96.3

Intact plants, grown as described in Fig. 1, were removed to a photosynthesis chamber and flushed with 400 μl l^{-1} CO_2, 21% O_2 and bal. N_2 at 25 °C, 75% relative humidity (RH), at 300 μeinsteins m^{-2} s^{-1} for 2 min. At this time the system was closed and $^{14}CO_2$ introduced. After 2 min of $^{14}CO_2$ incorporation, the plants were quickly (3–5 s) removed to liquid nitrogen, then extracted by grinding in 4 M formic acid. The CO_2 concentration in the chamber remained at 350–400 μl l^{-1} during the period of incorporation. The extract was lyophilised and the residual water-soluble compounds were fractionated by ion exchange and paper chromatography as described previously[3] except that the acid fractions were recovered from a Dowex-1 (formate) column by successive elution with 10 ml 4 M formic acid (acid-1 fraction), 10 ml 8 M formic acid (acid-2 fraction) and 6 ml 4 M HCl (acid-3 fraction). Phosphoglycolate was identified by co-migration with authentic samples in several solvent systems, before and after phosphate ester hydrolysis.

* Inhibition of *in vivo* glycolate oxidase activity was accomplished by submerging the roots of intact plants in 5 mM 2-hydroxy-3-butynoic acid (HBA)[16] for 20 min in the elevated CO_2 regime described in Fig. 1. Following treatment, the plants were labelled, extracted and fractionated as above. Control experiments, in which identically treated wild-type and mutant plants were assayed for glycolate oxidase activity, indicated that the HBA treatment resulted in >95% inhibition of enzyme activity.

† Values indicate % of total water-soluble counts recovered, and represent the mean of two independent experiments.

A preliminary survey of the gas exchange characteristics of the M5–M7 generations revealed that most of the putative mutants exhibited altered photosynthesis characteristics. The mutant strain CS119 was particularly striking in this respect. In a gas regime of 355 μl l^{-1} CO_2, 2.0% O_2 and balance N_2, the mutant assimilated CO_2 for extended periods at a rate about equal to that of the wild type (Fig. 1*a*). In an atmosphere containing 21% O_2, the photosynthesis rate of the mutant initially approached the wild-type rate but then rapidly declined to zero (Fig. 1*b*). This reduction of photosynthetic capacity was completely reversed by a 15–20-min dark treatment imposed at the time of 50–70% inhibition (data not shown). The rapidity of onset and the reversibility of inhibition suggest that the response is caused by a reversibly altered metabolite pool rather than photo-oxidative damage. Although we have not measured photosynthesis in 1% CO_2, normal growth of the mutant in such conditions implies that high levels of CO_2 can alleviate the deleterious effects of O_2 on photosynthesis. Thus, the mutant is

capable of sustained photosynthesis only in conditions which limit RuBP oxygenase activity[1,5].

The mutant also exhibited a greatly reduced rate of CO_2 release during illumination and did not show the post-illumination burst of CO_2 release commonly ascribed to photorespiration[5] (Fig. 1*c*). The rate of CO_2 release by the mutant in 2% O_2 (Fig. 1*d*) is about 30% of that observed in 50% O_2, suggesting that a substantial proportion of residual respiration in light is due to incomplete suppression of dark respiration during illumination[6]. Compared with other species[5], both wild-type and mutant plants exhibited a disproportionately high rate of CO_2 evolution in the dark. We have tentatively ascribed the relatively high dark respiration rates to the 24-h photoperiod used in cultivation of the plants.

The probable site of the lesion in the mutant was deduced from the pattern of $^{14}CO_2$ incorporation into early products of photosynthesis (Table 1). After 2 min of labelling in normal atmospheric conditions, 20% of the ^{14}C incorporated by the mutant was found in phosphoglycolate. In contrast, phosphoglycolate was barely detectable in wild-type *Arabidopsis*, as it is in other species examined under similar circumstances[3,7]. The increased labelling of the phosphoglycolate pool was accompanied by a large decrease in the labelling of serine and glycine, products of photorespiratory glycolate metabolism[2,8]. These results suggested that the mutant was deficient in phosphoglycolate phosphatase, a chloroplast enzyme which specifically hydrolyses phosphoglycolate to glycolate[9]. This conclusion was confirmed by assaying phosphoglycolate phosphatase activity in crude S-30 leaf extracts. The activity in extracts of the mutant was less than 5% of that found in extracts of the wild type (Table 2). We consider the presence of approximately wild-type levels of RuBP carboxylase activity in the mutant extracts to suggest that the chloroplast enzymes were recovered to a similar extent in the various enzyme preparations. Mixing extracts of mutant and wild-type plants did not result in inhibition of wild-type activity. Thus, we ascribe the reduction in phosphatase activity either to reduced enzyme synthesis or to the synthesis of a defective enzyme.

A single recessive nuclear mutation seems to be responsible for both lack of viability of the mutant and enzyme deficiency. F_1 progeny from the WT × CS119 cross were phenotypically wild-type in the normal gas regime, and leaf extracts contained ~50% of the wild-type ratio of phosphatase to carboxylase activity (Table 2). The restoration of one-half of wild-type activity in the heterozygote is consistent with the expectation for a mutation within the structural gene for the phosphatase[10]. Of 646 F_2 progeny, 153 exhibited the mutant phenotype. The 3:1 segregation (χ^2 0.60; $P < 0.4$) indicates a single recessive mutation. Several thousand M5 mutant seeds failed to produce any phenotypically wild-type plants, indicating purity and stability of the genotype. The co-segregation of phenotype and enzyme deficiency was tested by scoring 85 randomly chosen F_2 plants for a high CO_2 requirement at an early stage of growth by the method previously described. The plants were then revived in high CO_2, grown to the floral initiation stage, and assayed for phosphatase activity. We observed an absolute correspondence between low phosphatase activity and lethality (data not shown). Thus, both effects seem to be due to the same mutation, which we have designated *pcoA*-1.

Table 2 Phosphoglycolate phosphatase activity in wild-type and mutant leaf extracts

Strain	Phosphoglycolate phosphatase activity*	RuBP carboxylase activity*
Wild type	0.22	0.30
CS119	0.01	0.28
F_1 (WT × CS119)	0.08	0.23
Mixture†	0.11	0.29

Leaf tissue from 8–10 plants was ground at 4 °C in 10 mM HEPES (*p*H 7.0) and clarified by centrifugation at 30,000*g* for 20 min. Phosphatase activity was determined in 40 mM cacodylate (*p*H 6.3), 5 mM HEPES, 5 mM citrate, 5 mM $ZnSO_4$ and 0.5 mM EDTA[9]. The reaction was initiated with 2 mM phosphoglycolate dissolved in the same buffer. After 5 min at 25 °C, the reaction was terminated with acid molybdate reagent[18] and the release of phosphate determined colorimetrically. RuBP carboxylase activity was determined in anaerobic conditions by measuring the incorporation of $^{14}CO_2$ into acid-stable products[1]. The reaction mixture contained 50 mM Bicine (*p*H 8.1), 10 mM $MgCl_2$, 0.1 mM EDTA, 10 mM $NaHCO_3$ (0.1 μCi μmol^{-1}), 0.4 mM RuBP and 1 mM HEPES. The reaction was initiated with enzyme activated by preincubation in a similar mixture (no RuBP), and terminated after 2 min at 25 °C. All assays were carried out in triplicate on two independent extracts. Protein was determined colormetrically with the Folin–Ciocalteau reagent.

* Enzyme activity is expressed as μmol per min per mg protein.

† Equal volumes of wild-type and mutant extract were mixed immediately before assay.

Most of the properties of the *pcoA* mutant can be explained on the basis that phosphoglycolate is a potent inhibitor of triose phosphate isomerase[11]. When the mutant is illuminated in normal atmospheric conditions, phosphoglycolate produced by the RuBP oxygenase reaction[12,13] may accumulate at levels which inhibit the conversion of glyceraldehyde-3-phosphate (GAP) to dihydroxyacetone phosphate. This would reduce synthesis of RuBP and concomitantly reduce photosynthesis. The restoration of photosynthetic capacity in the mutant following a brief dark treatment is attributed to the dissipation of accumulated phosphoglycolate by residual phosphatase activity. Because GAP is a major export product of photosynthesis[14], inhibition of the isomerase would result in increased export of GAP as a substrate for cytoplasmic sucrose synthesis. We attribute the increased labelling of the neutral fraction in the mutant (Table 1) to such a mechanism. Also, increased labelling of the alanine pool in the mutant suggests that a proportion of the GAP may be converted to pyruvate by glycolysis.

Several properties of the mutant are directly relevant to the continuing controversy regarding the source of photorespiratory glycolate and CO_2 (refs 5, 15). First, it is now clearly established that phosphoglycolate is the major, if not sole, precursor of glycolate, as shown from an experiment in which glycolate metabolism was blocked by feeding the plants hydroxybutynoic acid (HBA), a potent inhibitor of glycolate oxidase (Table 1)[16]. The pattern of $^{14}CO_2$ incorporation in the wild-type in the presence of HBA is consistent with similar studies with other species[3,17] in that a large proportion of the label is found in glycolate, associated with a corresponding decrease in serine and glycine labelling. In contrast, the HBA-treated mutant does not accumulate significant amounts of labelled glycolate. Second, the low level of CO_2 evolution in the light and the absence of a post-illumination CO_2 burst in the mutant indicates that phosphoglycolate is a major precursor of photorespiratory CO_2. Third, reduced labelling of the glycine and serine pools supports the proposed flow of photorespiratory carbon through phosphoglycolate to glycolate, glycine and serine[2].

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Possible function of juvenile hormone-dependent protein in larval insect diapause

JUVENILE HORMONE (JH) has been shown to control the synthesis and storage of a diapause-associated protein (DAP) in the larval fat body of the southwestern corn borer, *Diatraea grandiosella* Dyar[1]. Our results indicate that DAP is synthesised *de novo* in pre-diapausing southwestern corn borers, reaches a maximum titre in newly diapaused larvae, and is used gradually during diapause. In addition, we have demonstrated recently that the protein has a molecular weight of about 35,000, and an isoelectric point of about 5.9 (unpublished). Since JH controls the larval diapause of this insect[2,3], DAP may be involved in diapause maintenance. Some properties of DAP are described here and the evidence for its function is appraised. Possible functions include a nutritive store of amino acids or peptides, a reservoir of a proenzyme, or a JH-binding protein. From the available evidence, the most likely model is that of a latent store of a high affinity JH-binding protein[4–7] which is released and activated during diapause.

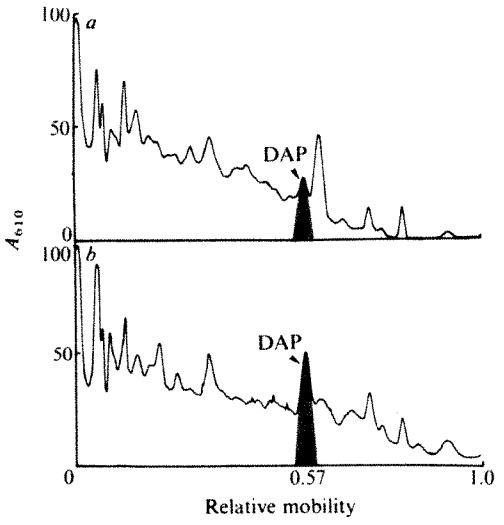


Fig. 1 Densitometric scans of electropherograms showing the effect of topical C_{17}-JH treatments on the proteins of the fat body of *D. grandiosella* larvae. *a*, Larvae (19 days old) at the beginning of the treatments. *b*, Larvae (28 days old) after 10 daily treatments (6 μg C_{17}-JH per 3 μl acetone per day). Larvae were reared in the diapause-averting conditions (25 °C; 16 h light, 8 h dark) until 19 days and then transferred to the diapause-inducing conditions (25 °C; 12 h light, 12 h dark). Untreated and acetone-treated controls pupated within 10 days without accumulating the diapause-associated protein (DAP), whereas those treated daily with C_{17}-JH accumulated DAP and entered a diapause-like state within about 24 days. After fat body homogenates were centrifuged at 21,000*g* for 30 min at 4 °C, proteins were subjected to electrophoresis in 7% acrylamide gels using a discontinuous buffer system with Tris-glycine (*p*H 8.3) as the bath buffer (2 mA per gel at 4 °C). Equal portions of the fat body were analysed to show changes in the total protein concentration. Based on estimates of soluble proteins present in the perivisceral fat body, the DAP content of a newly diapaused larva is about 400 μg.

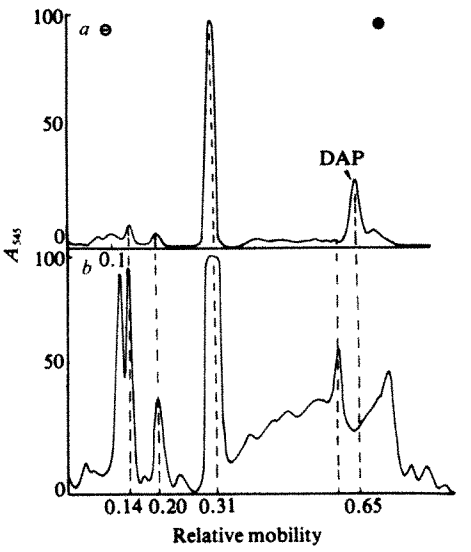


Fig. 2 Densitometric scans of electropherograms showing the relationship of proteins released from the perivisceral fat body to those present in the haemolymph in 110-day-old diapausing larvae of *D. grandiosella*. *a*, Incubation medium (100 μl, ~15 μg protein) after incubating one right and one left fat body sheet in 500 μl macromolecule-free Grace's medium (Grand Island Biological Co.) for 10 min. at 21 °C. *b*, Haemolymph (0.5 μl, ~40 μg protein). Electrophoretic conditions as described in Fig. 1, except that 5% acrylamide gels were used.

The effect of C_{17}-JH on the induction of larval diapause and the accumulation of DAP was examined (Fig. 1). Larvae which were reared on an artificial diet in diapause-averting conditions long enough to programme them for metamorphosis were transferred to diapause-inducing conditions and treated with C_{17}-JH, the principal JH homologue present in diapausing larvae[8]. The treated larvae continued to feed, and about 70% moulted into pigment-free immaculate morphs and entered a diapause-like state, whereas about 90% of the untreated larvae pupated directly. Daily C_{17}-JH treatments induced the perivisceral fat bodies to accumulate substantial amounts of DAP within 10 days. Only small amounts of DAP were present in the fat body at the beginning of the JH treatments, and little, if any, DAP was present in the fat body of pupae. These findings extend earlier observations that showed a similar pattern of accumulation of DAP in the fat body of non-diapausing larvae which had been treated with a JH mimic[1]. This JH regulation of protein synthesis in pre-diapausing larvae seems comparable to the JH control of vitellogenin synthesis in some adult insects[9].

The fat body of diapausing larvae was incubated *in vitro* to study protein release (Fig. 2). Larvae were reared in diapause-inducing conditions (23 °C; photoperiod of 12 h light, 12 h dark. In these conditions they passed through six larval stages, completed feeding between 35 and 40 d, transformed into immaculate morphs, and entered diapause[10]. The release of DAP from the fat body of 110-day-old diapausing larvae was detected within 10 min of incubation in macromolecule-free Grace's medium. Since the release was partially inhibited by potassium cyanide (10^{-2} M) and ouabain (5×10^{-3} M), it appears to be an energy-requiring process. Our results also showed that DAP was more readily released from the fat body of mid-diapausing larvae (110 days old) than from that of a last stage pre-diapausing larva (35 days old), even though larvae of

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both ages have a high titre of DAP in their fat bodies. In spite of the rapid discharge of DAP *in vitro*, and its gradual disappearance from the fat body of diapausing larvae *in vivo*, DAP does not accumulate in the haemolymph[1], suggesting that it may be structurally modified in the haemolymph.

To test if this structural modification of DAP occurs, incubation medium containing released DAP was treated with several concentrations of protease-containing midgut extracts. Electrophoretic examination indicated no decrease in the concentration of DAP attributable to midgut enzyme activity. Subsequently, the effect of the proteolytic enzyme, trypsin, was tested on the hydrolysis of DAP. Although incubation at 37 °C for up to 3 h resulted in the complete hydrolysis of proteins at relative mobility 0.10 and 0.20 (Fig. 2), DAP remained intact, thereby showing that DAP is relatively resistant to trypsin. Consequently, tritiated DAP was incubated for 60 min at 37 °C in a medium containing haemolymph from pre-diapausing larvae. The medium was then subjected to electrophoresis in 5% and 7% acrylamide gels and the distribution of radioactivity in

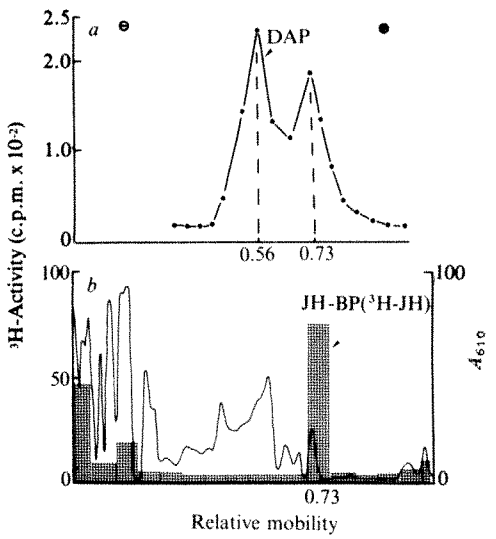


Fig. 3 Relationship between the diapause-associated protein (DAP) and the haemolymph proteins of *D. grandiosella*. *a*, Radioactivity in gel slices showing modification of a portion of prelabelled DAP following an incubation *in vitro* in macromolecule-free Grace's medium containing haemolymph from pre-diapausing larvae. Radiolabelled DAP was obtained by injecting 34-day-old pre-diapausing larvae with L-[4,5-^{3}H(N)]leucine (3.30×10^6 d.p.m., specific activity 5.0 Ci mmol^{-1}, NEN). After 48 h fat body proteins (21,000*g* supernatants) were extracted and subjected to disc electrophoresis. The zone containing DAP was homogenised in distilled water and centrifuged. The supernatant was lyophilised and tritiated DAP (200 μg per 100 μl distilled water) was incubated with Grace's medium (350 μl) and haemolymph (50 μl) from 36-day-old pre-diapausing larvae for 60 min at 37 °C. The medium was electrophoresed and the gels were sliced into 2–4-mm sections. Proteins were eluted with Soluene-350 and after a stable background had been reached the radioactivity in the slices was counted in Aquasol-2 (10 ml per vial) using a Beckman LS-100C liquid scintillation counter. The formation of the modified fraction seems to depend on the concentration of haemolymph and the incubation temperature, decreasing markedly at 23 °C. *b*, Densitometric scan of electropherogram and the distribution of radioactivity in gel slices showing *in vitro* binding of tritiated C_{17}-JH to haemolymph proteins of 36-day-old pre-diapausing larvae. Haemolymph (50 μl) was preincubated for 10 min in 450 μl macromolecule-free Grace's medium containing dichlorvos (10^{-4} M) as an esterase inhibitor. The medium was then charged with [10-^{3}H(N)]-C_{18}-JH (1 μCi, specific activity 13.5 Ci mmol^{-1}, NEN), and incubated for 20 min at 37 °C. After electrophoresis was carried out in 7% acrylamide the gels were sliced, and the radioactivity in the slices was measured as described under *a* (hatched boxes). Solid line, densitometric scan of electropherogram. JH-BP, Juvenile hormone-binding protein.

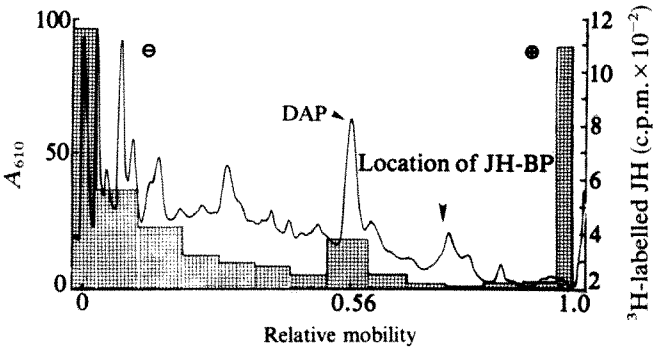


Fig. 4 Densitometric scan at 610 nm of fat body proteins (150 μg) of 39-day-old newly diapaused female larvae of *D. grandiosella* showing the binding of C_{18}-JH to protein fractions. The fat body homogenate was centrifuged at 21,000*g* for 30 min. The clear supernatant (300–500 μl) was treated with dichlorvos (10^{-4} M) to inhibit esterase activity, and then incubated for 20 min at 37 °C with 1 μCi of ^{3}H-labelled C_{18}-JH. After electrophoresis in 7% acrylamide the gels were sliced into 5-mm sections and the radioactivity was measured to 1% error as described under Fig. 3*a*. Solid line, densitometric scan; hatched boxes, radioactivity. JH-BP, Juvenile hormone-binding protein.

gel slices was examined (Fig. 3*a*). In these conditions a portion of DAP was converted to a fraction with a higher relative mobility, whereas incubation without haemolymph yielded only a single peak of radioactivity corresponding to DAP. The relative mobility of the modified fraction of DAP corresponded to that of the high affinity JH-binding protein present in the haemolymph (Fig. 3*b*). Subsequently, incubation of fat body homogenates with tritiated JH indicated that DAP showed only slight affinity for JH. No binding of JH occurred in fat body proteins corresponding to the JH-binding protein present in the haemolymph (Fig. 4). Since only enzymes present in the haemolymph appear capable of modifying DAP, the binding activity of the modified fraction has not yet been measured in the absence of haemolymph JH-binding protein.

The evidence obtained on the characteristics of DAP may be summarised as follows: (1) DAP accumulates in the fat body of pre-diapausing larvae in response to their high JH titre and is gradually used during diapause[1]; (2) DAP is released intact from the fat body *in vitro*, but it does not accumulate in the haemolymph of diapausing larvae *in vivo*; (3) DAP is resistant to hydrolysis by trypsin and midgut enzymes; (4) a portion of DAP is structurally modified during incubation *in vitro* in a medium containing haemolymph; (5) the amino acid composition of DAP bears little resemblance to that of the free amino acids present in the haemolymph[1]; and (6) DAP shows only a weak affinity for C_{18}-JH *in vitro*.

With the exception of the sex-linked vitellogenin[11] relatively little is known of the functions of the exportable proteins of the insect fat body. Although DAP could serve as a store for amino acids or peptides to be used during diapause, such a function is doubtful. Early and mid-diapausing larvae rely mainly on reserve triglycerides[12], and mid-diapausing larvae already show considerable loss of DAP from their fat bodies[1]. The molecular weight of DAP is also small compared with those of suspected haemolymph storage proteins[11]. Electrophoretic evidence did not reveal any relationship between DAP and the corn borer's vitellogenin[1]. Although a proenzyme function for DAP has not been ruled out, such a high requirement for a single enzyme seems unlikely (Fig. 1).

The following observations are relevant to the possible function of DAP as a latent store of JH-binding protein. Insects which undergo a JH-maintained diapause would seem to derive an adaptive advantage by storing an inactive JH-binding protein. The storage protein would not sequester JH, yet it would be readily available for release into the haemolymph during diapause when protein synthesis is at a minimum[13,14], thereby

ensuring the maintenance of a sufficient titre of JH in the haemolymph. The JH-binding protein present in the haemolymph of last instar pre-diapausing larvae of *D. grandiosella* has a molecular weight of 28,000, an isoelectric point of approximately 4.85, an apparent dissociation constant of approximately 3×10^{-7} M, a concentration of about 3.3×10^{-6} M, and a relative mobility of 0.73 in 7% acrylamide gels. The content of JH-binding protein in these larvae was estimated to be 11 μg, based on a haemolymph volume of 100 μl (unpublished). Although the half life of the binding protein is unknown, a store of about 400 μg DAP in a newly diapaused larva (Fig. 1) would seem to fall within the limits for a JH-binding protein store required over at least a six-month period. In addition, unlike the larval fat body of two other lepidopterous insects, *Manduca sexta* and *Plodia interpunctella*[15,16], that of *D. grandiosella* does not seem to contain or to release an active JH-binding protein. Neither the fat body nor the midgut of the southwestern corn borer shows JH binding corresponding to the high affinity JH-binding protein, yet the available evidence implicates the insect fat body as the source of the binding protein[15,16]. The present study suggests a critical function for a fat body protein in the maintenance of larval diapause.

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Evidence that murine pre-B cells synthesise μ heavy chains but no light chains

THE first identifiable member of the B-cell line, termed the pre-B-cell, has been shown by immunofluorescent techniques to contain small amounts of intracellular IgM, but to lack detectable surface immunoglobulin[1]. These cells have been found in several species at sites of B-cell generation, most notably fetal liver and adult bone marrow[1,2] and considerable evidence now exists which supports this precursor–progeny relationship of pre-B cells and B lymphocytes[1–5]. However, conflicting data exist with regard to pre-B cell expression of μ and light chains. Although these cells were originally described in mice as containing both intracellular heavy (μ) and light chains[1], using the same immunofluorescence techniques we have subsequently been unable to demonstrate either κ or λ light chains within these cells (P.B. and J.K., unpublished). Moreover, the presence of light chains in murine pre-B cells cannot be confirmed with the original anti-κ antibodies used in ref. 1 (M. Cooper, personal communication). These findings are also contrary to immunochemical data which suggest the presence of complete IgM molecules both intracellularly and on the surface of early fetal liver cells[6]. We have used somatic cell hybridisation to approach this controversial issue. We have now obtained, after fusion with fetal liver, hybridomas which synthesise large amounts of intracellular μ chain in the absence of any light chain (LC) synthesis detectable by immunofluorescent or immunochemical techniques. The immunoglobulin phenotype of these hybridomas is identical to that observed in immunofluorescence studies of pre-B cells from fetal liver or adult bone marrow, and suggests that synthesis of μ chain before light chain expression is a normal event in the early differentiation of the B-cell line.

Mouse fetal liver cells were obtained from BALB/cN and C57BL/6J fetuses of different gestational ages. These cells were fused with one of three different variants of the myeloma P3×63Ag8 (γ1, κ) by a modification of methods described by Lemke *et al.*[7]. These lines were (1) NS-1 (−, κ) which no longer synthesises the myeloma γ1 heavy chain but only the κ chain[8], (2) Ag8(6), which is similar to NS-1 (−, κ), and (3) Ag8653, a variant which has lost the ability to synthesise both myeloma γ1 and κ chains even after fusion. The isolation and characterisation of the latter two cell lines are described elsewhere[9].

The hybridomas isolated after fusion with Ag8653 are described in Table 1. It can be seen that the ratio of recoverable μ chain-synthesising hybrid cells compared with the total number of hybridomas obtained per fusion increased with the age of the fetuses, in parallel with the number of pre-B cells in fetal liver[1]. Hybrids were obtained from fetal liver fusions as early as 15 days of gestation, 2 days before we can detect $sIgM^+$ B cells[10]. The most striking observation is that of the 28 μ chain-synthesising hybridomas only 1 expressed either cytoplasmic or secreted light chains. SDS-polyacrylamide gel electrophoresis of biosynthetically labelled intracellular proteins

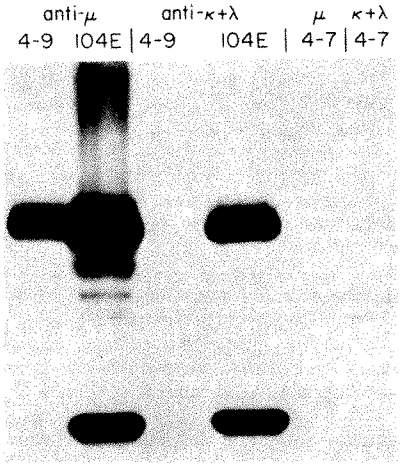


Fig. 1 Fluorogram of Ig synthesised by fetal liver hybridomas. MOPC 104E myeloma ascites cells and fetal liver hybridoma cells were cultured for 4 h in the presence of ^{14}C- and ^{3}H-labelled amino acids (Schwarz-Mann; protein hydrosylate), respectively. Cells were washed, lysed with 0.5% NP-40, and immunoprecipitated with purified goat antibodies to mouse μ or κ and λ chains[9] by the *Staphyloccocus aureus* technique[20]. After elution from the bacteria with SDS-urea, the immunoprecipitates were reduced, alkylated and electrophoresed on 8–12% SDS-diallyl tartardiamide (DATD)-acrylamide gels[21]. Fluorography was by the method of Bonner and Laskey[22].

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Table 1 Ig-Synthesising fetal liver hybridomas derived from fusion with the non-producer myeloma Ag8653

Age* and strain of fetus	No. of fetal liver cells fused	No. of hybrids No. of wells	Immunoglobulin phenotype† of hybridomas	Frequency
15 day (C57BL/6) timed by vaginal plug	1.5×10^8	240/840	μ^+, light chain$^-$; non-secretor	4/230
17 day (C57BL/6)	0.7×10^8	40/264	μ^+, light chain$^-$; non-secretor	1/40
17–19 day pooled fetal livers (BALB/cN)	1.5×10^8	83/240	μ^+, light chain$^-$; non-secretor	19/83
			$\mu^+\kappa^+$; secretor	1/83
18–19 day (BALB/cN)	0.7×10^8	30/240	μ^+ light chain$^-$; non-secretor	3/30

Cells were fused using polyethylene glycol (PEG) by methods described in Lemke *et al.*[7]. Briefly, dissociated fetal liver cells were mixed with an equal number of myeloma cells washed twice in pre-warmed RPMI 1640 without serum. The cell pellet was resuspended by gentle pipetting in 40% pre-warmed PEG. After ~1 min the PEG was diluted by slow dropwise addition of RPMI 1640 at 37 °C. The cells were pelleted and distributed directly in 1 ml of medium containing hypoxanthine, aminopterin and thymidine[18] into 24-well Costar plates so that each well contained $\sim5\times10^5$ fetal liver cells per well. Frequency = number of Ig^+ hybridomas/total number of hybridomas.

* Unless stated the ages of the fetuses were estimated.

† Intracellular immunoglobulin was detected by two methods: direct immunofluorescence using purified antibodies to mouse μ, κ and λ chains[19], or a modified enzyme-linked immunosorbent assay (ELISA). Briefly, an aliquot of hybridoma cells was detergent (NP-40) lysed *in situ* within the wells of plastic plates coated with purified goat anti-mouse μ chain antibody. Wells containing bound mouse μ chains were detected by addition of alkaline phosphatase-labelled goat anti-μ followed by the substrate *p*-nitrophenyl phosphate. A bright yellow reaction product was generated in positive wells. A more detailed description of this technique is given in ref. 9. Positive ELISA results were immediately confirmed using direct immunofluorescence. Secreted immunoglobulin was detected by analysis of tissue culture supernatants by either an ELISA, using anti-μ-coated microplates or immunoprecipitation and SDS-polyacrylamide gel analysis of biosynthetically labelled secreted protein.

immunoprecipitated from two representative fetal liver hybrids (4–9 and 4–7) is shown in Fig. 1. Two bands of radioactivity, corresponding to a μ chain and a light chain, were immunoprecipitated from the control, MOPC 104E (μ, λ plasmacytoma), by either anti-μ, or a mixture of anti-κ and anti-λ antibodies. In striking contrast, only μ chains were precipitated from 4–9 with anti-μ, and nothing with anti-κ and anti-λ. The hybridoma line 4–7 was derived from the same fetal liver fusion but was negative for immunoglobulin synthesis by immunofluorescence, a finding confirmed by biosynthetic labelling. The μ chain of 4–9 seems to be slightly larger than that of MOPC 104E on these gels. Simultaneous electrophoresis of ^{3}H-labelled 4–9 and ^{14}C-labelled 104E μ chains on the same gel, which was then sliced and counted after electrophoresis, confirmed this size difference (Fig. 2). The small peak of radioactivity in the light chain region of 4–9 is probably due to normal plasma cells in the peritoneal exudate feeder cells on which 4–9 was being maintained at this stage, as it has not subsequently been seen in the absence of feeder cells (data not shown). The 4–9 μ chain is also larger than the intracellular μ chain of a μ, κ hybridoma derived from adult spleen (not shown).

Although fusion of fetal liver cells with Ag8653, which contributes no heavy or light chains of its own, almost invariably resulted in hybrids with the phenotype μ^+LC^- non-secretor, this did not occur when NS-1 or Ag8(6), both of which synthesize intracellular κ chains, was used. Most of the μ^+ hybridomas also synthesized κ light chains and secreted both μ and κ chains. To determine whether, in these hybrids, the light chains were derived from the myeloma or pre-B cell parent, the radiolabelled secreted IgM was isolated, reduced and separated into light and heavy chains by slab gel electrophoresis. The light chain region was cut directly from the gel and subjected to isoelectric focusing[11] (Fig. 3). The light chains from a fetal liver μ, κ hybridoma derived from fusion with NS-1 were identical in focusing patterns to the κ chains of the parent myeloma, P3 × 63Ag8. Therefore, the κ chains which these hybrid cells assemble with μ chains and secrete as IgM molecules are derived from the parental myeloma and no light chain is contributed by the pre-B cell.

Our results indicate that these μ^+LC^- hybridomas are derived from fusion events between pre-B cells and myelomas. Alternate possibilities, that they are derived from B lymphocytes or that their phenotype is due to aberrant regulation of immunoglobulin synthesis unique to these kinds of hybridomas, are unlikely for the following reasons. (1) The phenotype of these fetal liver hybridomas (μ^+LC^-) reflects that which we have observed by immunofluorescence to be normal for mouse pre-B cells. (2) We have obtained these hybridomas from fetal liver before the ontogenetic appearance of $sIgM^+$ B lymphocytes. (3) There are well documented observations that the myelomas used for these fusions support synthesis of all heavy and light chain classes of immunoglobulins by hybrids made from adult spleen or lymph node cells[9,12,13]. This mitigates against the possibility that there has been a loss of some type of regulatory mechanism from these myelomas involved in κ chain synthesis

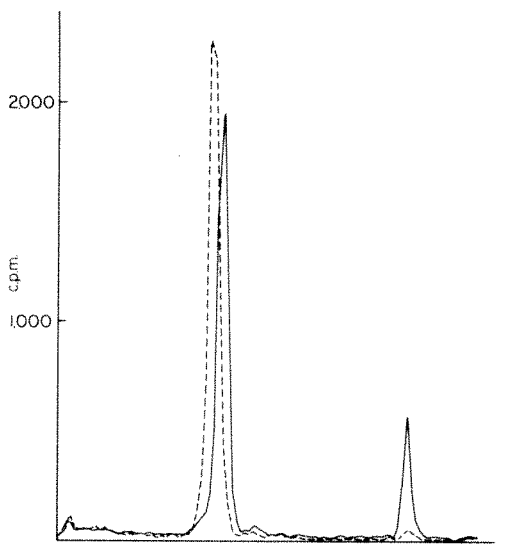


Fig. 2 SDS-polyacrylamide gel analysis of intracellular μ chains from the fetal liver hybridoma 4–9. Tritium-labelled 4–9 (– – –) and ^{14}C-labelled MOPC 104E (——) cell lysates were immunoprecipitated with anti-μ as in Fig. 1 legend. Both samples were electrophoresed on the same 8–12% SDS-DATD-acrylamide gel. One-mm gel slices were dissolved in 2% periodate, and ^{3}H and ^{14}C counted. The 4–9 μ chain peak was 3 × 1 mm slices larger than the μ chain of MOPC 104E.

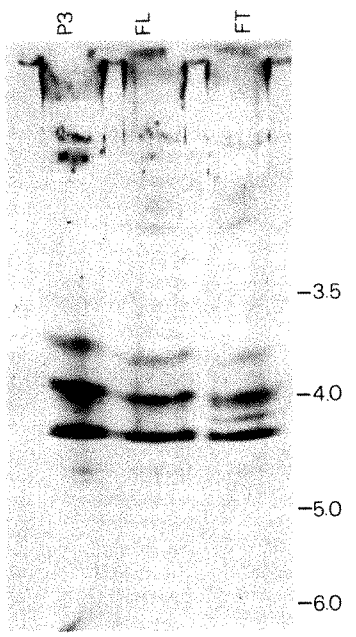


Fig. 3 Fluorogram of light chains separated by analytical isoelectric focusing in polyacrylamide gel. Purified ^{14}C- or ^{3}H-labelled secreted Ig was isolated as in Fig. 1, reduced and electrophoresed on 10% SDS-polyacrylamide gels. 10 μg of purified, fluorescamine-labelled, reduced and alkylated MOPC 104E IgM was included in each sample well as a marker. After electrophoresis, the light chain band was located by fluorescence of the MOPC 104E light chains on exposure of the gel to UV light. A small section (3 × 7 mm) of the gel containing the light chains was cut out and transferred to the well of an IEF gel[11]. The gel slice was then overlaid with 6 M urea and 1% NP-40. IEF gels contained in a final concentration 4.6% acrylamide, 0.12% NN'-methylene bisacrylamide, 16.6% glycerol, 4.3 M urea, 0.5% NP-40 and 2.3% carrier ampholytes (*p*H 3.5–10). Polymerisation was initiated by adding 0.00016% riboflavin, 0.025% tetramethylethylenediamine and 0.00125% ammonium persulphate and exposing the casted gel to fluorescent light for 3–4 h at 4 °C. Light chains from the following cell lines are shown: (1) P3 = P3 × 63Ag8. This is the parental myeloma (γ1, κ) from which NS-1 (ref. 8) and Ag8(6) (ref. 9) were derived. (2) FL = FL-26-13. A μ, κ fetal liver hybridoma derived from fusion of 18–19-day fetal liver cells with NS-1. Comparison with lane 1 reveals that the light chains were derived from NS-1. (3) FT = FT-93-4. A hybridoma secreting μ in association with both κ and λ light chains. The κ chains seem to be provided by NS-1, whereas the extra band probably represents the λ chains of the antibody forming cell. Tissue culture supernatants from (1) were immunoprecipitated with anti-γ1. Those from (2) and (3) were precipitated with anti-μ.

which cannot be reconstituted by the pre-B cell alone. (4) In an examination by double immunofluorescence of over 2,000 individual cells from 24 hybridoma-containing wells derived from a fusion of Ag8653 with spleen cells from hapten-immunised C57BL/6 mice, none was found that expressed μ chains without κ or λ chains (J.K. and P.B., unpublished observations). These results also agree with other findings that normally after fusions with mouse myelomas, heavy chain loss precedes light chain loss (refs 12, 13 and J.K. unpublished). (5) In these experiments individual cells of fetal liver-derived hybridomas were screened by immunofluorescence 14–15 days after fusion, directly from the original tissue culture wells. Thus, there was little opportunity for the development of light chain negative variants. If selective chromosomal loss were the explanation, it would have had to occur simultaneously in all cells in the clone (which we have never observed) or at the time of fusion. (6) In addition, it has recently been shown that some Abelson virus and chemically transformed cell lines believed to be derived from early B cells also express only μ heavy chains with no concomitant light chain synthesis[14,15]. (7) Direct immunochemical analysis of radiolabelled murine fetal liver cells also supports our hypothesis that μ chain synthesis normally precedes light chain expression (D. Levitt, unpublished observations).

As we have obtained predominantly μ^+LC^- hybridomas from fetal livers even at a time of development, when there are at least as many $sIgM^+LC^+$ B lymphocytes present as there are pre-B cells, how are we to explain the bias towards hybridomas with a pre-B cell phenotype? We have previously shown that in fusions of lipopolysaccharide (LPS) activated B cells the maximum fusion frequency correlated with peak cell proliferation[16]. The finding that many pre-B cells have been shown to be rapidly dividing while most newly generated B cells are not[3,4,17] may explain why two cell types representing such extremes of differentiation, that is, a pre-B cell and a myeloma, can fuse successfully. The immunoglobulin-negative fetal liver hybridomas (for example 4–7), which are frequently obtained, would by the same reasoning be derived from other proliferating fetal liver cells. Whether these cells are lymphoid or are derived from non-lymphoid embryonic cells, their further analysis should prove interesting.

The biological significance of asynchronous onset of μ and light chain synthesis is, of course, open to speculation but could be related to mechanisms involved in the generation of antibody diversity. If variable-region genes are expressed in an orderly, preprogrammed manner, it would be advantageous for a pre-B cell with extensive self-renewal capacity to fix a particular $C\mu V_H$ gene combination. In this sense a single pre–B cell would become the stem cell for a given V_H, and its offspring could then select from the entire V_L repertoire by a similar mechanism. In this way extensive combinatorial associations between V_H and V_L would be possible in the immunocompetent B cells generated from their pre-B cell precursors.

Because pre-B cells synthesise μ chains but apparently secrete little, if any, immunoglobulin, fusion of these cells with appropriate myelomas has allowed us to expand the clonal product of these cells. We will then be able to study pre-B cell μ chains before any kind of positive or negative selection can be exerted through the specificities of these immunoglobulins, as these cells do not seem to express stable receptors[1,5]. For these reasons the use of hybridomas made from fetal liver pre-B cells provides a unique means by which the functions, immunoglobulin structure and specificity of pre-B cells can be studied.

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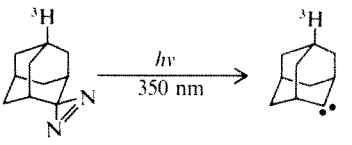
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Selective labelling of the hydrophobic segments of intrinsic membrane proteins with a lipophilic photogenerated carbene

HYDROPHOBIC photogenerated reagents are attractive candidates for the labelling of those parts of membrane proteins that are buried within the lipid bilayer. Recently, nitrenes generated from aryl azides have been used to distinguish intrinsic from extrinsic membrane proteins[1-4] and to label glycophorin A in or close to the transmembrane hydrophobic sequence[5]. We have questioned the use of aryl nitrenes on the basis of their evident lack of reactivity towards carbon–hydrogen bonds in



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single-bilayer phospholipid vesicles[6]. Because the lipid binding sites of intrinsic membrane proteins are believed to contain chemically unreactive hydrophobic amino acids, a reactive species that is less selective than a nitrene is needed to increase the probability that all the protein segments that penetrate the lipid bilayer will be labelled. In general, carbenes are more reactive than nitrenes (ref. 7 and refs therein); indeed, we have shown that carbenes generated within lipid bilayers do insert into saturated fatty acyl chains[8]. It is important to characterise the reactivity of lipophilic photogenerated reagents with well studied membrane proteins, whose membrane-associated regions have been identified by other experiments. We report here that the carbene, ^{3}H-adamantylidene (II), generated photochemically within biological membranes from ^{3}H-adamantane diazirine (I), labels three such intrinsic membrane proteins in or close to the hydrophobic peptides that are believed to lie within the hydrocarbon region of the lipid bilayer.

The carbene precursor 1-[^{3}H]spiro[adamantane-4,4′-diazirine] (I) is a stable compound which absorbs in the near UV (λ_{max} 372.5 nm, ε 245; the previously reported value[8] of 167 is incorrect). In the presence of phospholipid vesicles, the concentration of the diazirine within the bilayer is about 6,000-fold higher than the concentration in the surrounding solution[9]. Photogenerated ^{3}H-adamantylidene, which unlike most other dialkyl carbenes does not rearrange to unreactive molecules, labels the intrinsic proteins of the human erythrocyte membrane far more heavily than the extrinsic proteins[9]. Details of the

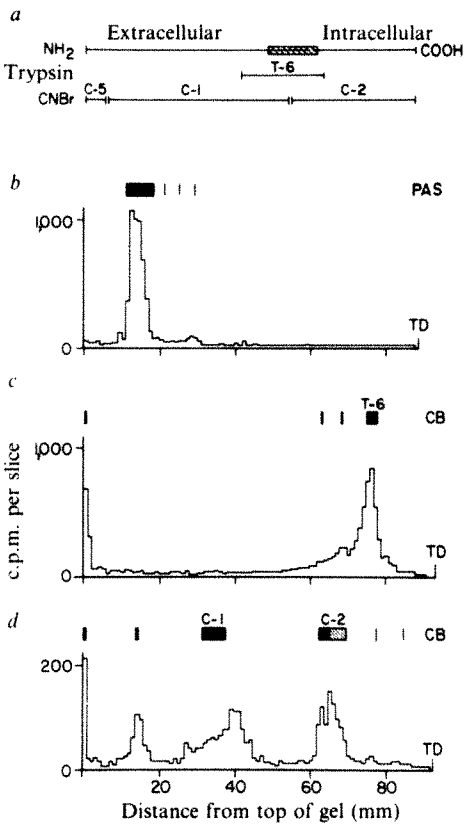


Fig. 1 Location of the sites in glycophorin A labelled by ^{3}H-adamantylidene. *a*, Schematic diagram of glycophorin A indicating the hydrophobic region (shaded), the T-6 tryptic peptide and the cyanogen bromide fragments (C-5, C-1 and C-2)[12,13]. *b*, SDS–PAGE profile of purified, labelled glycophorin A. ^{3}H-adamantane diazirine in ethanol was slowly added to a stirred suspension of human erythrocyte membranes (3–4 mg ml^{-1} of protein) in a glass-walled tube. The membrane preparation had been thoroughly purged with N_2 before the addition. The final concentration of the reagent was 12 μM (10 μCi ml^{-1}, specific radioactivity 840 mCi mmol^{-1}) and of ethanol, 1.0–1.5% (v/v). The solution was incubated on ice for at least 30 min, and then it was photolysed while stirring for 15 min at ~10 °C, 3 cm from a Rayonet RPR 3,500 Å UV lamp. The labelled membranes were diluted 10-fold into bovine serum albumin (10 mg ml^{-1}) in 5 mM Na_2HPO_4, *p*H 8.0, allowed to stand for 10–15 min at 4 °C and then recovered by centrifugation. After four such washes, the membranes were washed four times with 5 mM Na_2HPO_4, *p*H 8.0. The final pellet contained 35–40% of the radioactivity added initially. Glycophorin A was purified from the washed membranes by the LIS–phenol method[14], followed by gel filtration on Sepharose 6B-CL (Pharmacia) in 0.1% Ammonyx-LO (Onyx Chemical Co.), 5 mM Na_2HPO_4 and 25 mM NaCl, *p*H 7.4 (ref. 15). *c*, SDS–PAGE profile of the hydrophobic peptide, T-6. Purified glycophorin A was treated with trypsin and the T-6 peptide was recovered by acid precipitation. The radioactivity co-migrates with the broad Coomassie Blue-stained band characteristic of the T-6 fragment[11,16]. *d*, Distribution of radioactivity and Coomassie Blue stain after SDS–PAGE of the fragments generated by treating glycophorin A with cyanogen bromide for 48 h (ref. 12). All samples were subjected to electrophoresis on 12% acrylamide, 0.4% bisacrylamide tube gels. The electrophoresis buffer was 0.1 M Tris-Bicine, 0.1% SDS, *p*H 8.3. The gels were stained with periodic acid-Schiff's reagent (PAS) or with Coomassie Blue (CB). Stained gels were cut into 1-mm slices which were incubated in Protosol/Liquifluor/toluene (5:4:91, v/v/v) at 40 °C for 12 h and then counted in a liquid scintillation counter. The position of the tracking dye (bromphenol blue) is indicated by TD.

synthesis of the reagent and its full characterisation will be reported elsewhere.

The first intrinsic membrane protein that we have investigated in detail is glycophorin A, the major sialoglycoprotein of the human erythrocyte, which contains a single polypeptide chain that spans the plasma membrane[10-11]. It has been proposed that residues 73–95, which form a predominantly hydrophobic sequence of amino acids, lie within the bilayer[10]. Glycophorin A was purified from human erythrocyte ghosts that had been labelled with ^{3}H-adamantylidene. The distribution of periodic acid–Schiff's reagent stain, Coomassie Blue stain (not shown)

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and radioactivity on SDS–polyacrylamide gel electrophoresis (PAGE) showed that the isolated glycophorin was more than 95% pure (Fig. 1*b*). The hydrophobic peptide (T-6, Fig. 1*a*) that spans the bilayer was isolated from the labelled glycophorin A by trypsin treatment followed by acid precipitation[11,16]. Most of the radioactivity (70–85%) was recovered in the precipitate which gave a single broad band of radioactivity on SDS–PAGE that corresponded to the Coomassie Blue-stained T-6 peptide[11,12] (Fig. 1*c*). The small amount of radioactivity that did not precipitate with the T-6 peptide could not be traced to a specific region of the glycophorin molecule. In particular, the sialoglycopeptides contained no detectable radioactivity when analysed by electrophoresis. The distribution of label within glycophorin A was unchanged when labelling was carried out in the presence of glutathione (40 mM), a water-soluble scavenger of reactive intermediates[6,8].

The cyanogen bromide fragments of glycophorin A, C-1 and C-2, that overlap the hydrophobic segment[12,13] were approximately equally labelled (Fig. 1*d*). Adamantylidene therefore reacts at more than one site in glycophorin A. Furthermore, the overlap between the C-terminal fragment, C-2, and the hydrophobic peptide, T-6, represents (except for Arg 96 and Arg 97) 14 of the hydrophobic residues[13], suggesting that the label in C-2 arises from attack by adamantylidene from within the lipid bilayer. The overlap between C-1 and T-6 contains the 11 hydrophilic residues at the N-terminus of T-6 in addition to 9 hydrophobic residues[13]. It is therefore possible that part of the label in C-1 represents labelling of hydrophilic residues just outside the hydrophobic sequence.

In a second study, we have labelled the human major histocompatibility antigens (HLA-A2 and HLA-B7), which are complexes of glycosylated transmembrane heavy chains [molecular weight (MW) 44,000] and extracellular β_2-microglobulin molecules (MW 12,000)[17]. The amino acid sequences of the heavy chains of HLA-A2 and HLA-B7 are highly homologous (H.T. Orr, personal communication, and ref. 18): each molecule has a large N-terminal, extracellular sequence (~280 residues), a penultimate hydrophobic sequence (26 residues) and a C-terminal hydrophilic sequence (32 residues)[19,20]. As the C-terminus is intracellular[21], the hydrophobic sequence is likely to lie in the interior of the membrane. Papain cleaves native, detergent-solubilised HLA-A2 and HLA-B7 antigens in two steps[22]. In the first step, most of the C-terminal hydrophilic sequence is removed. In the second step, the hydrophobic sequence and a small number of the adjacent amino acids are cleaved from the large hydrophilic N-terminal domain (Fig. 2*a*). When HLA-A2 and HLA-B7 in lymphoblastoid cell membranes were labelled with ^{3}H-adamantylidene, tritium was incorporated into the heavy but not the light chains. Labelled, purified HLA-A2 and HLA-B7 were treated with papain (Fig. 2*b–d*). The first cleavage product carried ~85% and the second cleavage product <15% of the label found in the uncleaved heavy chain. The label must therefore lie in or close to the transmembrane sequence of the HLA-A2 and HLA-B7 antigens.

Finally, we have examined the labelling of influenza virus haemagglutinin, HA_2, the major intrinsic protein of the viral envelope[26,27]. When influenza virus is treated with bromelain, HA_2 (MW 25,000) is cleaved, yielding a water-soluble fragment, BHA_2 (MW 20,000), and a membrane-bound 'tail' (refs 28, 29). Intact influenza virus (JAP/305/1957) was labelled with ^{3}H-adamantylidene. About one-fifth of the protein-bound label was found in the matrix protein (M) and the remainder in the haemagglutinin subunit, HA_2. Little or no label was found in the neuraminidase, the nucleocapsid protein, or HA_1, which is attached to the outside of the virion by disulphide linkages to HA_2 (ref. 30). When the labelled, washed virus was treated with bromelain, at least 75% of the radioactivity in HA_2 was not present in BHA_2. Electrophoresis of the bromelain-digested, rewashed virus showed that a small radioactive peptide (MW ~5,000) was retained by the membranes[31]. Similar results have been obtained with a nitrene precursor[29].

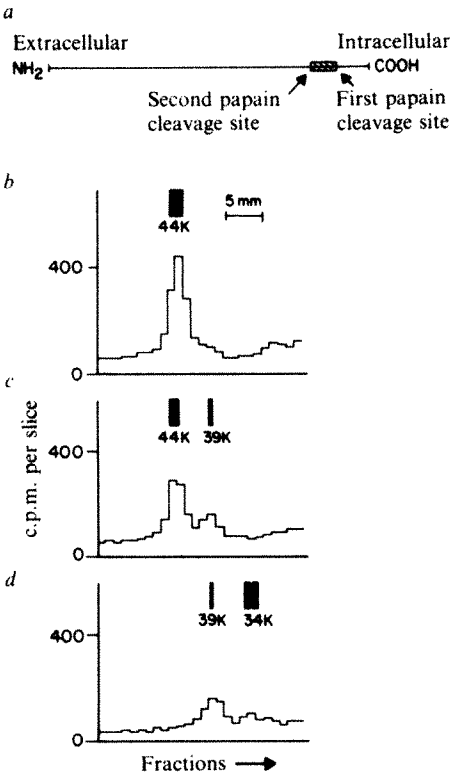


Fig. 2 Location of the sites in the heavy chains of HLA-A2 and HLA-B7 antigens labelled by ^{3}H-adamantylidene. *a*, Schematic diagram of the heavy chain of HLA-A2 and HLA-B7 antigens showing the approximate positions of papain cleavage and (shaded) the hydrophobic region of the polypeptide[19,20,22]. *b–d*, Labelled HLA-A2 and HLA-B7 heavy chains and their major cleavage products analysed by SDS–PAGE. Membranes, prepared from JY lymphoblastoid cells (1 g, homozygous HLA-A2 and HLA-B7)[23,24], were suspended in phosphate-buffered saline (0.1 ml), and labelled as described in Fig. 1 (but in air) with 1.0 mCi of ^{3}H-adamantane diazirine (final ethanol concentration 1.5%). The labelled membranes were washed twice with phosphate-buffered saline and extracted into 2% Nonidet P40 (ref. 21). The extract was adsorbed onto rabbit anti-human β_2-microglobulin IgG coupled to Sepharose beads, as described previously[21]. Immunoadsorption both purifies and concentrates the antigens. Aliquots of HLA-A2 and HLA-B7 (10 μg in 150 μl of buffer) eluted from the beads with β_2-microglobulin, were subjected to proteolysis with various amounts of papain for 1 h at 37 °C. The products were analysed by SDS–PAGE[25]. 80% of each sample was subjected to electrophoresis on tube gels which were subsequently fractionated into 1-mm slices with a Gilson Aliquogel apparatus. The slices were minced in 2% SDS, incubated with 10 ml of Biosolve/Liquifluor/Toluene (10:5:85 v/v/v) and assayed by liquid scintillation counting. The remainder of each sample was run on a slab gel which was stained with Coomassie Blue. Only the relevant portions of the gels are shown . *b*, (No papain) uncleaved heavy chain (MW 44,000; 100%); *c*, (0.05 μg papain) uncleaved heavy chain (~75%), and the first cleavage product (MW 39,000; ~25%); *d*, (0.5 μg papain) no uncleaved material, the first cleavage product (~25%) and the final cleavage product (MW 34,000, ~75%). The doublet of the final product is caused by separation of the HLA-A2 and HLA-B7 specificities. Fluorography of the slab gels confirmed the exact coincidence of the radioactive peaks with the Coomassie Blue-stained bands. The apparent MWs of the antigens and their fragments are probably not accurate as their differences do not correspond to the sizes of the fragments released on proteolysis[19,20,22].

It is clear from the present results that adamantylidene is an effective reagent for the identification of hydrophobic, membrane-bound segments of intrinsic membrane proteins. The greater reactivity of the carbene offers a significant advantage over the more commonly used lipophilic nitrenes. However, several points remain to be determined. First, the label within the hydrophobic sequences may be attached only to the more reactive amino acids (for example, Thr, Ser and Tyr in the case of glycophorin A). Second, we have not shown whether all the label is located within the hydrophobic sequences of the labelled proteins. Part of the label may lie just outside those

sequences. Even carbenes exhibit some chemical selectivity (ref. 7 and refs therein) and the rate of reaction of adamantylidene with the rather inert groups within the bilayer might be slower than diffusion to the membrane surface and reaction with the nucleophilic groups present near the interface. Furthermore, we have shown previously that, when irradiated, adamantane diazirine rearranges in part to 2-diazoadamantane[8]. It is possible that some of the labelling that we have observed is derived from this reactive molecule. It is interesting that the neuraminidase of influenza virus, which is regarded as an intrinsic protein[32], is barely labelled compared with HA_2 even when the higher number of copies per virion of the latter[33] is taken into account. It may be that the hydrophobic region of the neuraminidase is unreactive towards the carbene, that the hydrophobic region is shielded by tightly bound lipid or protein, or that this protein is actually only indirectly attached to the bilayer.

The three intrinsic proteins that we have studied are of a common structural type. They each contain a single hydrophobic peptide that interacts with the lipid bilayer. When the sites of labelling by adamantylidene have been elucidated at the level of individual amino acids, we will be able to use the reagent for structural studies of more complex intrinsic proteins (for example, those with polypeptide chains that span the membrane more than once) and membrane proteins of completely unknown structure. Even at the present stage of development, labelling with adamantane diazirine is useful as a convenient and rapid method for the preliminary characterisation of membrane proteins.

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A novel coenzyme from bacterial primary alcohol dehydrogenases

METHYLOTROPHIC bacteria are able to use methane derivatives as their sole source of carbon and metabolic energy and so can sustain growth on methane, methanol and other organic compounds which lack carbon–carbon bonds[1]. They are not autotrophic and, being unable to use carbon dioxide, rely ultimately on their ability to oxidise these substances. Although the primary alcohol dehydrogenases from a variety of methanol-grown bacteria have been purified and compared, and despite the increasing commerical importance of these organisms as sources of 'single cell protein', the essential features of these oxidative pathways remain unknown. The dehydrogenases exhibit a rather broad substrate specificity for primary alcohols and formaldehyde, and seem to contain a common and novel cofactor[2–6], which may be more generally associated with the oxidation of single-carbon compounds. The structure of the cofactor is reported here.

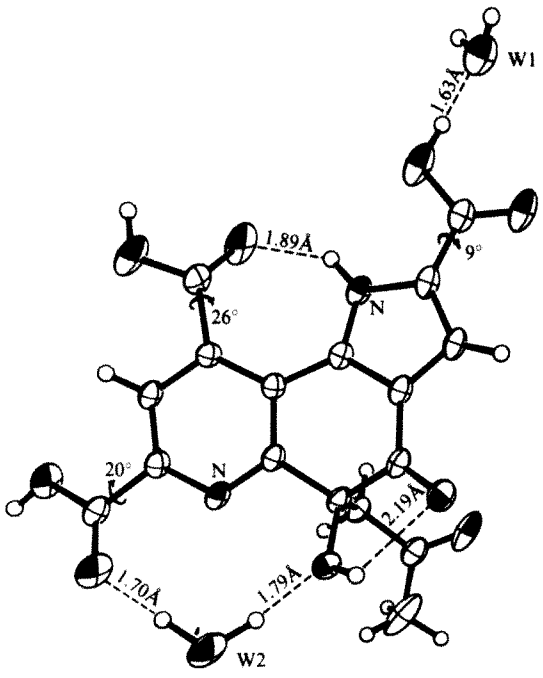


Fig. 1 A view of the methoxatin molecule[10] in the same orientation as the chemical diagram. Two water molecules are also shown. O atoms are shaded, N atoms shaded and labelled. Dotted lines indicate hydrogen bonds, and arrows the sense of the torsion angles marked on the diagram.

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The presumptive cofactor, obtained by denaturing the holoenzymes by heat or addition of acid, base, potassium bromide or organic solvents[5], slowly but spontaneously decomposes in water[7], a process which is apparently accelerated by the addition of acetone. To characterise the cofactor, which was presumed to be a pterin[8], we devised a procedure for isolating this degradation product from intact bacteria using, as the best source, cell paste of the facultative methylotroph *Pseudomonas* TP1 (ref. 9) grown on methanol. On treating aqueous solutions of the partially purified cell extract with equal volumes of acetone a product was obtained which, although very sensitive to base, could be separated by ion exchange chromatography using acidic solutions, in which it was almost indefinitely stable. The pale yellow solid isolated in this way in a yield of 5 mg per kg cell paste was identical to the material obtained from homogeneous dehydrogenase preparations after similar acetone treatment. It crystallised as effluorescent orange needles from supersaturated aqueous solution.

The limited quantities of material available and the difficulties encountered in achieving specific degradations made this an unsuitable approach to the elucidation of the compound's chemical structure, and spectroscopic data alone provided insufficient information. The structure could, however, be determined by X-ray diffraction analysis using two single crystals.

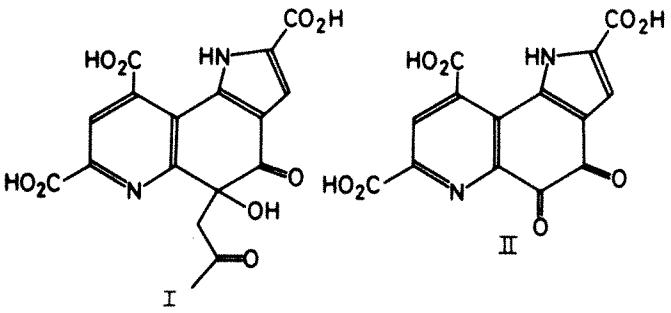
The crystals were triclinic, space group $P\bar{1}$ with unit cell dimensions: $a = 8.593(1)$, $b = 10.702(2)$, $c = 10.265(2)$ Å; $\alpha = 101.78(2)$, $\beta = 107.77(2)$, $\gamma = 91.48(2)°$. By assuming a molecular weight of 366, suggested by rather equivocal mass spectra, and $Z = 2$, the density calculated for a dihydrate, $D_c = 1.61$ g cm^{-3}, compared well with that obtained by flotation measurements on the crystal, $D_m = 1.61$ g cm^{-3}. Using a crystal mounted in a thin-walled glass capillary, diffractometer data were collected for 2,982 independent reflections with $2\theta_{max} = 130°$ and CuKα radiation. The intensities were empirically corrected for absorption and processed in the conventional manner.

The structure was solved by direct methods using a locally modified version of SHELX76 (G. M. Sheldrick, personal communication). A set of 28 peaks in the original E map which were within chemically reasonable bonding distance were assigned as carbon atoms and later identified (as C, O or N) by the interpretation of Fourier difference maps and refinement of the thermal parameters. All hydrogen atoms were unambiguously located from difference maps during the initial cycles of anisotropic refinement. The final reliability factor, with hydrogen atoms refined isotropically and other atoms anisotropically, was $R = 4\%$.

The structure obtained from this analysis is shown in Fig. 1. The cofactor derivative is thus 4,5-dihydro-5-hydroxy-4-oxo-5-(2-oxopropyl)-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid (I) and crystallises as the dihydrate with the formula $C_{17}H_{12}N_2O_9.2H_2O$ (formula weight 424).

This structure has an unusual network of hydrogen bonds. There is a strong intramolecular hydrogen bond between the pyrrolo-nitrogen atom and the carbonyl oxygen atom of the 9-carboxy substituent. A weaker bifurcated intramolecular hydrogen bond occurs between the 5-hydroxy and the 4-keto substituents. The two water molecules have very different roles in the structure: whereas W1 is important in maintaining the crystal integrity by participating in three intermolecular hydrogen bonds with symmetry-related molecules, W2 forms a tight intramolecular hydrogen-bonded ring by bridging the keto oxygen atom of the 7-carboxy-substituent and the hydroxyl oxygen atom.

Lack of structural information about this cofactor has probably contributed to the slow progress of studies of bacterial primary alcohol dehydrogenases. The crucial relationship between the structure of the artifact I, presented here, and that of the coenzyme itself must remain equivocal until the identity of the latter can be established by reconstitution of the holoenzyme from apoenzyme and cofactor. However, the comparatively mild and diverse conditions which bring about the reverse of this process and concomitant inactivation suggest that the green-fluorescing compound obtained by denaturing aqueous solutions of the dehydrogenases differs, if at all, only very slightly from the actual cofactor. The formation of I from this compound may be more straightforward; the presence of a 2-ketopropyl moiety at a racemic centre (a consequence of the space group) points strongly to a non-biological origin. We believe it arises during the isolation from the addition of acetone to the orthoquinone (II), 4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid, for which we propose the trivial name 'methoxatin'. The similar appearance of the product without acetone might then be explained by hydration which would alter the chromophore in an almost identical manner.



Methoxatin, although it is a quinone, cannot be simply categorised as such because other parts of this novel and surprisingly complex molecule are likely to contribute directly to its presumed function as a catalyst in biological oxidations. Apart from the relative orientations of the nitrogen atoms and carbonyl groups, the presence of three carboxylic acid functions is also remarkable and offers a ready explanation for the difficulties encountered in reconstituting the holoenzyme.

Experiments to reconstitute the holo-dehydrogenase and so test the view that II is indeed the cofactor, together with a total synthesis of this compound, are now in progress. Details of the isolation and properties of I, and a discussion of the possible mechanism of enzymatic catalysis involving II will be presented elsewhere.

We thank Shell Research Division and ICI for supplying, respectively, spray dried cells of *Pseudomonas extorquens* and Pruteen, the 'single cell protein' product from *Methylophilus methylotrophus*. We acknowledge the contribution of Dr G. Sperl to early work on isolation of the cofactor. This work was supported by NSF grant PCM 76–04988, the Robert A. Welch Foundation and the MRC.

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matters arising

Variation with time of a Sun–weather effect

THE reported[1] influence of the solar and interplanetary magnetic sector structure on terrestrial atmospheric vorticity at the 500-mbar level during the winters of 1963–73 has been disputed by Williams and Gerety[2]. We consider, however, that the response has been remarkably constant if an apparent decrease during the past few years of the intensity of tropospheric circulation is properly accounted for.

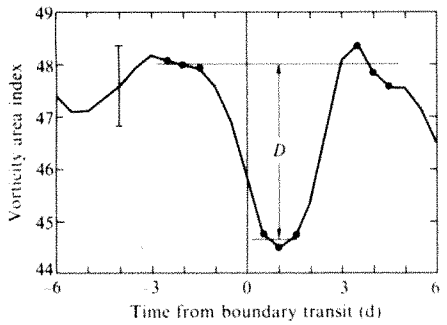


Fig. 1 A superimposed epoch analysis of the vorticity area index at 500 mbar about 162 times of interplanetary magnetic sector boundary transits during the winters in the interval 1 November 1963 to 31 March 1976 for which spacecraft observations of boundary transits are available. A typical error bar (twice the s.e.m.) is shown for the point at day −4.

First, Hines and Halevy[3] concluded that "If solar sector boundary crossings are to be employed in the determination of key dates, their times of occurence must be identified to at least an accuracy of ±24 h on average for the analysis to be successful. This constraint is not serious when spacecraft data are employed for the determination of field reversals, but it can become significant when indirect determinations are made from ground-based geomagnetic measurements"[4,5].

Second, Hines and Halevy[3] introduced the excursion x, defined as the difference between the maximum and minimum values of the vorticity area index (VAI) found in a 12-d interval centred on the time of boundary transit. The amplitude of the Sun–weather influence was small when the excursion was small and large when the excursion was large. In the past few years the observed excursions have been considerably smaller than in the previous years.

The interval during which spacecraft observations of the interplanetary magnetic field are available is the winter months from 1 November 1963 to 31 March 1976. The response of the VAI at 500 mbar to 162 sector boundary transits past the Earth during this spacecraft interval is shown in Fig. 1. The previously reported[1] minimum in VAI approximately one day after boundary transit is clear. A measure D of the depth of this minimum is defined as the average VAI near days −2 and +4 (w.r.t. day 0 as the boundary transit time) minus the average VAI near day 1. For each specified day the average VAI during three adjacent half-days is computed. In Fig. 1, $D = 3.30 \times 10^5$ km^2.

D is found[3] to increase as x increases; x_s divides the 162 boundary transits during the spacecraft interval into two equal groups, one with larger excursions and one with smaller. The Sun–weather effect is examined separately for each group.

Consider now the three-winter interval from 1 November 1963 to 31 March 1966. Figure 2 shows, for this interval (plotted at 1965), the average value of D associated with the group of boundary transits having excursions $>x_s$, and the average D for the group of boundary transits having excursions $<x_s$. The analysis is repeated one year at a time between 1 November 1975 and 31 March 1978 (plotted at 1977), the last for which data are available.

Between 1963 and the present shown in Fig. 2, the size of the Sun–weather associated with the group of boundaries having larger excursions is rather constant. For each interval shown in Fig. 2, D is larger for the group of boundaries associated with larger excursions than for the group of boundaries associated with smaller excursions, thus confirming in a much larger data set the result of Hines and Halevy[3]. Except for the winters 1968–71, D associated with the group of boundary transits having smaller excursions is close to zero.

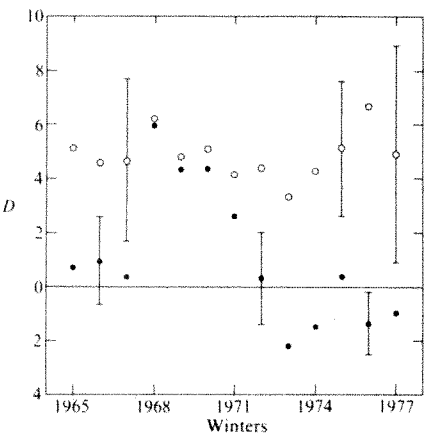


Fig. 2 D for the groups of boundary transits having larger excursions (○) and for the groups of boundary transits having smaller excursions (●). The results for the last winters should be considered preliminary until spacecraft observations of boundary transits are available. The total length of the error bar is twice the s.e.m.

During the winters of the spacecraft interval shown in Fig. 3 the number of boundary transits whose excursions were $>x_s$ and the number of boundary transits with excursions $<x_s$ are approximately equal (note x_s was defined to make them equal during the entire spacecraft interval). In recent winters the magnitude of the excursions has declined considerably such that in the last interval only seven boundary transits had excursions $>x_s$ while 31 transits did not. If this decline is not an artefact of the meteorological data processing, an important change in the large-scale tropospheric circulation in the Northern Hemisphere has occurred in the past few years.

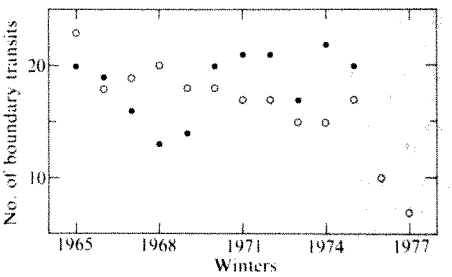


Fig. 3 The number of boundary transits in each three-winter interval for which the excursions are in the larger group (○) and in the smaller group (●). Note that in the last two intervals the number of boundary transits with larger excursions is considerably decreased.

In the winters before the spacecraft interval only indirect determinations of boundary transits made from ground-based geomagnetic measurements are available. Using these indirect boundary transits no consistent Sun–weather effect similar to that shown in Fig. 2 is found. It is quite possible that there was a variation in the Sun–weather effect in these earlier years, but the situation is complicated by the lack of spacecraft observations.

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WILLIAMS REPLIES—The biggest weakness of the study by Wilcox and Scherrer is immediately apparent. In subdividing the keydates according to their excursion, Wilcox and Scherrer have allowed themselves freedom to select the data *a posteriori*. Despite the work of Hines and Halevy[1] there is no known physical reason for selecting the data in this manner. In this context, it must be remembered that the data has already been carefully selected, for example the wintertime, 500 or 300 mbar VAI is chosen since it exhibits the best result. Unfortunately, in the absence of a physical understanding this is one of the few ways in which we can progress. Indeed, I have been working along similar lines[2].

A lesser weakness has been pointed out by Hines and Halevy[1]. If one hypothesises that the 'signal' is the remnant of the 'noise' in the VAI dataset, then one might also expect the signal to increase as the noise increases. However, one would not expect the consistency claimed by Wilcox and Scherrer. A new point along these lines concerns the definition of the excursion. If the signal is real, then the strength of the signal can hardly depend on the meteorological noise after day 0 since then one would merely be correlating the signal with itself. Therefore, I would like to see the analysis repeated, this time selecting keydates according to the excursion before the start of the signal.

With regard to more minor points, I would like to see the results of the same analysis at 300 mbar; and how sensitive is Fig. 3 to a small change in x_s?

If the signal is real and does depend on the intrinsic variability of the atmosphere, then this is an important result which ought to have strong implications for the search for a mechanism. In this case the study of Wilcox and Scherrer should be taken further to answer some important questions. For example is there a critical excursion below which no effect occurs? Is there a proportionality between the excursion and the amplitude of the signal? (The winters 1968–71, which could be interpreted as 1967–71 in view of the 3-y average employed and the '1967' and '1972' results, seem to suggest this is not so.) Can the winter–summer asymmetry of the result be explained in this manner, since one could expect the excursion to be less in summer?

The simplest interpretation of the VAI–SSB effect still seems to be statistical chance. This belief has been reinforced by an interpretation of this effect in terms of the tropospheric energetics in the wavenumber domain[3].

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Anionic copolymerisation of episulphides with elemental sulphur

PENCZEK *et al.*[1] believe that the anionic copolymerisation of propylene sulphide (PS) with elemental sulphur gives the high-molecular weight polysulphide polymers $-CH_2-CH(CH_3)-S_x-$ (% S up to 90%, that is, $x = 12$). Nevertheless, PS mainly yields copolymers with $x \leqslant 2$ (refs 2, 3). We believe that this contradiction[1–3] is caused by the presence of residual sulphur in the PS copolymers[1], which can be detected in the elementary analysis and does not show up in the NMR spectra. This assumption is based on our experimental data[2] and also confirmed by ^{13}C NMR data[1] which indicate that PS copolymers with $x \leqslant 2$, and not polysulphide polymers with x up to 12 (ref. 1), are being formed. Indeed, polypropylene sulphide ($x = 1$) is characterised by the signals δ20.7 for CH_3, δ38.6 for CH_2 and δ41.0 and 41.2 for CH[4], whereas polypropylene disulphide ($x = 2$)[5] is characterised by the signals δ19.05 for CH_3, δ44.6 and 45.0 for CH_2 and δ46.0 and 46.4 p.p.m. for CH[6]. The spectra of PS copolymers with $2 > x > 1$ also have the signals of conjunctive units $-SS-CH_2-CH(CH_3)-S-CH_2-CH(CH_3)-SS-$ at δ39.9 and 39.5 for CH, δ37.4 for CH_2 and δ19.4 p.p.m. for CH_3 (ref. 6). Therefore the increase in the intensity of signals 45.8, 45.6, 39.5, 39.1 and 19.4 p.p.m. with the increasing sulphur content in PS copolymers[1] are due to the increase in the amount of disulphide bonds ($x = 2$), and not polysulphide bonds ($x > 2$) as other signals in the spectra are absent (for example, CH_3 signal of trisulphide units should appear at δ18.1 p.p.m.[6]). It follows that the apparent values of $x = 2$ and 5 in the spectra of PS copolymers[1] conform to $x \leqslant$ 1.5 and 1.7, respectively. Like the ^{13}C NMR data[1,2], ^1H NMR spectra of PS copolymers[2] correspond to that of PS polymers with a varying ratio of mono- and disulphide units[6].

We have shown previously[2,3] that the probability of a polysulphide polymer formation increases as the nucleophilic reactivity of episulphides decreases. Very reactive ethylenesulphide is predominantly converted into polyethylene sulphide ($x = 1$). Polysulphide polymers ($x > 2$) are mainly produced from a less reactive cyclohexene sulphide, isobutylene sulphide and trimethylethylenesulphide. The latter forms the copolymers with x up to 7 whose ^1H NMR spectra show a gradual downfield shift of proton signals as x is increased in the case of more reactive PS. The probability of homopolymerisation and formation of stable disulphide units increases as unstable polysulphide chains must split at hard reaction conditions[1]. The stereochemical effect of substituents and more mild copolymerisation conditions[2] apparently decrease the homopolymerisation rate of substituted episulphides and thereby increase the probability of their copolymerisation with sulphur as well as stabilise and restrict the participation of polysulphide chains (at least for $x \leqslant 5$) in nucleophilic splitting reactions because of the sterically hindered growing thiolate anions. Thus, despite these limitations, which can be overcome by using active forms of sulphur, inorganic polysulphides or UV irradiation[2], this method offers broad possibilities in the synthesis of polysulphide polymers[2,3].

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PENCZEK *ET AL.* REPLY—Aliev *et al.* have confirmed a general principle of anionic copolymerisability of elemental sulphur[1], including copolymers with various cyclic sulphides as described in our patent[2]. However, Aliev *et al.* assume that copolymers of propylene sulphide with elemental sulphur could have merely be solid solutions of polysulphide polymers $-CH_2-CH(CH_3)-S_x-$ with an average $\bar{x} < 2$ and elemental sulphur. This was based on their failure to prepare in their system copolymers of propylene sulphide with higher sulphur content, and on their interpretation of the ^{13}C-NMR spectra. However, great care must be taken with the interpretation of the NMR spectra of polysulphides, because we observed recently that the upfield signals can be caused by the presence of the low-molecular weight cyclic polysulphides. These products were removed from our polymers by an extensive extraction with boiling methyl alcohol (unreacted sulphur was removed in the same way)[3].

Had Aliev *et al.* attempted to repeat our experiments, leading to the true copolymers with high content of the built in sulphur, it would have become clear that, for example, a 50:50 mixture of polypropylene sulphide and elemental

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sulphur (this mixture gives an apparent average $\bar{x} = 4.3$) dissolved in a common solvent (such as, CS_2) gives on evaporation of the solvent an opaque, heterogeneous mixture, from which good crystals of sulphur crystallise out. This behaviour sharply contrasts with that of copolymers with $\bar{x}$ even as high as $\bar{x} = 8$ (85.9% of sulphur) which give transparent, non-opaque films from solutions.

Another equally powerful proof of the absence of nonreacted sulphur in copolymers comes from our recent studies of these and related copolymers[3] by laser Raman method (Cary 82). Elemental sulphur shows two strong peaks in benzene solution, at 217 cm^{-1} and 475 cm^{-1}, well separated from other signals in the intentionally prepared mixtures of polysulphides with elemental sulphur. These peaks are absent in copolymers of sulphur with propylene sulphide, prepared as previously described[1].

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Short-range structure of amorphous alloys

WANG'S problem of the structures of amorphous intertransition metals[1] is twofold. First, what is the nature of the short-range chemical ordering? It is generally considered that it is probably related to the ordering in the corresponding crystal, although quantitative evidence, based largely on neutron scattering[2,3] and EXAFS[4] data, is still inadequate. There is no guarantee that the particular clustering of type A atoms about type B observed in the crystal will still be consistent with stability when the 'surrounding matrix' is disordered rather than crystalline, especially where the chemical forces are relatively weak. At the very weak extreme, the evidence is strongly against either crystalline or icosahedral (polytetrahedral) short-range ordering in ideal single component glasses[5–7].

The second, more interesting problem of structural modelling is: how can we construct an extended, dense, non-crystalline model which is both stable and consistent with the short-range chemical ordering (refs 5, 6)? We need to consider, (1) the topology of the connectedness between like and unlike component atoms, and (2) the packing constraints consistent with dense, extended non-crystalline systems. Neither of these is understood at all well, but we do know the problems are of a very different nature in two and three dimensions. For example, the triangulation of dense, disordered two-dimensional assemblies is straightforward and can be done with only minor distortions, as Wang shows empirically. The equivalent tetrahedral division of three-dimensional assemblies without the presence of additional octahedra or other more complex polyhedra is very much more difficult, and probably impossible. We cannot pack polytetrahedral units together without serious boundary misfits which cannot be accommodated by small distortions[5]. Second, the connective topology problems in two dimensions are also much simpler than the equivalent three-dimensional problem. Thus, whereas the misfits at polyhedron boundaries and the atomic disorder consequent on non-crystallinity, are relatively small in Wang's two-dimensional model, we learn very little about the equivalent problem in three dimensions, which is the space in which real amorphous alloys are found. It is unfortunate, though understandable, that the discussion is of a two-dimensional model. We cannot extrapolate into three dimensions, where the connective topology and packing constraints (Bernal's 'statistical geometry' (ref. 8)) are so poorly understood.

Wang comments that dense random packing of hard spheres (DRPHS) models fail to explain experimental data on these systems. This assumes an over-narrow interpretation of such models. Unfortunately, the specific structure[10] of the hard-sphere model, rather than the underlying concept of a dense, non-crystalline organisation consistent with the interatomic interactions[5], has often been applied incorrectly to binary alloys[9]. The value of the original model[10] was largely conceptual, focusing attention away from crystallites to models that could be discussed within an inherently non-crystalline framework. To apply such a simple, idealised model unaltered to a variety of binary systems is clearly incorrect[5]: even weak chemical ordering and slight softening of potential functions are known to result in a variety of non-crystalline structures, all conceptually related to DRPHS by their non-crystalline nature, but different from each other in detailed local structure[6]. Moreover, most computer-built so-called DRPHS models are not dense random packings; because of problems of accessing the high-density states by computer construction[5,6], most models are of a relatively low density[5,11], and hence will fail to reproduce the necessary packing constraints in the real system. The failure that Wang quotes[1] is not of the DRP concept, but of its inadequate realisation in a particular system.

The problem of binary amorphous alloy structure is how to feed in the chemical ordering when constructing a dense model so that the resultant model is consistent with both the ordering and the packing constraints consequent upon the high experimental density. This is far from easy, but it needs tackling quantitatively in three dimensions. The work of Gaskell[12], Boudreaux[13] and von Heimendahl[14] are useful attempts to solve these difficulties.

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WANG REPLIES—Like the original DRP concept, the proposed short-range structure of amorphous intertransition metal alloys is largely conceptual. This structural concept can be verified by experiments and structural modelling, but at present, the experimental evidence, based on limited scattering and EXAFS data, is inadequate to prove or disprove the proposed concept. Concerning structural modelling, Finney's past work can be summarised as "one cannot pack idealised polytetrahedra or spherically-symmetrical atoms to reach a high packing density" (ref. 1). New structural modelling techniques should be developed using extremely soft potentials to account for the mixing of irregular tetrahedra and the asymmetrically shaped atoms as suggested in the structural concept. Furthermore, if one analyses Samson's nine modes[2] for connecting truncated tetrahedra in crystalline compounds, one can see that the boundary problem in three dimensions may not be as hopeless as Finney suggests.

The main question is: to what extent is crystalline-like short-range order preserved in the real amorphous structure? This is difficult to answer because both composition and preparation method can influence the short-range structure. The real structure should be a mixture of metallic and covalent bondings with localised coordination numbers varying from 8 to 16. So far, the experiments and structural modelling do not uniquely define a real 'amorphous' structure. As Rustum Roy said, "for non-crystalline phases in general, it is only our imagination that limits our models ... of homogeneous and heterogeneous structures." I

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hope, through this structural concept, that we can systematise our knowledge about the formation, stability and unique properties of the amorphous intertransition metal alloys and relate these properties to those of the crystalline counterparts.

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Circular hydrogen bonds

SAENGER[1] rightly stresses both cooperativity and large deviations from tetrahedrality in water-involved hydrogen-bonding networks. Similar lattice unconstrained structures were observed previously when examining the hydration of biological macromolecules, especially proteins [2–4], although the α-cyclodextrin data examined by Saenger is of significantly better quality, and therefore more quantitative.

However, although I agree strongly that cooperative effects are relevant in OH-group hydrogen bonding [3–7], the existence of rings—be they homodromic or heterodromic—does not of itself conclusively indicate that cooperative effects are operating. Given even a purely pair-additive potential with an average coordination of more than two, we would expect ring structures to be a topological consequence. Current ideas on the structures of trigonal[8] (for example, B_2O_3) and tetrahedral[9] (for example SiO_2) glasses necessarily involve rings without any known cooperativity. As in aqueous systems, simulation calculations yield extensive ring structures using purely pair-additive potentials[10]. The wide glass-forming ability of the silicate glasses, and the many crystalline forms of silicates, underline the structural versatility of tetrahedral coordination; the ring structures are a consequence of the (non-cooperative) interaction potential and the need to fill space within the constraints of the potential.

The α-cyclodextrin crystal data are valuable for the quantification of deviations from ideal tetrahedrality, an effect previously noted with respect to poorer quality protein data[4,11]. The conventional wisdom of tetrahedrality in water is based on (1) the geometry of sp^3 hybridisation; and (2) the structures of the ices. The ice structures are, however, subject to the lattice constraint; extrapolation to small disordered clusters of molecules is dangerous. Concerning (1), most recent large basis set *ab initio* calculations on the water molecules [12] show the lone pairs poorly separated; the deviation from tetrahedrality is large. This is backed up by neutron diffraction studies of hydrates, which show the directional control of the lone pairs to be relatively poor[7,13]. Strong tetrahedrality is also inconsistent with recent neutron scattering data on liquid water (J. C. Dore and I. P. Gibson, in preparation; refs 14, 15); pair correlation functions predicted by non-cooperative tetrahedral models of the water molecule show far more short range tetrahedral structure than is observed[15]. In contrast, a cooperative potential which makes no such geometrical assumptions[5,6] is in agreement with the data (P. Barnes, J. L. F., J. E. Quinn and J. D. Nicholas, in preparation).

Cooperativity and imperfect tetrahedrality are indeed important aspects of aqueous systems which are normally ignored [3–7]. Saenger's data give quantitative support to imperfect tetrahedrality, but does not of itself conclusively indicate that cooperativity is operating.

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SAENGER REPLIES—From a topological point of view, it is certainly justified to assume that water molecules and hydroxyl groups could form circular H-bonded structures without invoking the cooperative effect. The quadrupole functionality of water in combination with purely pair-additive interaction potentials could be sufficient to arrange water molecules into circular aggregates, similar to the formation of circles in SiO_2 or B_2O_3 glasses, as pointed out by Finney. However, we should be aware that in Si—O—Si and B—O—B bonds the directions of the bonds (from donor to acceptor) are predetermined because O is the electron acceptor and Si or B are the donors. It is one of the special properties of H-bonds between hydroxyl groups O—H···O that both oxygen atoms can function either as donor or as acceptor. This duality is clearly resolved if H-atoms are located and, because the direction of the H-bonds is then known, the existence or absence of cooperativity between several such H-bonds is directly evident.

In the absence of a cooperative effect, we should expect near-random directions of O—H···O hydrogen bonds, be they in 'endless' chains or in circular structures (which then would be heterodromic). Because the neutron data on α-cyclodextrin·hexahydrate have enabled us to locate all H-atoms unambiguously, the directions of all the H-bonds are known. We find that in the two chain-like structures as well as in the homo- and antidromic circles all the H-bonds are undirectional, a strong indication for the existence of the cooperative effect. Obviously, both topological and cooperative effects favour and stabilise circular H-bonded structures.

Bogdan Lesyng (Warsaw University) has performed PCILO calculations on chain-like and circular H-bonds (in preparation). He compared H-bonding energies of these structures with those of the respective pairwise, isolated O—H···O interactions. The influence of the cooperative effect is marginal, about 1% of the total H-bonding energy, in the two antidromic circles, but 7.3% in the homodromic circle and 9.8% in the chain-like structure. The data suggest that cooperativity does exist in these H-bonded structures but its magnitude depends strongly on the arrangement of the H-bonds, one of the bonds in the antidromic circle III even being bifurcated. Another interesting result of these calculations concerns the dipole moments, 2.73 *D* for the homodromic circle but ≥ 6 *D* for the antidromic circles and chain-like structure, suggesting that in water clusters, homodromic circles should be the preferred species.

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reviews

Participation as an ideology

Eric Ashby

Technological Decisions and Democracy: European Experiments in Public Participation. By Dorothy Nelkin. Pp. 111. (Sage Publications: Beverley Hills and London, UK, 1978.) £6.

IF you have an hour or two to wait in Chicago airport between flights you are likely to be approached by earnest young people asking you to sign a protest against the use of nuclear power in America, following the incident at Harrisburg. It is ironic, in the light of the recent air tragedy there, that you would not be asked to sign a protest against the use of passenger aircraft. This is a symptom of the basic irrationality in public opinion about the applications of technology in America. It is a cause for concern among policy-makers. They know that in a pluralistic democracy the pressure for participation cannot be resisted and they know that satisfactory patterns for participation have not yet been worked out. It is clear that in all western democracies people are no longer content to leave decisions to experts; but the alternative is disquieting because the decisions cannot possibly be made without experts. If popular views about risks, costs and benefits coincided with the calculations of statisticians there would be some hope for rational discourse. But they don't. And the dilemma is compounded by the disturbing fact that protests against nuclear power, the siting of airports, and even research into genetic recombination easily slide into a much deeper protest against the concentrations of power inevitable in representative democracy. Given that participation has come to stay there is an urgent need for innovations to incorporate participation into our political system in such a way that it strengthens and does not clog up the machinery of government. The questions in need of answers are: Who should participate? How can those who participate be well enough informed to make a useful contribution? What pattern of participation (an adversary system or an inquisitorial system) would be more effective?

Dorothy Nelkin, in the Program on Science, Technology and Society at Cornell, has published several scholarly case-histories on this problem. In this brief essay she has turned to enquire how participation works in three European countries — Sweden, Holland and Austria — where decisions can be made in a much simpler political context than exists in the United States. Sweden is perhaps the most cohesive community in Europe, with a central government accustomed to a tradition of "consensus maintained by compromise". Holland is divided by religious and regional loyalties which may coalesce over technological and scientific issues, or may use such issues as ammunition for political conflicts. Austria is a federal republic which has recently succeeded in breaking away from the strong teutonic traditions of authoritarianism. All three countries have reacted to popular demands for participation in decisions about planning, the generation of energy, and science policy. In each country, public interest was ignited by incidents which were given saturation treatment in the media. In Sweden there were incidents like mercury poisoning, acid rain, and the energy policy for a nation without coal or oil but with a supply of uranium. In Holland action groups which had opposed the location of blast furnaces in Rotterdam, the location of an airport, and the building of a dam across the Oosterschelde combined to form the Foundation for Nature and the Environment, with some 100,000 members. In Austria, opposition to nuclear power culminated in a referendum last year in which some 64% of the electorate voted and which by a narrow majority rejected a newly built power station already charged with nuclear fuel.

Professor Nelkin describes how each of these countries has devised experiments in participation over issues in nuclear energy and science policy. The most significant experiment is the one devised in Sweden. For many years there have been study circles there, run by trade unions, religious groups, and other social organisations. In May 1974 the Riksdag approved a proposal to sponsor energy studies by these groups. Twelve thousand study circles, involving some 80,000 people, were subsidised to the tune of about £330,000. This did not produce any dramatic enlightenment among the Swedish people but it did mean that the government's Energy Policy Bill was more widely read, and — an unexpected result — the very complexity of the issues, when these were brought to public attention, increased the number of 'don't knows' in the pro- and anti-nuclear power controversy. In Holland the most interesting experiment is the organisation of courses in the University of Amsterdam to teach scientists how to popularise research findings, and in Utrecht University, courses for working journalists to broaden their knowledge of science and technology.

Professor Nelkin's essay is written with the clarity and precision characteristic of her earlier writings. Apart from its value as a summary of information not readily available elsewhere, it will stimulate the American and British reader to reflect on the arrangements for participation in their own countries. Political conflict and ambiguity, she writes, "are basic realities of technological decisions". To expect sweetness and light over Windscale, a third London airport, or coal mining in the Vale of Belvoir, is a delusion. It is a delusion also to expect technical decisions of this kind to remain technical: they are bound to become political. It is for this very reason that the public (in my view) have a right to participate. What, then, are reasonable conditions for participation?

First, if the authorities take the trouble (and it does involve trouble) to organise participation, then the participants ought to take the trouble to do their homework. Some preliminary education which is not just propaganda is essential. This means access to information and to the advice of experts who are not *parti pris* to the issue. Second, participation will not be acceptable if it is mere token consultation; there must be unambiguous assurances that the views of participants will be taken into account. (Enquiries under the Town and Country Planning Act do give the British public such assurances: witness the many enquiries in which the Minister has come down on the side of conservationists, from the siting of the Cambridge biochemistry department to the siting of a power station near Nottingham). Third, it is unrealistic to suppose that a majority of the public will take an intelligent interest in any major technological issue. There will be a spectrum of opinion, with persons of impenetrable prejudice at each end of the spectrum and a mass of apathetic people in the middle. But between these three groups are the people who matter. They already hold an opinion but they are willing to subject their view to further argument. They are sufficiently concerned to give time to attending meetings, reading articles, and joining pressure groups. These are the people who should be drawn into active participation. Their views ought to be taken seriously. It will not do to dismiss their opinions as coming from an élite minority, unrepresentative of the mass of opinion; for unless the mass takes the

trouble to express an opinion, it has to be assumed that it has no opinion. Nor will it do to say that these groups of concerned people do not understand the highly technical issues; it is the duty of the authority (the Central Electricity Generating Board, or the Ministry of Transport, or whoever proposes the project under discussion) to set out the issues in a way which can be understood by laymen.

We have an opportunity for an experiment in participation in England. The proposal to mine coal in the Vale of Belvoir is going to arouse savage opposition. The issues are highly complex. Would it be possible for the adjoining universities (Nottingham, Leicester, and Loughborough) to arrange extra-mural courses about the issue, conducted by experts in geology, energy technology, and economics who would not be regarded as in the pay of the Coal Board? And — but perhaps this is going too far — would it be possible for witnesses holding a class certificate from one of these extra-mural courses to be given preference in the public enquiry which will doubtless be held? A procedure like this might not lead to a wiser decision (though it might do that too), but it would be likely to increase confidence and sympathy for the decision. And in a pluralistic democracy the process is as important as the end product.

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Bumblebee economics

Bumblebee Economics. By Bernd Heinrich. Pp. 159. (Harvard University Press: Cambridge, Massachusetts, and London, UK, 1979.) $21.90; £11.40.

FIVE books dealing entirely with bumblebees have been published previously, in 1912, 1934, 1959, 1975 and 1978, and three other books, published in 1970, 1971 and 1974, have substantial parts devoted to bumblebees. The justification for producing another book on bumblebees would need to rest on significant advances in the subject or on a new approach. Certainly Bernd Heinrich gives us a refreshingly different and topical approach.

The central theme running through his book is that of energy economics, and most of the activities of individual bumblebees and of bumblebee colonies are examined in the light of profit and loss of energy resources. Bumblebees are particularly suited for such an examination. They live in temperate climates and often need to survive low temperatures. To ensure that the susceptible brood does not become chilled, the adults produce heat by metabolic activity and surround the comb with insulating material to retain it.

However, the ultimate object of a bumblebee colony is to convert the nectar and pollen collected by its foragers into sexually developed adults that mate and reproduce. Calculating the energy balances and the effects of likely changes in the collection, flow and expenditure of resources is shown to be fascinating but complex. For example, bumblebees are well known for their ability to fly and forage at relatively low temperatures and in adverse weather conditions, but the success of the currently in vogue phrase, 'foraging strategy', will depend on the energy they need to expend before flight is possible, the amount of fuel (nectar) they consume to reach the foraging area, whether sufficient nectar is being produced, and whether it contains a sufficient concentration of sugars; both these latter factors are also controlled by environmental temperature and relative humidity. It is easy to envisage circumstances in which a forager would use more sugar than it actually collected. Furthermore foraging in poor weather conditions can be hazardous as well as arduous, and so diminish longevity and the energy resources of the bee's colony. Even in favoured foraging conditions several trips are needed to produce sufficient nectar and pollen to rear one worker bee, and Heinrich calculates that to rear one queen requires the nectar and pollen loads collected by a worker during two days of continuous foraging.

We are still lacking information on the precise mechanisms that instigate queen production. Increase in colony size and decrease in brood/worker ratio release a greater proportion of bees for foraging so the collection of sufficient forage for queen production should become possible. However, larger colonies need larger foraging areas to support them, so individual foragers will need to forage further afield with diminished efficiency. As a colony becomes larger so does the average size of the worker so the larger forager and its greater load bearing capacity may more than compensate for an increased foraging area!

Heinrich also discusses how bumblebees interrelate with other animals and plants in their environment, and stresses especially the effect of bumblebee pollination on the production of fruits and seeds, that in turn provide the diet of much wildlife. In contrast to most insects that forage on plants, visits by bees actually increase the likelihood that the plants will reproduce, and so provide additional forage and energy requirements for succeeding generations of bees.

This book is written for the non-specialist; although easy to read the style is sometimes too colloquial for my taste. I see no point in calling empty cocoons "silken cradles" or to liken the adult bee to a "miniature helicopter" or "a small, furry, orange, black and yellow object". But few can avoid the temptation to try to wax poetic about the bumblebee.

Quite rightly, Heinrich pays particular attention to his own research and its implications. Although at times the reader is too frequently reminded to regard the bumblebee in terms of energy balance and allocation of resources, the central theme proves a useful one and serves the double purpose of binding the work into a coherent whole and allowing the author to return easily to his main line of thought after frequent digressions. There are two short appendices, on how to rear bumblebees and on the bumblebee species of North America, and a list of selected references.

This is a good book, packed with information. I recommend it as essential reading to those interested in social insects and hope it will find the wider readership it deserves.

John B. Free

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Fluid compositions in the Earth's crust

Fluids in the Earth's Crust: Their Significance in Metamorphic, Tectonic and Chemical Transport Processes. By W. S. Fyfe, N. J. Price and A. B. Thompson. Pp. 372. (Elsevier Scientific: Amsterdam and New York, 1978.) $49.75; Dfl.125.

THE authors in the Preface of this book say that it is an attempt to link metamorphic processes and rock mechanics, to provide a text unifying the fields of metamorphic petrology and structural geology. In one sense, they have admirably accomplished this task. The success of the book is that from the varied viewpoints and experiences of the authors, it is a synthesis of a portion of these complex and diverse fields. The general theme is the link between fluid compositions in the crust as controlled by equilibrium metamorphic mineral assemblages and kinetics, mass transport during metamorphic processes, and tectonics. The role of fluids in crustal processes is strongly emphasised. However, as the authors state, this text is not traditional or classic or all-encompassing.

Chapter 1 sets the stage, providing the focus for the link between fluid motion, geochemical and tectonic processes. It is a

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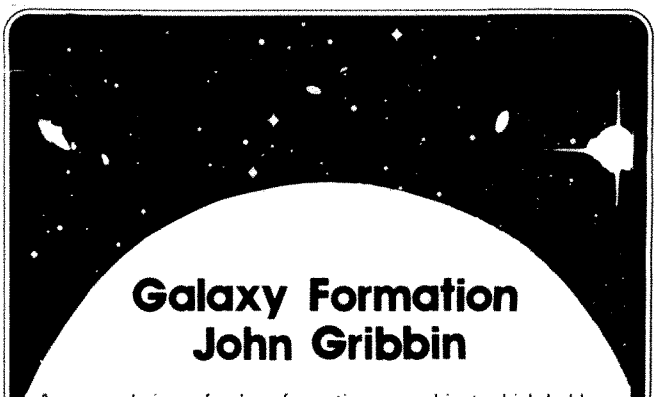
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John Gribbin

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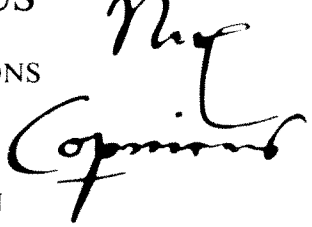
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short chapter, only 18 pages. In this, the authors point out that the question that concerns them in the book is how fluid flow is focussed, which is the question at the heart of economic gelology.

Chapter 2 describes the chemistry of natural fluids and the characteristics and role of water in transport processes. It is essentially a brief review of the chemistry of surface waters and subsurface fluids, including pore waters, oil-field brines, metamorphic and magmatic fluids, and fluid inclusions.

Chapter 3 is brief and deals with the volatiles bound in minerals. In Chapter 4, the solubilities of major rock-forming minerals are discussed in terms of P–T–X diagrams. The concept that fluid compositions are determined by mineral solubilities modified by kinetic and residence time considerations is initially developed. The conclusion is drawn that natural fluid compositions can be adequately described in the model system $NaCl-CO_2-H_2O$.

Some elementary theory governing metamorphic mineral reaction rates is presented in chapter 5, and applied to solution rates of SiO_2, $CaCO_3$, and alkali feldspars. Rates of mineral nucleation and growth and diffusion are considered from a theoretical and empirical standpoint. Finally, rates and mechanisms of metamorphic reactions are discussed.

Chapter 6 deals with the temperatures and pressures of metamorphism and metamorphic facies. It emphasises how, where and when water is released during metamorphism and the composition of metamorphic fluids.

Rock-dominated and fluid-dominated metamorphism are discussed in Chapter 7. The behaviour of H_2O and CO_2, O_2 and H_2, S and SO_2, halogens, and the behaviour of fluids during partial fusion, are emphasised. The role of fluid pressure and its relationship to P_{total} and P_{H_2O} are critically assessed.

In chapter 8, there is a sharp change in direction. The subject is rock deformation and rock mechanics, particularly the experimental aspects. The strength of rocks is discussed in terms of the environmental parameters of confining pressure, temperature, water and fluid content and pressure, and time. The authors conclude that earlier high-strength data on rocks were incorrect. Unfortunately, this chapter omits any account of recent experimental work on the weakening effect that the presence of water has on the creep strength of rock.

Using geological evidence, the authors attempt in chapter 9 to quantify the crustal conditions of temperature, confining pressure, pore fluid pressure, differential stress, and strain rate that control rock deformation. This is a very nice review of the 'state of the art'.

The concepts of permeability, hydraulic fracure, and elasticity are discussed in chapter 10, in preparation for the following two chapters. The treatment of fracture mechanics that is applied to hydraulic fracture is at an elementary level.

In chapters 11 and 12, controls on the escape of water during compaction, deformation and metamorphism, and igneous and salt diapirs and diapirism respectively, are considered. Very recent work on transport of magmas is not described in these chapters. In chapter 13, the role of fluids in tectonic and metamorphic processes is synthesised and regions of large-scale crustalmass transport delineated. The authors certainly succeed in showing that the dynamics of a wet Earth is quite different from that of a dry Earth. (It should be pointed out that the conversion factor for viscosity from c.g.s. units to S.I. units given in the book is wrong.)

We found this book to be a pleasure to read, perhaps because it dealt principally with fields only somewhat allied to ours. It is well written and syntheses in a single package 'pieces of the puzzle' involving crustal fluids, and metamorphic and tectonic processes. We recommend it.

Fred T. Mackenzie
J. Weertman

Fred T. Mackenzie is Professor of Geological Science and J. Weertman is Professor of Materials Science and Engineering and Professor of Geophysics at Northwestern University, Evanston, Illinois.

- *The Pursuit of Nature: Informal Essays on the History of Physiology* (for the review, see *Nature*, **270**, 117; 1977) has been published in paperback by Cambridge University Press (Cambridge, UK) at £2.95.

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Applied geology

Industrial Geology. Edited by J. L. Knill. Pp. 344. (Oxford University Press: Oxford, 1978.) £9.50.

THE most important aspect of this book is that it will bring to the attention of both teachers of geology and students alike, the industrial applications of the subject, of which many of them will have little practical experience or knowledge. Such a text would seem to be long overdue.

In fact the book arose from a series of lectures, given to students at the Department of Geology and Mineralogy at Oxford, by various industrial speakers whose contributions have now been put together to form this volume. Universities are not in a position — nor is it their function — to produce applied geologists, yet somehow undergraduates and especially postgraduates must be exposed to the practical aspects of the subject. The philosophy behind this concept is very clearly put down in Professor Vincent's foreword, and the first three paragraphs are recommended to everybody using this book. The artificial barriers between academic geology and applied geology as practised in industry are indeed attitudes of mind.

Almost every facet of industrial activity (but not Government) in which geologists are involved receives a chapter and the more important ones are given two; but the main aim is clearly to provide students with an introduction to the particular applications of geology in the mining, oil and civil engineering industries.

In the energy field, coal, oil and natural gas, the geologist has prime functions in both in the exploration and production phases, and the chapters point out the varied nature of the geologists role and the related disciplines with which he must become familiar, for example, geophysics reservoir, engineering economics.

In the minerals industry the geologist is no less important but here perhaps relies less on the seismic method and more on mapping and regional petrogenesis. Three chapters are included and these are concerned primarily with exploration and metallurgy in the UK.

The abstractive industries and the cement industry are then considered and these are followed in turn by descriptions of groundwater and the construction industry, geological hazards and conservation. It would be too lengthy a process to describe them in detail but even if the reader were expert in one field he would probably learn a great deal from reading the other chapters. So much more is to be gleaned, therefore, by the student coming fresh to the application of his subject.

Although not specifically intended as a careers guide, it will inevitably be used as one, not least to answer the question "what would I expect to do", but perhaps, more importantly, to show the student what particular facets (course options) of the subject will be best suited to the particular field eventually chosen.

The book deserves to be widely read not just by students and teachers, although they will have most to gain, but by all geologists.

J. R. V. Brooks

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Depositing thin films

Nucleation and Growth of Thin Films. By B. Lewis and J.C. Anderson. Pp.504. (Academic: New York, San Francisco and London, 1979.) £27.50; $57.

STUDIES of condensation of vapour atoms released in vacuum from localised sources can be dated from Knudsen's report in 1909 that mercury atoms did not condense on glass unless it was at a critical temperature, below -140°C; an observation reinforced by R.W. Wood who found a critical temperature effect for condensation of cadmium atoms on glass. Wood believed that the vapour atoms were reflected and Knudsen attributed this to a failure of the incident atoms to adjust to the surface temperature. Certainly the effects of reflection have been found with atomic and molecular beams and in thermal transpiration experiments, but the phenomenon witnessed by Knudsen and Wood arose from temporary sojourn of incident Hg and Cd atoms on foreign surfaces and their re-evaporation. Thus,incident atoms can thermally accommodate to a surface and still evaporate if their binding energy is weak.

It was Langmuir in 1917 who provided this latter explanation, showing by experiment and deduction that an adatom, unlike an elastically rebounding atom, would have an average surface lifetime before re-evaporating. As the lifetime rose or the impact rate increased so could the adatom density rise and migrating atoms meet to form stable clusters. Thus, onset of condensation depended on both the incident atom impact rate and the substrate temperature for a given atom/surface pair. When the surface binding forces for the adatom were high condensation ensued without these critical conditions and molecular beam experimentors some 50 years ago precoated surface detectors with silver to condense weak Cd-beams. The nucleating effect was turned to advantage by the Bosch Company in Germany who made Zn-coated paper for capacitors using an Ag-seed layer, a process which in contemporary times has been applied to fingerprint detection.

The emergence of the electron microscope made it possible to observe the cluster growth in condensates and observe the onset of condensation with a vapour source mounted in the instrument. This technical advance and the growing interest in thin solid films in industry brought into being a world based group of 'nucleation' workers. In the same period studies of the condensation of gases, cryo-pumping, have grown and these have contributed knowledge on physical condensation mechanisms.

The book under review effectively collects together the results of studies of physical condensation during the past two decades. The authors are known for their researches on film growth and physical properties. The reference text they have produced takes the reader through the logical sequence of physical adsorption, adatom migration, cluster growth and decay, nucleation kinetics and experiments on nucleation and epitaxial growth. The book is basically concerned with the physical condensation of atoms emitted at moderate thermal energies from a separate source, that is, non-equilibrium (or vacuum) evaporation. Thus, the title could be misleading particularly as films are now deposited by many different processes involving nucleation.

Deposition by sputtering is only briefly mentioned and an example given of film growth by ion beam sputtering. The high emission energy of sputtered atoms is referred to but other sources of energy transfer, such as backscattered energetic neutrals, are not considered. (On page 3 there is some confusion in use of the phrase "fairly high vacuum pressures" to describe the operating region for sputtering. Also this is not 10^{-1} to 10 torr as stated but 10^{-3} to 10^{-1}torr). However, this is basically a text dealing with atom impact at low energies in vacuum and we shall probably have to wait many years before all the conditions contributing to film growth by sputtering are unravelled although much is already known.

Why do workers in this field usually refer to Frenkel for the adatom/lifetime relationship but rarely give credit for the basic concept to Langmuir and his equally important equilibrium (hyperbolic) law for adsorption? Langmuir's work is not referenced in this text.

This is not an easy work to follow — the subject is complex and full of detail — and a table defining the many symbols used would save constant back searching. Several typographical errors and some of sense occur in the text (the index in at least one case did not agree with the text and is inadequate). The book will be of value to those entering this specialist field. However, a note of warning — nucleation not only depends on substrate material and impact conditions but also on surface state. Research in this field can require ultra-high vacuum apparatus with mass spectrometers to determine the mass distribution of the incident and desorbed vapour and the residual gas composition, and instruments to determine the substrate surface condition after *in situ* cleaning, for example, by ion etching. It is as costly as it is complex.

L. Holland

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Immobilised enzymes

Immobilized Enzymes: Research and Development. Edited by I. Chibata. Pp. 284. (Wiley: New York, London, Sydney and Toronto, 1979.) £24.50; $46.

IN recent years there have been many books published on various aspects of immobilised eyznymes. Nevertheless, this book is a welcome addition, coming as it does from Dr Chibata and his colleagues. Dr Chibata, as Director of the Research Laboratory of Applied Biochemistry at the Tanabe Seiyaku Co. Ltd, Japan, has contributed greatly to the understanding of immobilised enzymes and cells, and to the development of industrial processes using these novel catalysts. He is therefore internationally recognised as a leading expert on the topics covered in this book.

The book contains four chapters, of which the first is a short introduction to the subject. The second chapter is devoted to the preparation of immobilised enzymes and cells, with comprehensive lists of the techniques that have been used and the enzymes and cells that have been immobilised. It concludes with a useful section on immobilised coenzymes. The next chapter describes the effects of immobilisation on the properties of enzymes and cells, such as the *p*H and temperature dependencies of their activities and the operational and storage stabilities in various conditions. The last and longerst chapter is concerned with the use of these catalysts in chemical and food processes, and their analytical and medical applications. Naturally, there is considerable emphasis on processes developed by the authors but it is surprising to find so little about the production of high fructose-glucose syrups, which is by far the largest industrial application of immobilised biocatalysts. The final part of this chapter covers affinity chromatography and seems rather out of place in this book.

In the preface it is stated that the aim was to produce a comprehensive text, and to a certain extent the authors have succeeded. Unfortunately, presumably because of the interests of the authors, the book concentrates much more on the biochemical aspects and the engineering content is minimal. For instance, the section on reactors is very short and there is no consideration of the problems that arise from diffusional limiatation of the reaction rate. As a result, the book tends to be rather descriptive and lacks a quantitative approach. Despite these criticisms, this book is highly recommended as a good reference text.

M. D. Lilly

M. D. Lilly is Reader in the Department of Chemical and Biochemical Engineering at University College London, UK.

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RUDBACH, Jon A., and BAKER, Philip J. (edited by). Immunology of Bacterial Polysaccharides. (Proceedings of a Symposium presented at the Annual Meeting of the American Society for Microbiology, Las Vegas, Nevada, 16 May 1978.) (Developments in Immunology, Vol. 2) Pp.xi + 157. ISBN-0-444-00315-0. (New York, Amsterdam and Oxford: Elsevier/North-Holland, 1979.) $24.

RUDDON, Raymond W. (edited by). Biological Markers of Neoplasia: Basic and Applied Aspects. Pp.xii + 590. ISBN-0-444-00292-8. (New York and Oxford: Elsevier, New York, 1978.) np.

SHUTE, C. C. D. The McCollough Effect: An Indicator of Central Neurotransmitter Activity. Pp.147. ISBN-0-521-22395-4. (Cambridge, London and New York: Cambridge University Press, 1979.) £11.

STAMATOYANNOPOULOS, George, and NIENHUIS, Arthur W. (edited by). Cellular and Molecular Regulation of Hemoglobin Switching. (Conference, Seattle, Washington, June 19-21, 1978.) Pp.xxiii + 792. ISBN-0-8089-1159-7. (New York and London: Grune and Stratton, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1979.) $68.50.

STEWART, II, WILLIAM, E. The Interferon System. Pp.xii + 421. ISBN-3-211-81505-8 (Wien and New York: Springer-Verlag, 1979.) DM98; $53.90.

STUART, F. P., and FITCH, F. W. (edited by). Immunological Tolerance and Enhancement. Pp.viii + 196. ISBN-0-85200-189-4. (Lancaster: MTP Press, Ltd., International Medical Publishers, 1979.) £14.95.

TAYLOR, Angela E. R., and MULLER, Ralph (edited by). Problems in the Identification of Parasites and Their Vectors. (Symposia of the British Society for Parasitology, Vol. 17.) Pp.vii + 221. ISBN-0-632-00164-X. (Oxford and London: Blackwell Scientific Publications, 1979.) £9.60.

USDIN, Earl, BORCHARDT, Ronald T., and CREVELING, Cyris R. (edited by). Transmethylation. (Proceedings of the Conference on Transmethylation, Bethesda, Maryland, USA, on October 16-19, 1978.) (Developments in Nuroscience, Vol. 5.) Pp.xxii + 631. ISBN-0-444-00310-X. (New York, Amsterdam and Oxford: Elsevier/North-Holland, 1979.) $55.

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VYAS, Girish N., COHEN, Stephen N., and SCHMID, Rudi (edited by). Viral Hepatitis: a Contemporary Assessment of the Etiology, Epidemiology, Pathogenesis and Prevention. (Proceedings of the Second Symposium on Viral Hepatitis, University of California, San Francisco, March 16-19, 1978.) Pp.xxii + 748. ISBN-0-85626-182-3. (Tunbridge Wells, Kent: Abacis Press; Philadelphia: The Franklin Institute Press, 1978.) £38.50.

WELCOMME, Robin L. Fisheries Ecology of Floodplain Rivers. Pp.viii + 317. ISBN-0-582-46310-6. (London and New York: Longman Group, Ltd., 1979.) £19.95.

WINEK, Charles L., and SHANOR, Sydney P. (edited by). Toxicology Annual, Vol. 3. Pp.xii + 340. ISBN-0-8247-6773-X. (New York and Basel: Marcel Dekker, Inc., 1979.) SFr.90.

WINICK, Myron (edited by). Hunger Disease: Studies by the Jewish Physician in the Warsaw Ghetto. Translated from the Polish by Martha Osnos. (Current Concepts in Nutrition.) Pp.xiv + 261. ISBN-0-471-05003-2. (New York and Chichester: Wiley-Interscience, John Wiley and Sons, 1979.) £10.

WOOD, Clive (edited by). Tropical Medicine from Romance to Reality. (Proceedings of a Conference 12-14 December 1977.) Pp.ix + 286. ISBN-0-12-763360-X. (London: Academic Press; New York: Grune and Stratton, 1978.) £13.20; $27.25.

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Psychology

BOWER, T. G. R. Human Development. (A Series of Books in Psychology.) Pp.xiv + 473. ISBN-0-7167-0058-1. (San Francisco, CA.: W. H. Freeman and Company, 1979.) $15.

COHEN, John, and CLARK, John H. Medicine, Mind and Man: An Introduction to Psychology for Students of Medicine and Allied Professions. Pp.xviii + 401. ISBN-0-7167-1090-0. (Reading and San Francisco: W. H. Freeman and Company, 1979.) Hardcover £10; Softcover £4.95.

CONNOLLY, Kevin (edited by). Psychology Survey, No. 2. Pp.xi + 288. ISBN-0-04-150068-7. (London, Boston, Mass. and Sydney: George Allen and Unwin, 1979.) Hardback £7.50; Paperback £3.95.

FANTINO, Edmund, and LOGAN, Cheryl A. The Experimental Analysis of Behavior: a Biological Perspective. (A Series of Books in Psychology.) Pp.xiii + 559. ISBN-0-7167-1036-6. (San Francisco: W. H. Freeman and Company, 1979.) $15.

GOLDSTEIN, Arnold P., Monti, Philip J., SARDINO, Thomas J., and GREEN, Donald J. Police Crisis Intervention. (Pergamon International Library of Science, Technology, Engineering and Social Studies. Pergamon Genral Psychology Series.) Pp.vii + 175. ISBN-0-08-023874-2. (Oxford and New York: Pergamon Press, 1979.) £3.50.

JAYNES, Julian. The Origin of Consciousness in the Breakdown of the Bicameral Mind. Pp.467. ISBN-0-7139-1254-5. (London: Allen Lane, Penguin Books, Ltd., 1979.) £8.95.

KANDEL, Eric R. Behavioral Biology of *Aplysia*: A Contribution to the Comparative Study of Opisthobranch Molluscs. Pp.xiii + 463. ISBN-0-7167-1070-6. (A Series of Books on Psychology.) (San Francisco: W. H. Freeman and Company, 1979.) Cloth $40; Paper $20.

KLIX, Friedhart (edited by). Human and Artificial Intelligence. (Fundamental Studies in Computer Science, Vol. 8.) Pp.xv + 227. ISBN-0-444-85173-9. (Amsterdam, New York and Oxford: North-Holland Publishing Company, 1979.) Dfl.75; $36.50.

LAMB, Michael E., SUOMI, Stephen J., and STEPHENSON, Gordon R. (edited by). Social Interaction Analysis: Methodological Issues. Pp.x + 320. ISBN-0-299-07590-7. (Madison and London: The University of Wisconsin Press, 1979.) £12.25.

MIRON, Murray S., and GOLDSMITH, Arnold P. Hostage. (Pergamon International Library of Science, Technology, Engineering and Social Studies. Pergamon General Psychology Series.) Pp.170. ISBN-0-08-023876-9. (Oxford and New York: Pergamon Press, 1979.) Hard £7.50; Flexi £3.40.

SCHIZOPHRENIA: An International Follow-up Study. (World Health Organization.) Pp.xvii + 438. ISBN-0-471-99623-8. (Chichester and New York: John Wiley and Sons, 1979.) £16.

SHERMAN, Mark, Personality: Inquiry and Application. (Pergamon General Psychology Series.) Pp.x + 546. ISBN-0-08-019585-7. (Oxford and New York: Pergamon Press, 1979.) £7.95.

SNODGRASS, Joan Gay. The Numbers Game: Statistics for Psychology. Pp.xx + 647. ISBN-0-19-857179-8. (Oxford: Oxford University Press, 1977.) £5.95 net.

STAUB, Ervin. Positive Social Behavior and Morality: Socialization and Development, Vol. 2. Pp.xix + 317. ISBN-0-12-663102-6. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1979.) $14.50.

SZASZ, Thomas. The Myth of Psychotherapy: Mental Healing as Religion, Rhetoric, and Repression. Pp.xviii + 238. ISBN-0-19-217676-5. (Oxford and Melbourne: Oxford University Press, 1979.) £5.95 net.

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TECHNICIAN (GRADE 7)

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Please write to the Administrative Officer (Personnel), (Ref. S1352/N), The University, Sheffield S10 2TN. 3072(A)

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Applicants should send a curriculum vitae and names of three referees to Dr T. M. Scott, Chairman Anatomy Search Committee, Faculty of Medicine, Health Sciences Centre, Memorial University, St John's, Newfoundland, Canada, A1B 3V6. W323(A)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF CHEMISTRY

RESEARCH ASSSISTANT

Applications are invited for this post which is funded by ARC, and is available from 1 November 1979 for three years. The successful applicant will work on a project concerned with the synthesis of derivatives of steroids in meat.

Salary within Range 1B, £3,775 to £4,333, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 21 September 1979. 3037(A)

ELECTRON MICROSCOPIST

DEPARTMENT OF BIOCHEMISTRY

STANFORD UNIVERSITY

There will be an opening in the Biochemistry Department in April 1980 for a Research Associate with extensive background in EM applied to Cell Biology. The successful candidate will primarily collaborate with a group actively studying membrane biogenesis, intracellular transport of proteins, and secretion, and will utilize immunocytochemical and autoradiographic methods. He/she will also be responsible for managing the general use of the electron microscope and be available for consultation and collaboration with other research groups.

Expertise in conventional transmission EM techniques is essential, but an additional specialization (such as immunocytochemistry, or EM autoradiography) would be highly desirable. Suitable candidates could hold PhD, MS, or BA degrees. The extent of independence in this position, and the salary, will be commensurate with the individual's academic qualifications and experience. Send CV and bibliography to: Dr James E Rothman, Department of Biochemistry, Stanford University Medical Center, Stanford, California 94305.

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ROYAL POSTGRADUATE MEDICAL SCHOOL

DEPARTMENT OF MEDICINE

POST-DOCTORAL IMMUNOLOGIST

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Salary up to £5,077 plus £502 London owance.
Application forms are available from d should be returned to the Personnel fice, Royal Postgraduate Medical hool, 150 Du Cane Road, London 12 0HS quoting reference number 220. 2956(A)

UNIVERSITY OF LEICESTER

EXPERIMENTAL OFFICER

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Candidates should be graduates or have uivalent professional qualifications. lary on an incremental scale £3,689 to 108 pa according to qualifications and perience.
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ST. GEORGE'S HOSPITAL MEDICAL SCHOOL

(University of London)

POST DOCTORAL RESEARCH FELLOW

quired to work in Dr M.J. Clemens oratory in the Department of chemistry on a project funded by the ncer Research Campaign concerning the ulation of protein synthesis in culocytes and cultured cells and/or the chanism of action of interferon.
xperience in protein purification or ymology an advantage. It is expected the appointment will be for three years inning in 1980. Salary in University le IA. Further information from Clemens Tel. 01-672 1255 ext 4750.
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Application should be made by letter quoting Ref No 3/6T/79, giving full details of age, experience etc and addressed to the Personnel Office, University of Warwick, Coventry CV4 7AL by 10th September, 1979. 3057(A)

UNIVERSITY OF MANCHESTER

RESEARCH ASSISTANT IN PHARMACOLOGY

Applications are invited for this post from recent graduates with a first or upper second class degree in pharmacology, biochemistry or a related discipline. The appointee, who may register for a higher degree, will investigate the interaction of anti cancer drugs with the steroid receptor system in animal and human tissue. The project will be carried out in conjunction with the Christie Hospital and Holt Radium Institute.

Appointment is for one year initially extendable to three years at a salary commencing at £3,775 p.a. Range IB.

Applications, with curriculum vitae and the names of two referees should be sent as soon as possible to Dr I.D. Morris, Department of Pharmacology, Medical School, Manchester M13 9PT. 3023(A)

THE UNIVERSITY OF MANCHESTER

DEPARTMENTS OF PATHOLOGY AND MEDICAL BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

required for a joint project between the above departments, to investigate the functions of mast cells in lymph nodes, with special regard to the lymphatic spread of lymphoma cells. Starting salary up to £5,488 p.a. Duties commence as soon as possible.

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ST GEORGE'S HOSPITAL MEDICAL SCHOOL

(University of London)

RESEARCH STUDENT (PHYSIOLOGY)

required for a period of 3 years from October 1st to study nasal physiology in man and experimental animals in allergic conditions. Applicants require a good degree in biological sciences. The student will work for a PhD degree.

Applicants should send curriculum vitae with the names and addresses of two referees to Professor J.G. Widdicombe, Department of Physiology, St George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE. For further information please telephone Professor Widdicombe on 01-672 1255 ext. 4651. 3054(A)

School of Army Health, Mytchett, Aldershot

Chemist/ Biochemist

Occupational Health

The successful candidate will be fully responsible for providing scientific support to the Environmental Monitoring Team and will conduct a wide and expanding range of physical and chemical analyses of environmental and biological samples for research purposes. There will be opportunities for field investigations in army units, some of which may be overseas. During the first two years, work will be partly in the Army Occupational Health Research Unit at Farnborough.

Candidates (normally aged under 27) should have a degree or equivalent in an appropriate discipline. Some experience in Biochemistry, Clinical Chemistry or Occupational Hygiene would be advantageous.

Appointment as Scientific Officer. Starting salary (according to qualifications and experience) within the range £3,285 to £5,085 (to become £3,590 to £5,485 from 1.1.80). Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 21 September, 1979) write to Ministry of Defence, CM(S)1b3, Room 310, Savoy Hill House, Savoy Hill, Strand, London WC2R 0BX. *Please quote ref: SA/2/ET.*

Ministry of Agriculture, Fisheries and Food, Food Science Division

Chemist/ Biologist

London

This post is in the Food Surveillance Section, which assists the Division in providing an advisory service on all aspects of food science, food technology and human nutrition, both at home and abroad.

The chemist/biologist appointed will assist in the organisation of food contamination monitoring programmes. The work involves the production of data in liaison with industry, professional bodies and local organisations, together with the preparation of reports for official committees.

Candidates, normally aged under 30, should have a good honours degree in chemistry or a biological science. At least 2 years relevant postgraduate experience. An interest in administration and writing is essential.

Appointment will be as Higher Scientific Officer. Starting salary (according to qualifications and experience) within the range £5,505 to £6,990 (to become £5,875 to £7,515 from 1.1.80). Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 25 September, 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote ref: SB/42/AE.* 3075(A)

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College of Biological Science
DEPARTMENT OF NUTRITION

Applications are invited for the position of

Assistant Professor of Nutrition

Qualifications required are a PhD with training in experimental nutrition related to the field of aquaculture. The successful applicant will be required to teach aspects of comparative nutrition at the undergraduate and graduate levels, and to supervise graduate students and conduct research on applied and fundamental aspects of fish nutrition.

Curriculum vitae and the names of three references should be sent to Dr B L Walker, Chairman of Search Committee, Department of Nutrition, University of Guelph, Guelph, Ontario N1G 2W1 by December 1, 1979. Appointment subject to final budgetary approval. W325(A)

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)
DEPARTMENT OF PHYSIOLOGY

POST-DOCTORAL RESEARCH FELLOW

required to work on the nature and control of secretion of Clara cells in the lungs. Histological and biochemical techniques will be used and the research will be co-ordinated with existing studies of lung mucus secretions at the Medical School. The appointment is for 3 years funded by an MRC project grant. Emoluments £4,333 to £4,622 Bar £4,910 plus £502 London Allowance.

POST-DOCTORAL RESEARCH FELLOW

required for project to study airways mucus composition and factors controlling its secretion. Biochemical experience essential preferably of glycoproteins. The appointment is for 1 year funded by the Wellcome Trust. Emoluments £4,333 plus £502 London Allowance.

TECHNICIAN GRADE 5

urgently required to assist with research on nature and control of secretion in the lungs involving the application of biochemical and histological techniques. Experience in one of these fields essential. The appointment is for 3 years funded by the MRC. Emoluments £3,700 to £3,815 Bar £3,940 (under review) plus £275 London Allowance.

Further information regarding the above posts from Professor J.G. Widdicombe Tel. 01-672 1255 ext 4651.

Application forms from the Establishment Officer, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. Closing date 20th September 1979. 3056(A)

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

POST DOCTORAL RESEARCH FELLOW (IMMUNOLOGY)

required from 1 October 1979 if possible t join a group of five working under th direction of Dr B. Anderton on th structure and function of the cytoskeleto with particular reference t neurofilaments. Techniques used incluc protein chemistry and monoclon antibodies.

Applicants should possess experience i protein chemistry or immunochemistr The post which is for 3 years is funded by a SRC grant. Emoluments £4,910 to £5,1 bar £5,488 plus £502 London allowance.

Application forms and furth particulars from the Sector Administrato Sector 2, St. George's Hospital Medic School, Cranmer Terrace, Tooting SW 0RE. 3032(A)

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UNIVERSITY OF ESSEX

DEPARTMENT OF BIOLOGY

(a) POSTDOCTORAL RESEARCH FELLOW (Ref. AG/90)

(b) RESEARCH TECHNICIAN GRADE 5 (Ref. P/20)

ese MRC-supported posts are available up to 3 years from 1 October, to take 't in a study of the mitochondrial ıome of *Aspergillus nidulans* using ıetical, DNA and protein sequencing ɔroaches. The research is directed by ; R. Wayne Davies and C. Scazzocchio part of a larger group investigating ıtrol of gene expression.

Salary for post (a) at lower end of Range (£4,333 to £7,521 p.a.) and for post (b) Technician Grade 5 scale (£3,700 to 320 p.a.).

Applications, including a curriculum ıe and names and addresses of two 'erees, and quoting appropriate erence number, should reach the gistrar, University of Essex, Wivenhoe rk, Colchester C04 3SQ, from whom ther particulars may be obtained by 21 ɔtember 1979. 3069(A)

UNIVERSITY OF BRISTOL

Cosmic Ray Research

ɔplications are invited for three posts of

RESEARCH ASSISTANT

:h of 24 months duration, for work :hin the cosmic ray studies group under ofessor P.H. Fowler, FRS. These posts ll be concerned with experiments to :asure the high energy component of the tra-heavy cosmic rays and the nomalous" component of the low ergy cosmic rays.

Experience with nuclear or spacecraft trumentation, especially the use of drift ambers and microprocessor based data quisition sytems, would be appropriate.

Salaries will be on the scales for research ff grades 1A (2 posts) £4,333 × £289 and (1 post) £3,775 × £280. (Scale effective ɔm 1st October, 1979 and subject to 'ther review).

Applications, naming two referees and ing details of education and relevant ɔerience should be made by 20th ɔtember, 1979 to Dr R.M. Redfern, H. Wills Physics Laboratory, Tyndall enue, Bristol BS8 1TL. Telephone stol 24161 ext 110, from whom further ormation can be obtained. 3047(A)

MOLECULAR BIOLOGY AND IMMUNOLOGY OF TRYPANOSOMES

program for the study of antigenic riation in African trypanosomes ticipates the possibility of several enings at the level of graduate students, st doctoral fellows and technical staff. pects of study include (1) the molecular is of antigenic variation, (2) antigenic alysis and production of monoclonal ibodies, and (3) response of the host nune system to parasitic invasion. perience in either immunology, ombinant DNA technology and/or tein biochemistry are desirable.

Please submit curriculum vitae with tement of research goals and 3 letters of ɔmmendation by October 1, 1979 to Dr ıa Agabian, Dept. of Biochemistry 70, University of Washington, Seattle, n 98195. An Affirmative Action/Equal portunity Employer. W326(A)

Scientists for the Confectionery Industry

We are looking for scientists to strengthen our Divisional Research Department where we have vacancies in both our chemistry and physics sections.

Our business is making top quality confectionery such as Kit Kat, Polo, After Eight, Rolo and Black Magic. In the Research Department our work is varied and includes the development of a basic understanding of the complex systems involved in food manufacture and their application to practical problems. But to maintain our high standards, and adapt to a changing world, we need more understanding of the science underlying the art of making confectionery: we need to explore the application of new developments in other areas as well. Our work involves bringing together a wide variety of specialisms and is itself a complex blend of basic and applied research.

Our standards are high and we are looking for men or women who have a good degree in scientific subjects and at least three years' experience in either research (basic or applied) or industrial development. For both these posts the main requirement is an ability to develop ideas fully and to apply them in different fields.

Scientific excellence and a willingness and ability to work in a variety of fields, some of which may be outside your current speciality, is of more importance than your specific training; for the chemical vacancy, some training in chemistry is essential and an interest in mathematics would be useful.

The positions are based in York, an attractive city which provides reasonably priced housing, good schools and easy access to coast and countryside.

We offer a competitive salary and run profit sharing, pension and life assurance schemes. Where appropriate, generous help will be provided towards the cost of moving.

If you are interested please write for an application form quoting ref. U.674 to Miss D.M.N. Dick, Staff Office, Rowntree Mackintosh Ltd., York YO1 1XY.

Rowntree Mackintosh 3067(A)

THE OPEN UNIVERSITY

Earth Sciences Department

Technician (£3,474 to £4,056) under review

A vacancy exists in the new Mass Spectrometry Laboratory for a Technician. The successful applicant will be responsible for the day to day running of the ultra-clean laboratory area. Duties will include distillation and calibration of ultra-clean reagents, preparation of samples for the solid-source mass spectrometer, and stock control of chemicals and laboratory equipment. There will be considerable opportunity to work closely with academic staff in the development of new techniques and their implementation in a wide variety of projects in the geological sciences.

Applicants should possess a degree in Chemistry with at least four years relevant experience.

Application forms and further particulars are available from The Recruitment Office (JD3790/3), The Open University, P.O. Box 75, Walton Hall, Milton Keynes, MK7 6AL or telephone Milton Keynes 63404: there is a 24 hour answering service on 63868.

Closing date for applications: 13th September. 3062(A)

SHEFFIELD AREA HEALTH AUTHORITY (Teaching)
Southern District (Teaching)

CHIEF MEDICAL LABORATORY SCIENTIFIC OFFICER

PAEDIATRIC HAEMATOLOGY

Due to retirement, a vacancy occurs at the Children's Hospital, and applications are invited from suitably qualified and experienced Fellows of the Institute of Medical Laboratory Sciences.

The Department is well equipped and deals with all aspects of Paediatric Haematology, being a recognised haemophilia centre and leukaemia centre.

An active interest in management and training is essential and a positive contribution in a range of technical skills will be expected of the successful candidate.

Intending applicants are encouraged to visit the laboratory by prior arrangement with Dr. J. S. Lilleyman, Consultant Haematologist Tel. No. Sheffield 71111 Ext 329 who will supply a Job Description.

Applications stating age, qualifications and experience, together with names of two referees to the address below.

CHIEF MEDICAL LABORATORY SCIENTIFIC OFFICER

HISTOPATHOLOGY

Applications are invited from Laboratory Scientists who must also be experienced State Registered Fellows of the Institute of Medical Laboratory Sciences for the above post at the Children's Hospital.

The department is well-equipped and deals with routine and research in Paediatric and Developmental Histopathology, and offers a unique opportunity for persons interested in this work. An active interest in training of subordinate staff and positive contribution in a range of technical skills will be expected of the successful candidate.

Intending applicants are encouraged to visit the laboratory by prior arrangement with Professor J.L. Emery, Consultant Pathologist, Tel. No. Sheffield 71111 Ext. 254 who will supply a job description.

Applications stating age, qualifications and experience together with names of two referees to

District Personnel Officer, B Floor, Hallamshire Hospital, Glossop Road, Sheffield S10. 3034(A)

Ph.D BIOCHEMIST

FOR BIOTECHNOLOGY RESEARCH

The Fermentation Research and Development group of Pfizer Central Research carry out research on new products and processes for making chemicals by fermentation.

As a result of a recent promotion we now need an additional Ph.D., to join us as a Senior Research Scientist to work on a variety of fermentation projects including antibiotics, organic acids, and new products.

We are looking for a graduate in biochemistry or related subject who has a good knowledge of microbial biochemistry, and ideally, experience of fermentation techniques. The person appointed will join an established multi-disciplinary team of experienced scientists, be expected to be able to work independently and will be given technical support to allow this.

The starting salary for a Ph.D. with 3 years post-doctoral experience will be not less than £6,450 per annum and in addition to excellent career prospects there is good bonus potential. Pension, death benefit and salary continuance schemes are in operation, and there is generous assistance with re-location expenses to a pleasant coastal/rural area of East Kent. Flexible hours are worked and the social/recreational facilities are first class.

Pfizer

Applications giving brief details of age, qualifications and experience should be sent to: **D. W. Sells, Personnel Manager, Pfizer Central Research, Sandwich, Kent.**

3076(A)

News Editor

A senior vacancy exists for News Editor of *Nature*, the international and interdisciplinary journal for scientists and science policy-makers. This vacancy has occurred with the move of the present News Editor to the new post of *Nature*'s Chief European Correspondent.

The News Editor will have responsibility under the Editor for about twelve news pages in each week's issue covering policies, events and personalities of concern to, or influenced by, the international scientific community.

He/she will have the full time services of an American and a European staff correspondent as well as help with lay-out and sub-editing.

The successful candidate will have several years experience as a journalist, a strong interest in international affairs and an enthusiasm for planning, co-ordinating and commissioning. Science background not essential but familiarity with the demands of professional/academic readership an advantage.

Write as soon as possible to — The Editor, *Nature*, Macmillan Journals Limited, 4 Little Essex Street, London WC2R 3LF, enclosing a curriculum vitae.

3048(A)

MACMILLAN

THE OPEN UNIVERSITY

Faculty of Science

Course Co-ordinator for the Science Foundation Course

Applications are invited for the permanent post of Course Co-ordinator to work on the Science Foundation Course (S101), which is in its first year of presentation, having been remade in 1978.

The main duties of the post are to provide a range of academic, administrative and editorial services to the members of the large Course Team concerned with the production and smooth running of this full-credit multi-disciplinary, multi-media undergraduate course.

Applicants for this post should hold a Science Degree in one of the following disciplines: Biology, Chemistry, Physics or Earth Sciences. An ability to work as part of a team is essential and an interest in adult education would be an advantage.

Appointment will be within the Course Co-ordinator salary range of £3,689 to £6,108 pa with U.S.S. benefits.

Application forms and further particulars are available from The Recruitment Office (JD2914/3), The Open University, P.O. Box 75, Walton Hall, Milton Keynes, MK7 6AL or telephone Milton Keynes 63404: there is a 24 hour answering service on 63868.

Closing date for applications: 13th September. 3029(A)

UNIVERSITY OF EDINBURGH

DEPARTMENT OF MOLECULAR BIOLOGY

POST-DOCTORAL RESEARCH FELLOWSHIPS

Applications are invited for the post of Postdoctoral Research Fellow to work on project concerned with the isolation of DNA sequences of development importance in *Xenopus* by molecular cloning procedures. Experience of nucleic acid or microbial genetic techniques would be an advantage though not essential.

There are two posts available; salary is on the scale 1A according to experience, and will be for three years starting as soon as possible.

Applications, including a curriculum vitae and names and addresses of two referees should be sent to Dr P J Fox, Department of Molecular Biology, King's Buildings, Mayfield Road, Edinburgh EH9 3JR. Please quote Reference 5052.

3043(A)

THE UNIVERSITY OF HULL WOLFSON INSTITUTE

Applications are invited for the post of

ANIMAL HOUSE SUPERVISOR (GRADE 6),

for the new Wolfson Institute of Gerontology. The work will involve the general supervision and administration of the animal house and laboratory, under the Director, Professor J.G. Phillips. The post carries full responsibility for providing the technical service, either by institute staff or by an agency agreement with the Zoology Department to which the institute is closely linked.

Qualifications required are HNC or equivalent and at least 10 years experience in animal husbandry. Salary on the scale £3,984 to £4,758 per annum (under review).

Applications, naming three referees, and giving full details of age, qualifications and experience should be sent to the Technical Staff Officer, University of Hull, HU6 7RX by 12 September. Further Particulars are available. 3021(A)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF GEOLOGICAL SCIENCES

RESEARCH ASSOCIATE IN MARINE GEOPHYSICS

Applications are invited for the post of Research Associate under the terms of a NERC grant. Duties are to assist the geophysics research group in the preparation of data and maps for publication. Degree in Physical Science required but prior knowledge of geophysics unnecessary.

The post is tenable for one year only. Salary will be on the RA1B scale £3,775 to £5,488 (October Scales) plus superannuation. Maximum starting salary will be £3,775.

Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham, PO Box 363, Birmingham B15 2TT, to whom applications (3 copies) including full curriculum vitae and naming three referees should be sent by Monday 17th September 1979.

Please quote ref: NG6. 3018(A)

THE MATHILDA AND TERENCE KENNEDY INSTITUTE OF RHEUMATOLOGY

POST DOCTORAL ESEARCH ASSISTANT IN

DIVISION OF BIOCHEMISTRY

)lications are invited for the post of earch Assistant for the further elopment of a project investigating the ecular basis of the pathological changes connective tissues associated with cular joint degeneration (osteo-ritis). The work is fundamental and ns part of an integrated programme of nective tissue research within the ision of Biochemistry (Head, Dr Helen ir, FRS). Previous experience of nective tissue or protein biochemistry, teolytic enzymes or tissue culture iniques would be an advantage.

he position is funded in the first ance for 3 years; salary in the range of 135 to £5,701.

.pplications enclosing a curriculum e and the names of two referees should ient to Dr Helen Muir, The Director, nedy Institute of Rheumatology, 6 e Gardens, Hammersmith London W6 N. 3035(A)

VICTORIA UNIVERSITY OF WELLINGTON

New Zealand

OLOGY: Lectureship in the artment of Geology. A petrologist is uired to teach mainly igneous ology. Experience in mineralogical, nical or analytical techniques would be dvantage in relation to the availability the JEOL-733 electron-probe oanalyser and the X-ray fluorescence infra-red spectrometers and action unit. From 1 February 1980.

)SING DATE: *17 September 1979.*

ALARIES: The salary scale for urers is NZ$11,894 to $14,615 p.a., uding the general wage order vance. It is expected that there will be tantial adjustment in the salaries of lemic staff announced shortly. Fares to New Zealand.

ONDITIONS OF APPOINTMENT: spective applicants should obtain litions of appointment and method of lication from the Association of mmonwealth Universities (Appts), 36 don Square, London WC1H 0PF. 3026(A)

AGRICULTURAL RESEARCH COUNCIL

INSTITUTE FOR RESEARCH ON ANIMAL DISEASES

cancy has arisen in the SPF production of the Institute for an experienced

SENIOR ANIMAL ECHNICIAN (FIAT,AIAT)

ct as Head of Unit under the Chief nal Technician.

thorough knowledge of breeding ms and the microbiological principles lved in the operation of an SPF lling will be required, together with ious experience in the supervision of .

ie appointment will be as a Process and eral Supervisory Officer Grade D. y scale (currently under review) £3,558 ,243 pa. Excellent working conditions easant rural surroundings with a staff urant and hostel accommodation for a le person if required. Non-ributory pension scheme.

pplications to the Secretary, Institute Research on Animal Diseases, pton, Newbury, Berkshire, RG16 , quoting Ref. 299. 3052(A)

SCIENTIFIC OFFICER POST

IN THE COMPUTER SIMULATION OF THE SOLID STATE.

The Computational Science Group of the Daresbury Laboratory has a vacancy for work on the computer simulation of solid state structures, both perfect and defective. It is intended that the programme be carried out in collaboration with ICI Corporate Laboratory, Runcorn, and University groups principally at Oxford and University College, London.

The post is offered in a permanent position at the level of Scientific Officer, in a salary scale of £3289 to £5085*depending on age, qualifications and experience. The successful candidate, who will have a good degree in Physics or Chemistry will be responsible for providing assistance to University groups wishing to make use of the computer programs made available by the project.

*(£3591 to £5486 with effect from 1.1.80)

Applicants, of either sex, should write (or telephone Warrington 65000, Ext.467) for an application form quoting reference DL/702 to:-

The Personnel Officer

DARESBURY LABORATORY

Science Research Council

Daresbury, Warrington WA4 4AD

Closing date - September 28th 1979.

3040(A)

Southampton THE UNIVERSITY

PHYSIOLOGY/ PHARMACOLOGY DEPARTMENT

BIOCHEMICAL AND INSTRUMENTATION TECHNICIAN GRADE 4

Applications are invited for an appointment to specialise in biochemical and instrumentation technical support for the Physiology/Pharmacology Group in the School of Biochemical and Physiological Sciences. The successful applicant will have experience and knowlege of basic biochemical instrumentation, including spectrometers, centrifuges, recording equipment etc. He will be responsible for providing specialist biochemical support for research and postgraduate training programmes. An interest in electronics would be an asset.

Minimum qualifications for this appointment are Science ONC or equivalent with at least 7 years appropriate experience, but candidates with degrees and research experience in Biochemistry, or other relevant experience, are encouraged to apply.

The appointment will be made at Grade 4 on salary scale £3,222 to £3,708 per annum (under review) with starting salary in accordance with qualifications and experience.

Applications giving date of birth, details of qualifications and experience and the names and addresses of two referees, should be sent to Mr C.N. Saull, The University, Southampton SO9 5NH, as soon as possible, quoting reference number 576/T. 3024(A)

Spectroscopy Section Head

A Spectroscopist is required to manage a small team providing spectroscopy services for the research and development chemists at our Chemotherapeutic Research Centre. The section will shortly acquire a Bruker 250 MHz Fourier transform nmr spectrometer and is also equipped with CW nmr, ir and uv spectrometers. The Section Head will be required to work closely with synthetic organic chemists to maximise the contribution of the spectroscopy facilities to the research effort. Candidates should preferably have 2 to 3 years post-doctoral experience and detailed knowledge of the application of carbon 13 and proton nmr spectroscopy to organic chemistry is essential.

This vacancy is at our Brockham Park site near Dorking, Surrey and free transport is available to and from local stations. Good starting salary, non-contributory pension scheme, flexible working hours, good sports and social facilities etc.

Please apply to: Miss E. A. Gunn, Site Personnel Officer, Beecham Pharmaceuticals, Research Division, Brockham Park, Betchworth, Surrey RH3 7AJ. Telephone: Betchworth 4111 Ext. 2271.

Beecham Pharmaceuticals

3017(A)

Royal Observatory, Edinburgh

Software Specialists

SCIENTIFIC OFFICERS/ HIGHER SCIENTIFIC OFFICERS

There are vacancies for software specialists at the Royal Observatory, Edinburgh.

The Observatory is responsible for three major national facilities — the 1.2m Schmidt telescope in Australia, the COSMOS high-speed measuring machine at Edinburgh and the 3.8m Infrared telescope in Hawaii. All facilities employ software specialists for developing control system software (for controlling telescopes, telescope instrumentation and high-speed measuring machine) and for developing scientific applications software for the reduction and analysis of data.

Initially, the successful applicants will be posted to the UK Infrared Telescope Unit. This 3.8m telescope is the largest of its kind in the world. The telescope and the ancillary instrumentation are controlled by two PDP 11/40 computers. The successful applicants will be responsible for the support and development of the telescope operating system, written in assembler, and the instrument control systems, written in higher level languages.

Applicants for these posts should have experience in one or more of the following fields: assembler language programming, FORTRAN, real-time software, and scientific applications packages or microprocessor systems. A good degree in Mathematics, the Physical Sciences or Computer Science is essential.

Because the telescope is situated at high altitude a special medical examination is given to all members of the UKIRT operating team on Hawaii. Success in that examination will be a pre-requisite for appointment to this post.

Salary will be in the range £4,218 to £6,211 (under review) depending upon age and experience. Non-contributory superannuation scheme.

SRC

Application forms and further details from: Personnel Officer, Royal Observatory, Blackford Hill, Edinburgh, EH9 3HJ. Tel: 031-667 3321.

CLOSING DATE: 21 SEPTEMBER, 1979

3059(A)

The Brewing Research Foundation requires a

PRINCIPAL SCIENTIST

as head of its

MICROBIOLOGY AND FERMENTATION SECTION

Candidates, aged between about 30 and 45 should have good academic qualifications and appropriate research experience. The successful candidate, who will be responsible to the Director for the development and organisation of the Section's research programme, will also be expected to carry out his own personal research.

The post offers and excellent opportunity for an alert and ambitious researcher to make a name in this important part of the food and drink industry.

The Foundation provides good conditions of service; the initial salary will be within the range £8,500 to £11,000.

Applications giving details of education, experience, papers etc. should be sent to the Assistant Director, Administration and Finance, Brewing Research Foundation, Lyttel Hall, Nutfield, REDHILL, Surrey, RH1 4HY. 3074(A)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF PHARMACOLOGY

Applications are invited from graduates for a temporary post of

RESEARCH ASSISTANT

in the above Department. The successful candidate will work on a project, supported by the MRC, investigating the role of O-methylation in the catabolism of circulating catecholamines. An interest in the application of biochemical techniques to pharmacological problems is essential. The appointment willbe made for a fided period of two years.

Starting salary in the range £3,775 to £4,333 on the 1B scale for Research and Analogous Staff (£3,775 to £5,488) (under review).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 103/3/D. Closing date for applications 21 September 1979. 3042(A)

ASTRONOMER ROYAL FOR SCOTLAND REGIUS CHAIR OF ASTRONOMY UNIVERSITY OF EDINBURGH

The Secretary of State for Scotland invites applications for the office of Astronomer Royal for Scotland and for the post of Regius Professor of Astronomy in the University of Edinburgh. The Science Research Council will participate in the selection of the successful applicant, so that he/she may be appointed by the Council as Director of the Royal Observatory, Edinburgh. It is hoped that the person appointed will assume responsibilities with effect from 1st October, 1980.

Further particulars may be obtained from the Secretary, Scottish Education Department, 8 George Street, Edinburgh EH2 2PF to whom 4 copies of applications, including the names of three referees, should be sent not later than Monday, 8th October 1979. 3044(A)

BRISTOL POLYTECHNIC

DEPARTMENT OF SCIENCE

RESEARCH ASSISTANTSHIP Ref R42/101

Applications are invited from graduates in biochemistry or a related subject for a

RESEARCH ASSISTANTSHIP

to study the pharmacological and biochemical properties of neurotransmitter receptors present in the central nervous system of insects. The position will be supported by an SRC research grant to Dr K J Cattell. Suitably qualified candidates will be encouraged to register with CNAA for a higher degree. The post is tenable for three years from 1 October 1979. Salary £3,480 plus £72 per annum.

Further details and application forms (to be returned by 10 September, 1979) from Personnel Officer, Bristol Polytechnic, Coldharbour Lane, Bristol BS16 1QY. Please quote appropriate Post Reference Number in all communications. 3046(A)

To place your advertisement in these pages Phone: 01-831-6901

ASSISTANT PROFESSOR

DEPARTMENT OF PHYSICS

UNIVERSITY OF BRITISH COLUMBIA

The University of British Columbia Vancouver expects, subject to the usu budgetary confirmation, to make a tenur track appointment at the Assistai Professor level in the Physics Departmei commencing July 1, 1980. Primai consideration will be given to applican with research interests in Intermedia Energy Physics centred around th TRIUMF meson facility situated on th U.B.C. campus. Outstanding candidates other fields are also invited to apply.

The successful candidate will be expecte to teach effectively at both the unde graduate and graduate levels. The closir date for applications is November 30, 197

Send resume, bibliography and th names of three professional references t Professor Garth Jones, Chairmai Committee on Initial Appointment Department of Physics, The University British Columbia, 2075 Wesbrook Plac Vancouver, BC, V6T 1W5 Canada.

W318(A)

FRESHWATER BIOLOGICAL ASSOCIATIO

SENIOR ANALYTICAL CHEMIST

Vacancy at Windermere Laborator Duties to include: general co-ordination analytical programme and development close liaison with biological staff; routi analysis; development of new metho oversight of radiochemical laborato including training of scientific staff techniques and safety.

Candidates should hold a first degree relevant industrial qualification and ha considerable practical experience. T pensionable post is graded HSO/SSO a starting salary would be £5,800 or abc (approx. 10% additional pay award is d 1st January 1980).

Further details and application for may be obtained from: The Secretai Freshwater Biological Association, T Ferry House, Ambleside, Cumbria LA 0LP. Closing date 14th September 1979 2979(A)

Required, starting October 1st,

Nuffield Department of Orthopaedic Surgery, University of Oxford

(1) BIOCHEMISTRY TECHNICIAN

for research project on glycoprotein metabolism in calcified tissues. Experience with chromatographic immunochemical and radioisotopic techniques useful, but not essential Salary on Whitley Council Medica Laboratory Scientific Officer scale £3,261 to £4,680, according to age qualifications and experience.

(2) TECHNICIAN

for research project on bone formatior in diseases of the skeleton. The work will include tissue culture photography and preparation o tissues for microscopy. Salary or Whitley Council Medical Laboratory Scientific Officer scale £2,169 to £3,26 (under review) according to age qualifications and experience.

Apply giving curriculum vitae and th names of two referees to Dr M Owen MRC Bone Research Laboratory Nuffield Orthopaedic Centre, Oxfor OX3 7LD.

3073(A)

CENTRAL PUBLIC HEALTH LABORATORY
COLINDALE

A Junior Medical Laboratory Scientific Officer is required by the Division of Microbiological Reagents and Quality Control.

Candidate must have two science 'A' levels, ONC in Sciences or a suitable degree.

Work could include Bacteriology, Serology and Virology and training for necessary examinations and state registration will be available.

Applications to Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London, NW9 5HT. Telephone 01 205 7041.
3019(A)

SIMON FRASER UNIVERSITY
Burnaby, British Columbia
DEPARTMENT OF CHEMISTRY
APPOINTMENT IN BIOCHEMISTRY

Applications are invited for a tenure track appointment at the assistant professor level, preferably with specialization in the area of macro-molecular biochemistry, starting September 1, 1980. The appointment is subject to budgetary approval. The successful applicant will be required to teach graduate and undergraduate courses in biochemistry and to establish a vigorous research program.
The current base of the assistant professor rank is $19,720 per annum.
Applications, including curriculum vitae, a concise statement of proposed research and the names of three referees, should be sent by December 31, 1979 to: C.H.W. Jones, Chairman, Department of Chemistry, Simon Fraser University, Burnaby, BC, Canada V5A 1S6.
W319(A)

THE LONDON HOSPITAL MEDICAL COLLEGE (University of London)
LABORATORY SCIENTIFIC OFFICER IN THE BONE AND JOINT RESEARCH UNIT

Laboratory Scientific Officer (state registered) required for this Research Unit. Duties will include studies of blood cell functions and studies of experimental models of arthritis in laboratory animals. Apply for further qualification encouraged. Salary in accordance with Whitley Council scales for medical laboratory scientific officers.
Details from Professor H.L.F. Currey, Bone and Joint Research Unit, The London Hospital Medical College, Turner Street, London E1 2AD, Tel. 01-247-5454 Ext. 422, quoting reference BJRU/7/79.
3065(A)

To place your advertisement in these pages Phone: 01-831-6901

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY
London SW7 2AZ
GRADE 5 TECHNICIAN

required to work with Dr D.M. Glover on the characterisation of segments of drosophila DNA cloned in E. coli. Experience in nucleic acid biochemistry and microbiology would be an advantage. Salary in the range £4,294 to £4,844 inc. (under review). The post is superannuable: there is a generous sick pay scheme. The working week is 37 and a half hours: 5 weeks' annual holiday plus several days in addition to public holidays at Christmas and Easter. There is a modern staff club and excellent facilities, with sports centre with swimming pool.
Application forms can be obtained from Mr I P Blench, Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ. Phone 589 5111 extn 1130. 3053(A)

UNIVERSITY OF SURREY
POSTDOCTORAL RESEARCH FELLOW
(Supported by G.E.C. Hirst Research Centre)
DEPARTMENT OF ELECTRONIC AND ELECTRICAL ENGINEERING

required for a two year appointment to work on the research of Ion Implantation applied to the production of GaAs field effect transistors. Applicants would have some experience of compound semiconductors and/or Ion Beam technology.
Salary according to experience will be in the region of £5,500 per annum on the Research and Analogous Staff Range 1A Scale.
Applications, consisting of a curriculum vitae and names and addresses of two referees, should be sent to Professor K.G. Stephens, Department of Electronic & Electrical Engineering, University of Surrey, Guildford, Surrey. GU2 5XH by 21st September, 1979. 3063(A)

UNIVERSITY OF OXFORD
DYSON PERRINS (ORGANIC CHEMISTRY) LABORATORY
POSTDOCTORAL RESEARCH ASSISTANT

A postdoctoral Assistant is required to join a group engaged in the synthesis of biologically active peptides. Experience is desirable but not essential. The appointment, which is for one year, renewable for a second year, will start as soon as possible. Salary (Grade 1A) in the range £4,776 to £5,850 (under review) according to age and experience, plus superannuation.
Applications, with curriculum vitae and the names of two referees, should be sent as soon as possible to Dr G T Young, The Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY. 2944(A)

UNIVERSITY OF ABERDEEN
DEPARTMENT OF MICROBIOLOGY
RESEARCH ASSISTANT

Applications are invited for the above post which is funded by SRC and is available for 3 years. The successful applicant will work with Dr G.W. Gooday on the characterisation of the enzyme chitin synthase from fungi. Candidates should have an Honours degree in Biochemistry, Microbiology or Biology.
Salary within Range 1B, £3,775 to £4,333, with appropriate placing.
Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 21 September 1979. 3036(A)

PHARMACOLOGISTS/ PHYSIOLOGISTS
— CARDIOVASCULAR

We are looking for two people to join a team involved in cardiovascular research at our R&D Laboratories situated in Loughborough.
The successful applicants will be involved in an expanding multidisciplinary project concerned with developing novel ideas for the treatment of heart disease and for evaluation of new compounds with potential therapeutic activity.
To fill one of these vacancies we are looking for a graduate who has about 3-4 years post-graduate, relevant experience (alternatively a recent PhD graduate). For the second vacancy we would like to appoint a more recent graduate.
The benefits of working for Fisons include a competitive salary, flexible working hours, pension facilities, an active Sports & Social Club and assistance with relocation expenses where appropriate.

Applicants (male or female) please contact Miss Cathy Mason, Personnel Officer, Fisons Limited, Pharmaceutical Division, Bakewell Road, Loughborough, Leicestershire. Tel Loughborough 66361, quoting reference number rd63/N.

FISONS
PHARMACEUTICAL DIVISION
3041(A)

Queen Charlotte's Maternity Hospital
Goldhawk Road, W6

This world famous Post Graduate Teaching Hospital has 161 beds and a high standard of patient care. It is situated within easy reach of shopping facilities at Hammersmith and Chiswick and is close to Stamford Brook tube station.

Basic Grade Scientific Officer
Department of Microbiology

Applications are invited from graduates in Nursing or Biological Sciences for the above post which is a Postgraduate Studentship. Previous experience is not essential. The successful candidate will be expected to supervise, develop and work in the Human Milk Bureau maintaining the Bank and to pursue a course of study leading to the research degree of PhD in the University of London. This special Postgraduate Studentship is offered for 4 years.

Applications with names of two referees to Professor R Harley at the above address. 3068(A)

THE NEW SOUTH WALES INSTITUTE OF TECHNOLOGY SYDNEY AUSTRALIA

School of Life Sciences

Principal Lecturer and Head

Department of Biochemistry

The New South Wales Institute of Technology is a corporate tertiary institution, established to provide a wide range of professional courses for those entering or already employed in industry, government, and technological fields.

The School of Life Sciences occupies a six-storey building at Gore Hill adjacent to the Royal North Shore Hospital. There are well equipped laboratories for teaching and research in Biochemistry, Pathology, Microbiology, Environmental Biology, Physical Biology and General Biology. The school also jointly operates the Gore Hill Research Laboratories and an Electron Microscopy unit with the Royal North Shore Hospital.

The appointee will be responsible to the Head of School for the teaching and development of undergraduate and postgraduate programs in Biochemistry. This will include the continuing development of a postgraduate course in Clinical Biochemistry.

It is anticipated that the appointee will possess substantial postgraduate qualifications and have relevant teaching and administrative experience. A professional interest in Clinical Biochemistry or Biochemistry related to Industrial applications would be an advantage.

The President of the Institute, Dr. R.L. Werner will be available in London for preliminary interviews from 10-21 September inclusive.

Further information on the Institute and conditions of employment may also be obtained from Miss Challoner, at the address below.

Conditions and Method of application:

The position carries a salary range of $A26,644 to $A28,809 P.A.

Fares and a contribution toward removal and initial accommodation expenses will be provided for an overseas appointee. A housing loan scheme is also available. With consent of council, academic staff are permitted to undertake limited consulting work.

Due to a change in the salary range the position is being readvertised, and the closing date for applications has now been extended to 1st October, 1979. Previous applicants will automatically be carried forward and need not re-apply.

Applicants should arrange for three confidential referees' reports to arrive by the same date. Applications should include, address, phone number, personal details, experience, qualifications, affiliations, publications, research work conducted, and the names and addresses of those referees' contacted. Applications and referees' reports are to be sent to: **The Official Secretary, N.S.W. Government Offices, 66 Strand, London WC2N 5LZ. Telephone 01-839 6651.** 3051(A)

EDINBURGH COLLEGE OF ART HERIOT-WATT UNIVERSITY

Department of Architecture

LANDSCAPE TECHNICIAN

Applications are invited for the post of Technician for the B.A. (Hons) Course in Landscape Architecture. Candidates will be expected to assist with the development of project work and should have a sound scientific background with experience of landscape planning or conservation work. People with training in animal or plant ecology should apply.

Salary scale — £3,222 to £4,758 with placing according to age and experience.

Further information and application forms may be obtained from:—

The Secretary and Treasurer,
Edinburgh College of Art,
Lauriston Place,
Edinburgh, EH3 9DF

3014(A)

CHAIR IN HYGIENE AND ENVIRONMENTAL HYGIENE

at the

RUPRECHT-KARLS-UNIVERSITÄT, HEIDELBERG

Applications are invited for the above position. The applicant should have a broad knowledge in the field of hygiene and environmental hygiene and have the requisite teaching experience. His/her work should already have found international recognition.

The microbiological-diagnostic laboratories of the University Clinic belong to the institute. The institute also has a medical examination unit. The applicant should have experience in the organisation and direction of such an institute as well as in the field of micro-biological diagnosis.

In addition to modern diagnostic apparatus the successful applicant will have at his/her disposal ample laboratory facilities for research as well as the necessary materials and personnel.

Applications should be sent to the Fakultät für Theoretische Medizin, Im Neuenheimer Feld 346, D-6900 Heidelberg, by 15th October, 1979. W327(A)

The Queen's University of Belfast

RESEARCH ASSISTANT

Departments of Pure and Applied Physics and Midwifery & Gynaecology

Applications are invited for a graduate research assistantship, supported by the Medical Research Council, to work on a collaborative investigation of human sperm motility using laser light scattering techniques. The appointment is tenable for up to three years and initial salary will be in the range £3,775 to £4,333 (under review). Applicants must hold a degree in the physical sciences, experience in computing would be advantageous. A suitably qualified person may register for a higher degree.

Applications with curriculum vitae and the names and addresses of two referees should be sent to the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. Closing date: *28th September, 1979.* 3016(A)

UNIVERSITY OF LIVERPOOL

POST DOCTORAL RESEARCH IN CONNECTIVE TISSUE

The Department of Veterinary Clinical Studies is to make an experimental study, over five years, of the changes in the biochemical structure of the uterine cervix at the time of birth in animals.

Applications are invited from suitably qualified candidates, preferably with post-doctoral experience in connective tissue chemistry, to lead the investigation of the interaction of collagen and proteoglycans in this tissue.

The post is tenable from 1st October, 1979, for one year in the first instance and is renewable.

The initial salary will be within the range £4,232 to £5,591 per annum (under review).

Applications, together with the names of three referees, should be received not later than 21st September, 1979, by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote ref: RV/752/N. 2969(A)

ST GEORGE'S HOSPITAL MEDICAL SCHOOL

(University of London)

POSTGRADUATE RESEARCH ASSISTANT

Required from 1 October in Department of Physiology for project on effects of alcohol on the central nervous system. Applicants should have an honours degree in biological science preferably with knowledge of biochemical techniques. The appointment is for 2 years funded by The Medical Council on Alcoholism. Emoluments £3,775 to £4,055 plus £502 London Allowance. Further information from Dr. T. Stone Tel. 01-672 1255 ext 4657.

Application forms from the Establishment Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. Closing date 20 September. 3055(A)

To place your advertisement in these pages Phone: 01-831-6901

AN FORAS TALUNTAIS

requires an **ENGINEER** at its Oak Park Research Centre, Carlow, for research in the area of solar energy in agriculture and mainly relating to biomass fuels.

ESSENTIAL: An honours University degree, or equivalent, in Engineering or related discipline.

DESIRABLE: An appropriate post-graduate qualification and/or suitable experience.

Appointment up to and including Senior Research Officer level depending on qualifications and experience. Marriage and children's allowances. Non-contributory pension scheme and provision for widows' and orphans' pensions.

Application forms and further particulars obtainable from the Personnel Department, An Foras Taluntais Headquarters, 19 Sandymount Avenue, Dublin 4 (Phone: 01-688188).

Latest date for receipt of completed forms Friday 21st September, 1979.

3049(A)

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY
DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL RESEARCH ASSISTANT IN MOLECULAR BIOLOGY

Applications are invited for the above position to work on the genome organisation and expression of a plant virus with a circular single-stranded DNA genome. The investigation is a prelude to utilisation of the virus DNA as a transforming vehicle for the genetic modification of plant cells. Experience in some aspect of nucleic acid or protein biochemistry would be desirable. The post, which is supported by the science research council, is available for a maximum of three years, with a starting salary on research grade 1A within the range £4,260 to £4,805 p.a. To be reviewed 1/10/79., plus London allowance of £502 p.a.

Applications including curriculum vitae and names and addresses of two referees should be sent to Dr K.W. Buck, Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ. 3027(A)

DEPARTMENT OF METALLURGY AND SCIENCE OF MATERIALS
UNIVERSITY OF OXFORD
RESEARCH FELLOW

Applications are invited for a research fellow to study the effects of radiation damage in ceramic insulators. These will be studied both *in situ* in a HVEM and in a conventional 100kV TEM. The project is in collaboration with AERE Harwell. Candidates should possess a PhD degree or equivalent. The post is tenable for up to 3 years. Salary range £4,232 to £7,145 p.a. Applications with brief curriculum vitae and names and addresses of two referees should be sent to the Administrator, Department of Metallurgy and Science of Materials, Parks Road, Oxford OX1 3PH as soon as possible. 3039(A)

ASSISTANTSHIPS

UNIVERSITY OF DURHAM
DEPARTMENT OF ZOOLOGY
POST-DOCTORAL BIOCHEMIST/PHYSIOLOGIST

ired for a three year Senior Research stantship tenable as soon as possible, vestigate the control of lipogenesis and ibrane biogenesis during hormone-ced cell differentiation in avian liver. arch-experience in biochemistry or a ed field is essential and experience of culture/enzymology/immuno-istry would be an advantage. Initial y in the range £4,333 to £4,910 per im within Range 1A plus super-ation.

pplications (3 copies) naming three ees should be sent by 28 September to the Registrar and Secretary, ice Laboratories, South Road, am, DH1 3LE, from whom further culars may be obtained. 3064(P)

UNIVERSITY OF EXETER
POST-DOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for the post of Research Assistant in connection with an investigation, supported by the SRC, of the stereochemistry of branched-chain amino acid biosynthesis. Applicants should have experience in organic synthesis and familiarity with radiotracer techniques would be an advantage.

The successful candidate will be appointed on the salary range £4,232 × £273 to £5,048 p.a. plus U.S.S. The post is tenable for two years.

Applications, giving the names of two referees, should be sent as soon as possible to Dr D.H.G. Crout, Department of Chemistry, University of Exeter, Stocker Road, Exeter EX4 4QD. 3066(P)

Re-advertisement

Centre for Applied Microbiology & Research, Porton Down
DIAGNOSTIC REAGENTS LABORATORY

Post-Graduate Research Assistantship

A graduate research assistant with at least a 2nd Class Hons. Degree is required to investigate the use of triazine chromophores in protein and enzyme purification, immobilisation, assay and structural analysis. The successful candidate will be expected to register for a PhD. The post is funded by a research contract and is tenable for up to 3 years.

Salary will be in the range £3,486 to £4,935 increasing to £3,807 to £5,316 on 1.1.1980. NHS terms and conditions of service.

This post may be discussed informally by contacting Dr A. Atkinson, Director, Diagnostic Reagents Laboratory. Tel. 0980-610391, Ext.406/365.

Applications, including curriculum vitae and two academic referees should be sent to Mrs M. Bushby, Personnel Officer, CAMR, Porton Down, Salisbury, Wilts. Closing date 14th September, 1979.

PHLS

3070(P)

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF APPLIED BIOLOGY

Applications invited for three-year A.R.C. financed

RESEARCH ASSISTANTSHIP

for work on intra-crop diversity for the control of brassica insect pests starting 1 October 1979 or as soon as possible thereafter. Appointee expected to register for PhD. Salary (to cover fees and living) according to age in range £3,418 to £4,776.

Further details from, and applications with full personal particulars and names and addresses of two referees to the Secretary, Department of Applied Biology, Pembroke Street, Cambridge, CB2 3DX, by 15 September 1979. 3038(P)

CHELSEA COLLEGE (University of London)
ROLE OF LIPIDS IN PHOTOSYNTHESIS

Applications are invited for a S.R.C. funded

RESEARCH ASSISTANTSHIP

for work on the role of lipids in the organisation of photosynthetic membranes. Applicants should have a good honours degree in biophysics, biochemistry or chemistry.

The post has a starting salary of £3,775 (plus £502 London Allowance) and is available for up to three years.

Further particulars can be obtained from Dr W.P. Williams, Department of Biophysics and Bioengineering, Chelsea College, 17a Onslow Gardens, London S.W.7. 3028(P)

FELLOWSHIPS

UNIVERSITY OF HULL
DEPARTMENT OF APPLIED PHYSICS
RESEARCH FELLOW

Applications are invited for a research fellowship, which is immediately available, for a period of two years (renewable). The post is suitable for graduates with at least three years' research experience in the field of solid state physics. The person appointed will be required to investigate the use of ion implantation for fabricating fast, photovoltaic, infrared detectors in materials such as HgCdTe. The study is supported by the Ministry of Defence (CVD) and is monitored by R.S.R.E., Malvern.

Salary scale: At an appropriate point on the scale £4,333 to £7,521 per annum (from 1 October 1979).

Applications (two copies) should give full details of education, qualifications and research experience, together with the names of two referees and should be sent by 10 September 1979 to Dr E.L. Thomas, Department of Applied Physics, The University of Hull, Hull, HU6 7RX from whom further details may be obtained. 3025(E)

RESEARCH FELLOWSHIP

A post-doctoral fellow with experience in immunology, somatic cell genetics or DNA cloning technology required for a project investigating the genetic control of development. Specific experiments planned include the genetic manipulation of murine teratocarcinoma stem cells and various stem cell hybrids.

Three year appointment. Salary range £5,800 to £7,100. For further information telephone Dr P. Goodfellow (01.242.0200 ext 265).

Applications with curriculum vitae and the names of two referees should be sent to the Personnel Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, WC2 quoting reference 371/79. 2980(E)

Fellowship in Telecommunications

Radio Research Board Australia

As part of its activities designed to foster research in telecommunications and radio science in the universities and other appropriate organizations, the Radio Research Board has established a Fellowship scheme. Under this scheme one Fellowship may be awarded each year for full-time research by young scientists or engineers of exceptional promise and proven merit. These are post doctoral awards tenable for a period of two years in an Australian university, approved research institute or industrial laboratory as determined by negotiation between a prospective Fellow and the Board. The agreed area of research will also be determined by negotiation but the Board is open to consider any proposal in the broad field of telecommunications and radio science.

Qualifications The applicant should have a PhD degree or its equivalent, and be preferably under 30 years of age.

Radio Research Board Fellowships are available to applicants of all nationalities but they are asked to indicate their future plans.

Stipend $A17,131 per annum — increased to $A18,403 per annum at age 28 years. Stipend is subject to income tax.

Allowances Payable in respect of a Fellow's dependent spouse ($500 p.a.) each dependent child ($200 p.a.), "superannuation-like" payments (up to ten per cent of stipend), appropriate insurance coverage and necessary travel expenses. Host institutions may be paid an allowance towards the cost of supporting the Fellow.

Applications Persons interested in applying for the above Fellowship should obtain application forms and a statement of the conditions from The Secretary, Radio Research Board, PO Box 225, DICKSON, ACT 2602. AUSTRALIA.

Applications close on 30 November, 1979 and in the normal course of events candidates may expect to be advised on the outcome of their applications by the end of March, 1980.

3071(E)

COLAISTE NA HOLLSCOILE CORCAIGH
UNIVERSITY COLLEGE CORK

W.K. KELLOGG FOUNDATION RESEARCH FELLOWSHIPS

Two Research Assistants with post graduate experience are required for research work in Food Chemistry, Food Engineering, Food Microbiology or Food Technology.

Candidates should preferably have a PhD in an area relevant to the research work and be available to start before December 31st, 1979.

The appointments are funded by the W.K. Kellogg Foundation and are of one year's duration. The value of each Fellowship is £4,400 (Irish pounds).

Curriculum Vitae with names of two referees should be sent *before October 31st 1979 to:-*

Dr S. Condon,
Dean, Dairy Science Faculty,
University College,
Cork (Ireland).

3061(E)

MEDICAL RESEARCH COUNCIL
National Institute for Medical Research
LABORATORY OF BACTERIAL INFECTIVITY

Applications are invited for two postdoctoral fellowships:

1. An organic chemist is required for a project involving the synthesis of a new type of antibacterial agent. Some experience in natural product chemistry would be an advantage.
2. A biochemist or microbiologist to study the mechanism of action of novel antibacterial agents. An understanding of bacterial genetics would be an advantage.

Both fellowships, to start as soon as possible, would be for a period of up to three years and will be in the present salary range £4,940 to £6,627 p.a. plus £502 p.a. London Allowance; superannuation provision.

Applications, including curriculum vitae and the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, LondonNW7 1AA, quoting reference B1.1. 3015(E)

FRIEDRICH MIESCHER-INSTITUT:
A Postdoctoral Fellowship

in Biochemistry is available for the area of DNA synthesis in cultured mammalian cells. Experience in purification of DNA enzymes, DNA binding proteins or *in vitro* DNA replication is desired. The fellowship will run for two to three years.

Candidates should send c.v. and three letters of reference to Dr. L. Jimenez de Asua, Friedrich Miescher-Institut, PO Box 273, CH-4002, Basel, Switzerland. W292(E)

UNIVERSITY OF HULL
DEPARTMENT OF PLANT BIOLOGY
POST-DOCTORAL RESEARCH FELLOWSHIPS IN PLANT DISEASE PHYSIOLOGY

Two postdoctoral research fellowships funded by the ARC are available for research in plant disease physiology under the supervision of Professor J. Friend. The posts are tenable for a maximum period of 3 years. Applicants for both posts should have completed three years postgraduate research and have, or expect shortly to be awarded, a PhD degree. The background experience required for each post is as follows:

1. Histochemical investigation of the resistance of potatoes to *Phytophthora infestans*. Applicants should have experience in histochemical and cytochemical techniques at the light and electron microscope level.
2. Cell wall degradation in onions infected by *Botrytis allii* and *Sclerotium cepivorum*. Applicants should have experience in plant biochemistry, preferably on either polysaccharides or enzymology.

Salary from 1 October 1979 will be on the scale £4,333 to £7,521 per annum.

Further particulars are available from Professor J. Friend, Department of Plant Biology, The University, Hull, HU6 7RX to whom applications in the form of a curriculum vitae, giving full details of research experience and naming two referees, should be sent by 14 September 1979. 3020(E)

ALL SOULS COLLEGE
Oxford

RESEARCH FELLOWSHI

The College intends to make an electio a Research Fellowship in the course of academic year 1979-80. It is anticip that the election will be made in or at February 1980.

The Research Fellowship (which is o to men and women) will be tenable f period of seven years. The College w normally renew the Fellowship if the evidence of satisfactory achievement. retiring age is 67. The stipend payab related to the age of the Fellow u appointment.

The Fellowship will be open to t working in the following fields of st Law, History, Philosophy, Poli Economics, English, Classics Mathematical Sciences.

Further particulars, including salary terms of appointment, may be obta from the Warden's Secretary, All S College, Oxford. Applications, w should contain the names of not more three referees, must reach the Warde later than 1 October, 1979, and shou marked "Research Fellowship".

3060(

UNIVERSITY COLLEGE LONDON
CHEMISTRY DEPARTMENT
POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a Postdoctoral Research Fellowship tenable from 1st October 1979 or as soon as possible thereafter. The appointment is for one year with the possibility of extension for a second year.

The initial salary will be in the range £4,673 to £5,576 inclusive of LA 1.10.78 scales + U.S.S. depending on age and experience. The successful applicant will work with Professor F. Sondheimer F.R.S. on the synthesis of non-benzenoid aromatic compounds and related substances.

Applicants should arrange for letters of recommendation from two referees as well as a curriculum vitae to be sent as soon as possible to Professor F. Sondheimer, Chemistry Department, University College London, 20 Gordon Street, London WC1H 0AJ. 3030(E)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF BIOCHEMIST

Applications are invited for an N financed Post-doctoral

RESEARCH FELLOWSH

to work with Dr. Ian P. Trayer on the of myosin light chains in actom interaction. The successful applican join a group investigating different as of the actomyosin interaction usi variety of chemical and phy techniques. The post is tenable fro October 1979, and is funded for years. Experience in general pr chemistry and enzymology would advantage. Starting salary will be bet the points £4,333 and £4,910 (plus US the Research Fellow 1A scale, depe on age and experience.

Further details can be obtained Miss L R Heyes, Senate Reg University of Birmingham, PO Box Birmingham B15 2TT to w applications, (two copies) together curriculum vitae and names of referees, should be returned not later 17th September, 1979. 3058

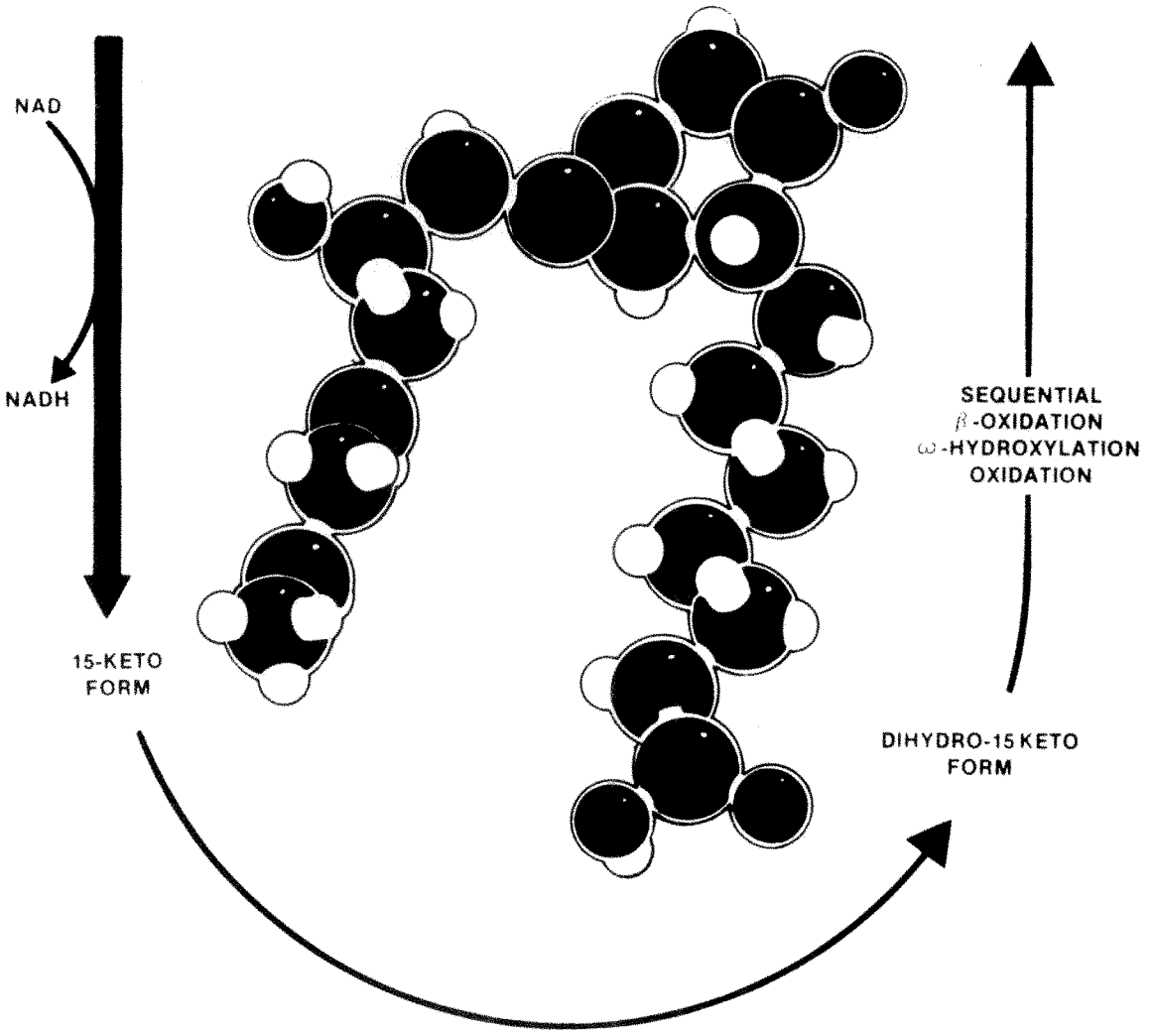
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